STUDIES ON ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITIES OF *CURCUM LONGA, MIMOSA PUDICA, EUPHORBIA SERRATA* MEDICINALLY USED IN NIGERIA.

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CERTIFICATION

We hereby certify that this Dissertation "Studies on antibacterial, antifungal and antioxidant activities of *Curcum longa, Mimosa pudica, Euphorbia serrata* medicinally used in Nigeria" was carried out by ASSUMPTA UGBONMA UGENYI with Reg.no. 20124771778, and in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Environmental Health Biology in the Department of Biotechnology of Federal University of Technology Owerri.

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DEDICATION

To my Late father Dr. Michael Ezeonyeulo Ezirike [Mee].

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TABLE OF CONTENTS

Title page

Certification i

Dedication ii

Acknowledgements iii

Table of contents v

List of tables xii

List of figures/ charts/plates xiii

Abstract xiv

CHAPTER I: INTRODUCTION

1.0 Background information 1

1.1 Statement of problem 3

1.2 Aim of study 3

1.3 Objectives of study 3

1.4 Research Questions 4

1.5 Research Hypothesis 4

1.6 Justification of study 4

1.7 Scope of the research work 5

CHAPTER II: LITERATURE REVIEW

2.0 Literature review 6

2.1 *Euphorbia serrata* 6

2.2 *Mimosa pudica* 8

2.3 *Curcum longa* 11

2.4. Phytochemicals 15

2.5 Effects of Phytochemicals 17

2.5.1 Antioxidant Activity 17

2.5.2 Antimicrobial Activity 18

2.5.3 Anti-inflammatory Activity 18

2.5.4 Phyto-hormones Activity 18

2.5.5 Alteration of Biotransformation Enzyme Activity 18

2.6 Phytochemical and their Roles 19

2.6.1 Alkaloids 19

2.6.2 Flavonoids 19

2.6.3 Saponnins 22

2.6.4 Tannins 23

2.7 Microorganisms 24

2.7.1 *Wohlfahrtiimonas chitiniclastica* 24

2.7.2 *Alcaligenes faecalis* 24

2.7.3 *Klebsiella pneumoniae* 26

2.7.4 *Proteus mirabilis* 26

2.7.5 *Candida tropicalis* 28

2.7.6 *Wickerhamomyces anomalus* 29

2.8 Toxicological Assessment of Medicinal Plants 30

CHAPTER III: MATERIALS AND METHOD

3.0 Materials and Method 32

3.1 Area of Study 32

3.2 Reagents and Chemicals Used 32

3.3 Equipment and Instrument Used 32

3.4 Collection of Sample Materials 32

3.5 Source of Test Microorganisms Used in the Study 33

3.6 Plant Preparation 33

3.6.1 Sample Extraction 33

3.6.2 Aqueous Extraction 33

3.6.3 Ethanol Extraction 33

3.6.4 Methanol Extraction 35

3.7 Phytochemical Screening 35

3.7.1 Qualitative Analysis of Plant Samples 35

3.7.2 Test for Saponins 35

3.7.3 Test for Alkaliods 36

3.7.4 Test for Cardiac Glycosides 37

3.7.5 Test for Terpenoids 37

3.7.6 Test for Steroids 37

3.7.7 Test for Tannins 37

3.7.8 Test for Flavonoids 38

3.7.9 Test for Phenol 38

3.8 Quantitative Screening of the Plants Samples 38

3.8.1 Estimation of Saponin 38

3.8.2 Estimation of Flavonoids 39

3.8.3 Estimation of Alkaloids 39

3.8.4 Estimation of Tannin by Titration 40

3.8.5 Estimation of Cardiac Glycosides 41

3.8.6 Estimation of Phytate 41

3.8.7 Estimation of Terepenoid 41

3.8.8 Estimation of Phenol 42

3.8.9 Estimation of Steroids 42

3.9 Sterilization of Materials Used 42

3.10 Isolation of Test Organisms 42

3.10.1 Identification of Isolates by DNA Sequencing 43

3.10.2. DNA Extraction 44

3.10.3 Polymerase Chain Reactions 45

3.10.4 DNA Sequencing 46

3.11 Media Preparation 47

3.11.1 Muller Hinton Agar 47

3.11.2 Sabouraud Dextrose Agar 47

3.11.3 Media Sterilization and Pouring 47

3.12 Inoculum Preparation 47

3.12.1 Standardization of the Inoculum for Bacteria Test 47

3.12.2 Standardization of the Inoculum for Fungi Test 48

3.13 Determination of Antibacterial activities of *E. serrata, M. pudica and C.longa* Extracts. 48

3.14 Determination of Antifungal Activities of *E. serrata, M. pudica and C.longa* Extracts. 48

3.15 Determination of the Antioxidant Activities of *E. serrata, M. pudica and C.longa*

Extracts. 49

3.15.1 Determination of Free Radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity. 49

3.15.2 Determination of Nitric Oxide Radical Scavenging Activity 49

3.15.3 Determination of Hydrogen Peroxide (H_2O_2) Radicals Scavenging Activity 50

3.15.4 Determination of Total Reducing Power 50

3.16 Toxicological Tests 50

3.16.1 Determination of Acute Oral Toxicity 51

3.16.2 Determination of Sub-chronic Oral Toxicity 52

3.16.3 Termination of the Experiment 52

3.16.4 Collection of Blood Sample 52

3.17 Biochemical Analyses 53

3.17.1 Determination of serum alanine Aminotransferase Activity 53

3.17.2 Determination of Creatinine 54

3.17.3 Estimation of Extent of Lipid Peroxidation (Malondialdehyde) 54

3.17.4 Determination of Aspartate aminotransferase (AST) 55

3.17.5 Determination of Catalase Activity 55

3.17.6 Estimation of Glutathione Peroxidase 56

3.17.7 Estimation of Reduced Glutathione 56

3.17.8 Determination Total Serum Proteins 57

3.17.9 Determination of Alkaline Phosphate (ALP) 57

3.17.10 Determination of Superoxide Dismutase (SOD) Activity 58

3.17.11 Determine of Urea 58

3.17.12 Determination of Lactate Dehydrogenase (LDH) 60

3.17.13 Determination of C-reactive Protein 60

3.17.14 Determination of Serum Albumin 61

3.18 Haematological Analyses 62

3.18.1 Determination of Erythrocyte Count by Haemocytometry 63

3.18.2 Determination of Total Leucocyte by Haemocytometry 63

3.18.3 Estimation of Packed cell volume (PVC) 64

3.18.4 Determination of Haemoglobin (Hb) concentration 64

3.18.5 Determination of Platelet 65

3.19 Histopathological analyses 65

3.20 Data analysis 65

CHAPTER IV: RESULTS AND DISCUSSION

4.1 Results 68

4.1.1 Qualitative and Quantitative Phytochemical Result 68

4.1.2 Identified Microorganisms Used in the Study 69

4.1.3 Antibacterial and Antifungal Activities of *E.serrata, M.pudica* and *C.longa* Extracts 78

4.1.4 Antioxidant Activities of *E.serrata, M.pudica* and *C.longa* extracts. 89

4.1.4.1 Effects of Ethanol and Methanol Extracts of *E.serrata, M.pudica, C.longa* and Ascorbic acid on Scavenging of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical. 89

4.1.4.2 Effects of Ethanol and Methanol Extracts of *E.serrata, M.pudica, C.longa* and Ascorbic acid on Scavenging of Nitrous Oxide Radical. 93

4.1.4.3 Effects of Ethanol and Methanol Extracts of *E.serrata, M.pudica, C.longa* and Ascorbic acid on Scavenging of Hydrogen Peroxide (H_2O_2) Radical. 97

4.1.4.4 Reducing Power of Ascorbic acid sSandard used with Ethanol and Methanol Extracts of *E.serrata, M.pudica, C.longa*. 101

4.1. 5 Acute Oral Toxicity 105

4.1.6 Sub-chronic Oral Toxicity 105

4.1.6.1 The Effects of Administration of Ethanol Extracts of *E.serrata M.pudica and C.longa* for28 days on Body Weight of Albino Rats. 107

4.1.6.2 The Effects of Administration of Ethanol Extracts of *E.serrata, M.pudica* and *C. longa* for 28 days on Liver Function Parameters in Albino Rats 111

4.1.6.3 The Effects of Administration of Ethanol Extracts of *E.serrata, M.pudica* and *C. longa* for 28 days on Renal Function Parameters in Albino Rats. 113

4.1.6.4 The Effects of Administration of Ethanol Extract of *E.serrata, M.pudica* and *C. longa* for on 28 days on Oxidative Stress Function Parameters in Albino Rats.115

4.1.6.5 The effects of Administration of ethanol leaf extract of *E.serrata, M.pudica* and *C. longa* for 28 days on C-Reactive protein 117

4.1.6.6 The Effect of Administration of Ethanol Extracts of *E.serrata, M.pudica* and *C. longa* on Hematological Profile in Albino Rats. 117

4.1.6.7 Relative Organ Weights of Rats Treated with Ethanol Extracts in Sub-chronic Oral Toxicity Study. 120

4.1.7. Histopathological Result 120

4.1.7.1 The Effect of 200 mg/kg and 400 mg/kg of Ethanol Extracts of *C. longa* (1a and 2a); *M. pudica* (3a & 4a); *E. serrata* (5a & 6a) on Liver Histomorphologies of Albino Rats in Sub-chronic Oral Toxicity Study. 120

4.1.7.2 The Effect of 200 mg/kg and 400 mg/kg of Ethanol Extracts of *C. longa* (1b and 2b); *M. pudica* (3b & 4b); *E. serrata* (5b & 6b) on Kidney Histomorphologies of Albino Rats in Sub-chronic Oral Toxicity Study. 122

4.1.7.3 The Effect of 200 mg/kg and 400 mg/kg of Ethanol Extracts of *C. longa* (1c and 2c); *M. pudica* (3c & 4c); *E. serrata* (5c & 6c) on Heart Histomorphologies of Albino Rats in Sub-chronic Oral Toxicity Study. 122

4.2 Discussion 126

CHAPTER V: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion 136

5.2 Recommendations 137

References 138

Appendices 168

LIST OF TABLES

3.1: DNA sequencing for microbial samples used in the study. 46

4.1: Qualitative phytochemical screening of the leaves of *E. serrata, M. pudica* and rhizome of *C. longa.* 70

4.2: DNA Sequence and Gene Bank Ascession numbers of the bacteria and fungi used 72

4.3: Comparative analysis of effects of ethanol and methanol extracts of *E.serrata, M.pudica*, *C.longa* and ascorbic acid on DPPH radical scavenging. 92

4.4: Comparative analysis of effects of ethanol and methanol extracts of *E.serrata, M .pudica*, *C.longa* and ascorbic acid on scavenging nitric oxide radical. 96

4.5: Comparative analysis of effects of ethanol and methanol extracts of *E.serrata, M .pudica*, *C.longa* and ascorbic acid on scavenging H_2O_2 radical. 100

4.6: Comparative analysis of reducing power of Ascorbic acid standard with ethanol extracts of *E. serrata, M. pudica* and *C.longa.* 104

4.7: Acute oral toxicity of ethanol extracts of *C. longa, M. pudica* and *E. serrata.* 106

4.8: Relative organ weights of rats treated with ethanol extracts in sub-chronic oral toxicity study. 121

LIST OF FIGURES/ CHARTS/ PLATES

Plate 2.1: A *Euphorbia serrata* plant. 14

2.2 : A *Mimosa pudica* plant. 14

2.3a : *Curcum longa* plant 14

2.3b & c: *Curcum longa* rhizome and grounded turmeric. 14

4.1: The photomicrograph of liver sections of all groups. 123

4.2: The photomicrograph of kidney sections of all groups. 124

4.3: The photomicrograph of heart sections of all groups. 125

Figure 2.1: Types of phytochemicals. 16

2.2a: The skeleton structure of the flavones. 20

2.2b: The skeleton structure of main classes of flavonoids. 22

2.3: Structure of Saponnin. 22

2.4: Structure of Tannin. 23

3.1: Map showing study area. 34

4.1: The percentage quantitative constituent of phytochemicals of *E.serrata, M. pudica* and *C.longa* 71

4.2: Antibacterial and antifungal activities of ethanol extracts of *E. serrata, M pudica, C.longa* and control at 50 mg/ml. 80

4.3: Antibacterial and antifungal activities of methanol extracts of *E. serrata M.pudica, C.longa* and control at 50 mg/ml. 81

4.4: Antibacterial and antifungal activities of aqueous extracts of *E.serrata, M.pudica, C.longa* and control at 50 mg/ml. 82

4.5: Antibacterial and antifungal activities of ethanol extracts of *E. serrata, M pudica, C.longa* and control at 37.5 mg/ml. 83

Figure 4.6: Antibacterial and antifungal activities of methanol extracts of *E. serrata*

M.pudica, C.longa and control at 37.5 mg/ml. 84

4.7: Antibacterial and antifungal activities of aqueous extracts of *E.serrata, M.pudica, C.longa* and control at 37.5 mg/ml. 85

4.8: Antibacterial and antifungal activities of ethanol extracts of *E. serrata, M pudica, C.longa* and control at 25 mg/ml. 86

4.9: Antibacterial and antifungal activities of methanol extracts of *E. serrata M.pudica, C.longa* and control at 25 mg/ml. 87

4.10: Antibacterial and antifungal activities of aqueous extracts of *E.serrata, M.pudica, C.longa* and control at 25 mg/ml. 88

4.11: Effects of ethanol extracts of *E.serrata, M. pudica, C. longa* and ascorbic acid on scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. 90

4. 12: Effects of methanol extracts of *E.serrata, M. pudica, C. longa* and ascorbic acid on scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. 91

4.13: Effects of ethanol extracts of *E.serrata, M. pudica, C. longa* and ascorbic acid on scavenging nitric oxide radical. 94

4.14: Effects of methanol extracts of *E.serrata, M. pudica, C. longa* and ascorbic acid on scavenging nitric oxide radical. 95

4.15: Effects of ethanol extracts of *E.serrata, M. pudica, C. longa* and ascorbic acid on scavenging hydrogen peroxide (H_2O_2) radical. 98

4.16: Effects of methanol extracts of *E.serrata, M. pudica, C. longa* and ascorbic acid on scavenging hydrogen peroxide (H_2O_2) radical. 99

Figure 4.17: Reducing power of ascorbic acid standard used with ethanol extracts of *E.serrata,M. pudica* and *C. longa* at 700nm. 102

4.18: Reducing power of ascorbic acid standard used with methanol extracts of *E.serrata,M.pudica,* and *C. longa* at 700nm. 103

4.19: The effect of ethanol extracts of *E.serrata on* body weights of albino rats. 108

4.20: The effect of ethanol extracts of *M.pudica* on body weights of albino rats. 109

4.21: The effect of ethanol extracts of *C. longa* on body weights of albino rats. 110

4.22: The effects of ethanol extracts of *E.serrata, M. pudica* and *C. longa* on liver function parameters in albino rats. 112

4.23: The effects of ethanol extracts of *E.serrata, M. pudica* and *C. longa* on renal function parameters in albino rats. 114

4.24: The effects of ethanol extracts of *E.serrata, M. pudica* and *C. longa* on oxidative stress function parameters in albino rats. 116

4.25: The effects of ethanol extracts of *E.serrata, M. pudica* and *C. longa* on C- Reacttive protein 118

4.26: The effects of ethanol extracts of *E.serrata,M. pudica* and *C. longa* on haematological profile in albino rats. 119

ABSTRACT

Three plants –*Euphorbia serrata, Mimosa pudica L.* and *Curcum longa L.* suggested from their ethno-medicinal uses to possess antimicrobial, and antioxidant activities were evaluated for their different biological activities. Phytochemical analyses were carried out using standard qualitative and quantative analytical methods. Antimicrobial activities were determined by well diffusion method for the isolates; *Proteus mirabilis, Alcaligenes faecalis, Klebsiella pneumoniae, Wohlfahrtiimonas chitiniclastica, Candida tropicalis* and *Wickerharmoyces anomalus i*dentified using DNA sequencing. The antioxidant potentials of the extracts were determined by their abilities to scavenge 2, 2-diphenyl-1- picrylhydrazyl radical, nitric oxide radical, hydrogen peroxide.The antioxidant potentials were further determined by the reducing power using standard methods. Acute and sub-chronic oral toxicity of the extracts were carried out with Lorke′s method using male albino rats. Pathology examinations of the organs (liver, kidney and heart) were also evaluated. Results of phytochemical analysis revealed the presence of Alkaloids (*E. serrata* 6.85%; *M. pudica 2*.94%; *C. longa* 5.28%), Tannins (*E. serrata,* 35.2%; *M. pudica,* 24%; *C. longa* 24.2%), Saponnins (*E. serrata* 5.77%; *M. pudica* 6.20%%; *C. longa* 7.26%), Flavonoids (*E. serrata* 9.80%, *M. pudica* 53.8%, *C. longa* 13.18%), %) and Cardiac glycosides *(E.serrata* 5.31%, *M. pudica* 3.22%, *C. longa* 3.30%). All the extracts inhibited the growth of both bacteria (effective zone of inhibition 7 to 26 mm) and fungi (effective zone 5 to 17 mm) compared to chloramphenicol and griseofulvin respectively. Aqueous extracts of *C.longa.* had no inhibition for *A. faecalis* and the fungi at 25mg/ml. The magnitude of bacterial inhibition by the extracts followed order (*M.pudica* > *C.longa* > *E.serrata*) and *(C.longa* > *E. serrata* > *M.pudica*) for the fungi. Scavenging of DPPH, nitric oxide, H_2O_2 radicals and reducing power ability were concentration dependent. Ethanolic extracts had a significant ($p < 0.05$) antioxidant scavenging activities for H_2O_2 and reducing power. *E. serrate, M. pudica, C. longa* exhibited a strong reducing power (R.P 0.5_{AU} = 15.01 µg/ml, 10.0 µg/ml, 9.38 µg/ml) (R.P 0.5_{AU} = 34.09 µg/ml, 21.77 µg/ml and 21.63 µg/ml) for ethanol and methanol extracts respectively and effectively scavenged DPPH, nitric oxide and H_2O_2 radicals. Threshold inhibitory concentrations (IC_{50}) of *E. serrate, M. pudica, C. longa* and ascorbic acid against DPPH radicals were (28.74 µg/ml,19.64 µg/ml,15.62 µg/ml and 15.62 µg/ml) for ethanol extracts and (2.92 µg/ml, 16.10 µg/ml, 12.50 µg/ml and 15.62 µg/ml) for methanol extracts. IC_{50} of *E. serrate, M. pudica, C. longa* and ascorbic acid for nitric oxide radicals were (22.67µg/ml, 50.02µg/ml, 48.34µg/ml and 15.46 µg/ml) for ethanol extracts and (16.55µg/ml, 16.17 µg/ml, 15.36 µg/ml and 15.46 µg/ml) for methanol extracts. IC_{50} of *E. serrata, M. pudica, C. longa* and ascorbic acid against H_2O_2 radicals were (15.31 µg/ml, 15.26 µg/ml, 15.26 µg/ml and15.15 µg/ml) for ethanol extracts and (15.48 µg/ml, 24.17 µg/ml, 35.03 µg/ml and 15.15 µg/ml) for methanol extracts. Mortality from the extracts at a single dose of 5000 mg/kg body weight, were revealed. Sub-chronic oral toxicity of the extracts 200 mg/kg and 400 mg/kg body weight revealed non-significant difference ($p > 0.05$) on the biochemical parameters (ALT, AST, ALP, LDH, SOD, GSH, TP, GPx, CAT, ALB, Urea and Creatinine). Haematology parameters (HB, WBC, RBC, PVC) values when compared with control, showed significant difference ($p < 0.05$).Similar results were also revealed for body weight when compared with control. Examination of the liver, kidney and heart showed no significant morphological changes except with *M. pudica,* where observed pathological changes include fatty degeneration and inflammatory cells (lymphoctyes and macrophages) irrespective of extract dosage.

Keywords: Phytochemicals, Antibacterial, Antifungal, Antioxidants, Toxicity.

CHAPTER I: INTRODUCTION

1.0 BACKGROUND INFORMATION

Medicinal plants are spread globally, but are more profused in tropical countries (Ziarati, 2012) like Nigeria. According to Calixto (2000), about 25 percent by estimation, of modern drugs are obtained from plants. Also according to Bodeker, Ong, Grundy, Burford and Shein, (2005), 65 to 80 percent of the world's population living in developing countries, depend mostly on medicinal plants for their health care arising from high cost of orthodox medicine and lack of access to modern medicine. Traditional medicines are now widely accepted due to compatibility to human body, effectiveness and less side effects (Shweta, Sinha, Tyagi and Kumar, 2011). Generally, it is considered that compounds produced naturally, rather than synthetically, can be biodegraded easily and therefore more encouraged to be environmentally friendly. In Africa, unavailability of synthetic drugs has made rural communities to depend on medicinal plants as source of primary health care (Ngari, Chiuri, Kariuki and Huckett, 2010). Plants have been used by man, for many years for a wide variety of purposes such as pharmaceutical alternative medicine, food preservative and natural therapies. Natural antioxidant, antibacterial, antiviral, antifungal agents, nutrients and cytotoxic agents, have gained popularity in recent years due to growing global concern about the alarming rise in the rate of infection caused by antibiotic resistant microorganisms. Several plant species used traditionally have good potential as antimicrobial and antiviral agents and these have raised the optimism of researchers on the future of phyto-antimicrobial agents (Ganhiraja, Sriran, Meena, Srilakshmi, Sasikumar and Rajeshwari, 2009). Many research groups have screened plant extracts to detect secondary metabolites with relevant biological properties in an effort to find new lead compounds. Plants contain substances such as alkaloids, aldehydes, peptides, essential oils, and phenolic compounds possessing high curative potential against human pathogens for example fungi, bacteria and viruses (El astral, Aera and Aam, 2005) and these compounds also act as potential antioxidants to inhibit free radicals and lipid peri-oxidation (Madson, Andersen, Jorgensen and Skibsted, 2000; Jagetia, Rosk and Babu, 2004; Alisi and Onyeze 2008; Kumawat, Gupta, Tara-Chand and Singh, 2012). Nigeria has a great variety of plants which are used in trado-medicine to cure various diseases (Egwaikhide and Gimba, 2007). According to Okigbo and Nneka (2005) to assess plants from the traditional African system of medicine gives us clues as to how they can be used in the treatment of diseases. The success story of chemotherapy lies in the continuous search for new drugs with

consistent potency and little or no side (toxicological) effects to take action in response to the challenge posed by resistant strains of microorganisms. Youyou Tu, a Chinese researcher won the Noble prize for Medicine and Physiology in 2015, for her work on *Arthemisia annum* L. which led to the characterization of arthemisinin used in the treatment of malaria. Nigeria has employed the Arthemisinin combination therapy (ACT) which proved effective in controlling malaria. This has given further impetus to the assessment of plants used in traditional medicine in different Nigerian societies.The present work has assessed the following plants: *Euphorbia serrata, Mimosa pudica*, and *Curcum longa*, for antibacterial, antifungal and antioxidant activities.

E. serrata also known as serrated spurge and Saw-tooth spurge, originated in Europe and Africa but has been introduced to some other places as weed. According to Zargari (1993) a significant percentage, mostly those native to Madagascar and Africa especially in Nigeria are succulent.

M. pudica is a shrub that is active through out the year (or perennial), grows to 50-70 centimeter; the stem 2-2.5 millimeter long and sparse prickles. It is cultivated for its uniqueness as the leaves fold inward and droop when touched and reopened within minute after. *M. pudica* originated in South America and Central America but is now a pan Tropical weed. According to Germplasm Resources information Network (2008), *M. pudica* is also known as humble plant, shame plant, touch me not, sleeping grass (Tropical Biological Association, 2015) and prayer plant. The leaves are bipinnately compound, with 1 or 2 pinnae pairs and 19-26 leaflets per pinna. The fruit is made of clusters of 2-8 pods of 1-2 centimeter long each. On the margin it is prickly, pod contains brown seeds 2.5 millimeter long and the petioles are also prickly and on close observation, upper part of the floret petals are red and the filaments are pink to lavender (US Forest Service, 2008).

Curcum longa a rhizome and a herbaceous (perennial) plant from the ginger family, Zingiberaceae (Chan, Lim, Wongs, Lim, Tan, Lianso and Yong, 2009). It originated in Southeast India, requires temperature 20^0 C - 30^0 C to thrive (Prasad, Aggarwal, Benzie,and wachtes-Galor, 2011). In Indian folk medicine, it is used for treating stomach and liver ailments, and can be applied topically to heal wound, basically due to its supposed antimicrobial properties (Chatarvedi, 2009). Other disease conditions like those of pulmonary, gastrointestinal systems, aches, pain, wounds, sprains, liver ailment and skin (eczema,shingles, allergy chicken pox and scabies)(Khalsa,2013) are cured with turmeric. In Nigeria, *C. longa* is used as food condiments

and culinary because of its nutritional and medicinal value within its rhizomes which contains curcumin (Diferuloyl methane), turmerol or turmeric oil and 1,7-bi, 6 hepta-diene-3, 5-dione. Its Curcumin content has anti-inflammatory and analgestic properties (Srimal, Khanna and Dhawan, 1971). As reported by Chopra, Nayar and Chopra, (1986), *C.longa* to possess antibacterial, antifungal, anti-inflammatory properties, contains proteins, fats, vitamins (A, B, C, etc); all these have significant roles in wound healing and regeneration.

In this research work, *Euphorbia serrata, Mimosa pudica and Curcum longa*, were evaluated for their antioxidant, antibacterial and antifungal properties, as well as their toxicity to albino rats.

1.1 STATEMENT OF PROBLEM

The rising prevalence of multidrug resistant strains of bacteria, fungi, and viruses etc, and the emergence of new strains arising from mutation have become pressing global challenge to various health institutions including World Health Organization (WHO). New drugs synthesized are not very affordable and available due to the high cost of production. These encourage many to go back to Mother Nature and its rich sources of natural remedies, thereby suggesting interest in exploiting plants for medicinal purposes. Though the therapeutic use of medicinal plant products has been supported by WHO, there is an indisputable need to generate corroborative evidence substantiating their proposed medicinal application. Exploiting and confirming the potentials of medicinal plants, particularly those employed in folk medicine could be a promising alternative as it could give a new source of antimicrobial agents with possible novel mechanisms of action.

1.2 AIM OF STUDY

The aim of this study is to evaluate the antibacterial, antifungal and antioxidant properties of some medicinal plants used in Nigeria.

1.3 OBJECTIVES OF STUDY

i. To evaluate qualitatively and quantitatively the phytochemical constituents of leaves of *Mimosa pudica*, *Euphorbia serrata* and *Curcum longa (*rhizome),

ii. To identify microbial clinical isolates using molecular methods – Polymerase Chain Reaction

(PCR), DNA sequencing and blasting analysis.

iii. To assess the antibacterial and antifungal properties of the plant extracts against some clinical microbial isolates.

iv. To evaluate the antioxidant activity of the plant extracts.

v. To evaluate the acute and sub chronic toxicity of these plant extracts on animal model, the effects on their organs –heart, liver and kidney

1.4 RESEARCH QUESTIONS

This study endeavours to answer the following research questions:

1. What kind and quantity of bioactive compounds are present in the plants extract?

2. Can these bioactive compounds inhibit the growth of microorganisms?

3. Can the inhibition by these bioactive compounds be compared to orthodox antibiotics?

4. Do these plant extracts exhibit antioxidant properties?

5. What effects do these plant extracts have on male albino rats?

1.5 RESEARCH HYPOTHESIS

1. There is no significant effect using these extracts in treatment of infections caused by bacteria and fungi.
2. There is no significant antioxidant property of these plant extracts.
3. There is no significant toxicity effect of these plant extracts on the test animals.

1.6 JUSTIFICATION OF THE STUDY

Medicinal plants are relevant in pharmacological research and drug development for the treatment of various diseases. Plants produce many secondary metabolites which constitute important sources of microbicides, pesticides and other pharmacetical drugs. Secondary metabolites from higher plants serve as defense agents against invading microorganism. Several plant species used traditionally have potential antimicrobial properties and this has raised the optimism of researchers about the future of phyto-antimicrobial agents. Many research groups

have screened plant extracts in an effort to detect secondary metabolites and discover new lead compounds having relevance to biological activities.

Nigeria has a great variety of natural vegetation used in trado-medicine to cure various ailments. These medicinal plants need to be conserved and preserved as we use them in treating ailments, and research to avoid complete extinction of the species from their natural habitat, for future generational use, and for biodiversity. Assessing plants from the traditional African system of medicine gives us with clues as to how they can be used in the treatment of diseases. Large investment and research in the field of anti-infective agents are now urgently needed if the public health crisis caused by these pathogens is to be averted.

1.7 SCOPE OF THE RESEARCH WORK

This work covers evaluation of qualitative and quantitative phytochemical constituents of the leaves of *M. pudica, E. serrata* and rhizome of *C. longa* used in folklore medicine in Nigeria and the molecular identification of clinical test isolates. Others include the assessment of the antibacterial, antifungal and antioxidant activities of the extracts on some molecularly identified clinical isolates. In addition to their acute toxicity, the pathology of the extracts on albino rats was evaluated.

CHAPTER II: LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 *Euphorbia serrata*

Euphorbia serrata belongs to the family Euphorbiaceae, a species of spurge known as serrated spurge, saw tooth spurge and also known as "ogwu garri" in Igbo. *E. serrata* plant is presented in plate2.1

Through-out history, the natural products of *Euphorbia* plant have played a signifcant role in the life of man especially as source of medicinal products (Leland, Ara, Peter, Kautman, James and Harry, 2006). It is an annual plant and has small size (Soforowa, 1993). It originated in Europe and North Africa but has been introduced some other places as weed specie. *Euphorbia* is one of the six largest genuses of the flowering plants having over 2000 species. It is a monoecious herb, shrub or tree and often succulent (Zargari, 1993). They contain latex, have unique flower structure and grow from 20 centimetres to about half a metre in height. The leaves are of three types, lower median, upper and connate. The leaves are sessile and toothed penni (Ali and Nasir, 1986). Has a spherical capsule fruit with gray seeds.

In Nigeria these species are found – *E. hirta, E. heterophylla, E. splendes, E. milli, E. prostata, E. thymifolia, E. tirucalli* and *E. desmondi* (Ekeke and Ndukwu, 2014). The natural products of *Euphorbia* plants and its natural products have played a significant role in the life of man throughout history as regards to individual use (Leland *et al*., 2006). *Euphorbia* species are used in traditional medicine for the treatment of various ailments like skin diseases, intestinal parasites and warts. The plant extracts have been found to have significant anti-inflammatory, analgesic, and haemostatic and wound healing properties. It has been reported that *Euphorbia* possesses anti-arthritic, anti-convulsant, anti-eczema, anticancer, antidiabetic, antimicrobial, anti-inflammatory, antispasmodic, antitumor, antitussive properties, hormonal and myelopoiesis properties (Eberle, Erb, Flamer and Meyer, 1999; Valente, Ferreira, Abrenu, Pedro, Cerquerira and Nascimento, 2003; Ferreira, Duarte, Gyemant, Radics, Cherepner, Varga and Molnar 2006; Luo and Wang, 2006). Various species of the genus *Euphorbia* are used in treating of cancer, diarrhea and bronchial asthma (Glavez, Zarzuelo, Crespo, Lorente, Ocete and Jimenez, 1993).

Euphorbia serrata have been used in the treatment of ailments such as skin diseases, gonorrhea, migraines, intestinal parasites and warts. The plant lattices are used in fish poisons, and

insecticides (Uzair, Coothar and Choudhary, 2009). The stem is used for the treatment of ashma, bronchitis and various lung complaints. This plant has analgesic, anti-inflammatory activities, and anti-mutagenicity (Loh, Er and Chen, 2009) and anti-oxidant (Basma, Zurriani, Latha and Sasidharam, 2011).

The result of different phytochemical analysis of *Euphorbia serrata* extracts revealed the presence of tannins, alkaloids, flavonoids and terpenes which may be responsible for antibacterial properties. Phyto-therapeutically active tannins are used to treat non-specific diarrhea. According to Tijjan, Sallau and Sunusi, (2011) there are tannins which are reported to have various physiological effects such as anti-irritant, anti-secretolytic, anti-phlogistic, anti-microbial and anti-parasitic.

Akujobi, Anyanwu, Onyeze and Ibe, (2004) reported the antimicrobial properties of *Euphorbia* plant inhibiting the growth of various microorganisms at different concentrations (Osadese and Ukwez, 2004; Perumal, Pillai, Cai, Mahmud and Ramanathan, 2012; Velasco, Morales, Diaz and Meccial, 2013; Venkatanagaraju and Goli, 2014). Nweze, Okafor and Njoku, (2004) opined that antimicrobial properties of the plant extracts were due to the presence of secondary metabolites.

Cowan (1999), and Draughon (2004) studied the antibacterial property of extracts of *E.serrata* and the results showed the antibacterial properties to be due to the presence of alkaloids, flavonoides and tannins. Duada, Saadullah, Hasnain, Hidayatullah, Mushtaq, Akahtar,and Khattak, (2014) assessed the antibacterial properties of *E. serrata* stem extract against common bacterial pathogens and revealed that methanolic and chloroform extracts showed antibacterial activity. According to Goncalves, Ramos, Soares, Mashronah and Silveira (2006) and Sudhakar, Rao, Rao, Raju and Ventateswarlu, (2006); antibacterial property has been reported for various *Euphorbia* species. Ahmad, Khan, Chaudhary, Janb, Zair, Akhtar and Gilani, (2012) evaluated the antifungal and antispasmodic properpties of dichloromethane and methanolic extracts of *E. granulata*. The results revealed that the crude dicholoromethane extract of *E. granulata* has the potential to be an antifungal against *M. canis* and *Aspergillus flavus,* and also the plant extracts show spasmolytic effect. Rauf, Ajmal, Uddin and Roothullah, (2013), investigated cytotoxic effect of aerial parts of *E. milli* and *E. pulcherrima* with the crude alcoholic and ethyl acetate extracts showing excellent cytotoxic activity.

Sunusi (2014) investigated *in vitro* synergistic activity *E. balsamifera* root extract and ampiclox against some bacterial isolates. This study revealed that the plant drug combination is

synergistically very active against the microorganisms *E. coli* and *Klebsiella pneumoniae*. The antibacterial test of *E. condylocarpa* revealed that methanol root extract of this plant was able to inhibit the growth of *Bacillus subtillis, Bacillus pumilis* and *Staphylococcus epidermidis* (Sepideh, Vahid, Seyed, Erfar, Hamidreza, Reza and Amir, 2015). Also past investigations, revealed that the root extract of *E. condylocarpa* has significant use in traditional medicine to treat skin diseases, gonorrhea and migraine (Jassbi, 2006).

According to Khan, Qureshi, Gillani and Dullah, (2011), crude extract of *E. hirta* effectively exhibited antibacterial activity against all the tested bacterial strains. Also, Khan, Drochner, Steingass and Islam, (2008) assessed the antibacterial properties of *E. hirta* plant extracts against enteric bacteria. Similar results were found by Abubakar (2009) that methanol extract of *E. hirta* could be beneficial in treating ailments caused by enteric organisms. Also similar studies illuminated the antibacterial properties of ethanol extract of *E. fusiformis, E. hirta* and *E. tirucalli* against *Staphylococcus epidermidis* and *Staphylococcus aureus* (Parekh, Jadeja and Chanda, 2005; Natarajan, Britto, Srinivasan, Nagamuruga, Mohanasundari and Perumal, 2005).

2.2 Mimosa pudica

Mimosa pudica (plate 2.2) commonly known as "touch and die" is a creeping annual or perennial herb, often grown for its curiosity value, as the compound leaves fold inward when touched and reopens within minutes. This plant has other names such as Shame plants, Humble plant, Touch me not (Germplasm Resource information network, 2008), Sleeping grass (Tropical Biological Association., 2015), Prayer plant and "oso aka" (Igbo name). *M. pudica* originated in South America and Central America. In Tanzania, South Asia, South East Asia and many pacific Islands it is an invasive species (Tropical Biology Association., 2015), but in Nigeria, Seychelles, Mauritius and East Asia not invasive.

It is also found in tropics and sub-tropics in Nigeria as common weeds widely distributed in open wet waste places and grass land.

Mimosa pudica, short prickly plant with its stem erect in young plant, but becomes creeping or trailing with age, slender and branched. It grows up to 0.5 meter height and spreads up to 0.3 meter with bipinnate leaves (Saraswat and Pokharkar, 2012), fern-like and pale green in colour with tendency of closing when disturbed..The fruit of *Mimosa* are pods, 1.5 – 2.5cm long, falcate and closely prickly on sutures (Saraswat and Pokharkar, 2012). The fruit consist of clusters of 2-

8 pods and breaks into 2-4 segments, with pale brown seeds 2.5 mm long (US Forest Service, 2008).

Mimosa plant has been used for the treatment of various ailments. In folk medicine, it is used in stoping bleeding and in skin disease. It contains mimosine (an alkaloid), free amino acids, sitosterol, linoleic acid and oleic acid. The studies of the phytochemical components of this plant have been carried out by several research works (Ahmad and Beg, 2001) and the antimicrobial property of the plant (Ojalla, Remes and Hans, 1999). The leaves and stem have been reported to contain the alkaloid, mimosine, mucilage and the roots contain tannins (Ghani, 1998). *Mimosa* is used for its hepato-protective (Gaurikarwani, 2011), hypolipidemic (Rekha, 2010), anti-convulsant (Ngo Bum, 2004), anti-depressant (Molina, 1999), antifertility (Ganguly, 2007), anti hepatotoxic (Nazeema and Brindha, 2009), wound healing (Dnyaneshwar, Taur and Patil, 2009) properties. Lozoya and Lozoya (1989) reported major chemical substances of interest from this plant as alkaloids and steroidal sapogenins. According to Gandhiraja, Sriran, Meena, Srilakshmi, Sasikumar and Rajeshwari, (2009), the methanol extract of leaves of *M .pudica* revealed the presence of phytochemicals like alkaloids tannins, quinines, coumarin, phenols, saponins, flavonoids, terpeniods and glycosides.

Ranjeet, Sathish, Seethalakshmi and Rao, (2013) observed in their study that ethanol extracts of *M. pudica* leaves and roots showed that tannin and protein are present in both samples. Seed of *M. pudica* have diuretic property (Krishnaraju, Rao, Sundararaju, Vanisree, Tsay, Gottumukkala and Susbaraju, 2006). Also the roots of *Mimosa* contain tannin, alkaloid mimosine, ash, calcium and oxalate crystals according to Oudhia, Chhui-Mui and Lajwanti, (2006). Gandhiraja *et al*., (2009) evaluated the antimicrobial property of *Mimosa* against *Aspergillus famigatus*, *Citrobacter divergens* and *Klebsiella pneumonia.*They observed that the methanolic extract of *M. pudica* leaves exhibited antimicrobial activity against the microorganisms. Kokane, More, Kale, Nehete, Mehendale and Gadgoli, (2009) observed in their investigation of wound healing properties of methanol extract of *M.pudica* root; revealed that the extract had good wound healing properties which may be due to phenolic contents. Ayurveda medicine reports usage of *M. pudica* in arresting bleeding and improving wound healing activity. Wound healing studies on roots of this herb showed that phenolic constituents (tannins) play a significant role in wound healing process (Muthusamy, Kirubanadan and Bripriya, 2008). Ngo-Bum, Dawalk, Schimut and Rakotronirina, (2000), reported that the roots have anti-convulsant property. Also Burn, Dawalk,

Scmutz, Rakotonirina, Rakotonirina, Portel, Jeker, Oipeh and Herrling, (2004) observed in their study that the decoction of *M. Pudica* has strong anti-convulsant action and that these activities could be the reason for its use in African trado-medicine. Nazeema and Brindha (2009) investigated the anti-hepatotoxic of mimosa pudica Linn on diabetes mellitus and its complication and antioxidant defense potential of *M. pudica*, the result revealed a considerable protection against the toxin - induced oxidative stress and liver damage. *M. pudica* aqueous root extract exhibited high inhibitory effect on the poisonous properties of venom of Indian snakes as observed by Mahanta and Mukherjee (2001). In line with this, Prashath, Vinutha and Salma, (2007) investigated preliminary screening of *M. pudica* extract, and show that it exhibited anti-venom activity against sea snake (*Enhydrina schistosa*) poisoning. The study revealed that *M. pudica* root has acetylcholine esterase inhihitory property.

Pawaskar and Kale (2006) investigated the antibacterial properties of extracts of *M. pudica* whole plant in various solvents against some bacterial strains. It shows that the plant can be used in treating infections caused by these microorganisms. Result of the study showed that the *M. pudica* whole plant extract possesses good antibacterial properties with inhibition of 7mm to 18mm against the microorganisms. The aqueous and methanol extracts of *M. pudica* were tested against different fungal, including *A. fumigatus* for their antifungal properties and the results showed significant antifungal properties as reported by Gandhiraja *et al.*, (2009).

Akinsinde and Olukoya (1995) investigated the vibrocidal activities of some local herbs, and result revealed that *M. pudica* has antimicrobial activity against *Vibrio cholera.* Ethanol extract of *M. pudica* leaves administered orally to mice at a dose of 250 mg/kg body weight revealed a significant hyperglycemic effect as observed by Amalrayi and Ignacimuthu (2002). Molina, (1999) observed in their study that aqueous extracts from *M. pudica* produced antidepressant effects in rat, compared to two tricyclic antidepressant used in the study (Clomipramine and desipramine).

Pande and Pathak (2009) investigated the effect on libido of sexually active Swiss albino male mice by the ethanol extract of roots of *M. pudica*. The result revealed that administration of the extract orally increased the libido and hormonal levels of testosterone significantly. *M. pudica* has several traditional usages which have been described. Root decoction is effective in treating gravel and other urogenital complaints (Chatterjee and Pakrashi, 2006). According to Prajapati, Purohit, Sharm and Kumar, (2003), *M. pudica* is also used in the treatment of sexually

transmitted diseases, dysentery, fever, leprosy, stomach worms, insect bite, insomnia, nervousness, and piles. This plant has great potential in terms of therapeutics and its exploration is highly demanding for its benefits to man.

2.3 Curcum longa

Curcum longa (plate 2.3) is a rhizome plant of the family Zingiberaceae (ginger family) (Chan *et al*., 2009). Commonly known as turmeric, *Curcum longa* (tumeric) a perennial herbaceous monocotyledon spice and also an important foreign earning crop and is a native to South East India, and needs temperature between 20^0C and 30^0C. (Prasad *et al*., 2011).

Although it grows in Asia, China and much tropical location, including Nigeria where the rhizomes are used as a spice in cooking to add fragrance, and flavor in their dishes. Most of *Curcum longa* is cultivated in India and used as a main ingredient in curry. The spice ingredient for curry contains approximately 20 -30% of turmeric. It grows up to 1 m high with dull yellow flowers. The rhizomes (roots), which are boiled, dried and then ground forms the yellow spice (Bharat, Anistree, Smanoj and Shishir, 2005). Tumeric has a bitter, somewhat sharp, taste and is the roots or rhizomes or the bulbs are used in food and medicine. Tumeric components include the three curcuminoids: demethoxycurcumin bisdemethoxycurcumin and curcumin (diferuloylmethane - primary constituent and responsible for its vibrant yellow colour). Other constituents include tumerone, atlantone, Zingiberoune, sugars, resins and proteins. *C. longa* apart from being used as a flavoring and colouring agent in Nigerian cusine is used for its various medicinal properties. Likewise in Asian cusine, it is used in cheese, butter, yogurt and other kinds of food according to Anun and Nalini (2002) apart from its flavoring and colouring properties it is also used for various medicinal properties. Tumeric are used for various purposes and administered through different routes.. Example applied on the skin for wounds, blistering diseases such as pemphigus and herpes Zoster, for parasitic skin infections, and for acne. It is also taken orally for common cold, liver diseases, and urinary tract diseases and as a blood purifier and for treatment of chronic rhinitis and Coryza it is taken through inhalation (Eigner and Scholz, 1999). Curcumin has numerous pharmacological properties including antioxidant and anti-inflammatory (Hsu Ch and Cheng, 2007) and antimicrobial properties (Negi, Jayaprakasha, Jagan and Sakariah, 1999).

Studies carried out by Chainani-Wu, (2003) revealed that curcumin affects cellular enzymes, and

angiogenesis. On toxicity study, it was found to be non-toxic with wide margin of safety (Annun and Wahl, 1991). Although it has been reported by Cheng, Hsu, Lin, Hsu, Ho, Shen, Ko, Lin, Lin, Ming-Shiang, Yu, Jee , Chen, Chen, Chen, Lai , Pu, Pan, Wang, Tsai, and Hsieh, (2001) that 8 g dosage administered per day for three months were not toxic to humans. Amit, Anup and Kajal, (2011) in their study of antifertility effect of the rhizome of *curcuma longa*, showed evidence that curcumin has anti-ovulatory effect. According to Apisariyakul, Vanittanakonm and Buddhasukh, (1995); Roth, Chandra and Nair, (1998) many *in vitro* and animal studies has been published describing the antifungal, antioxidant, anti- inflammatory, antiviral properties of curcuminoids (Ammon, Safayhi, Mack and Sabieraj, 1993; Bisset, 1994; Miller and Murrary, 1998;Young-joon,1999; White and Foster, 2000). An *in vitro* study measuring the activity of curcumim on an inducible stress protein (endothelial heme oxgyenase-1) carried out by Mortellini, Foresti, Bassi and Green, (2000), using bovine aortic endothelial cells incubated for 18 hours revealed improved cellular resistance to oxidative damage. Also other animal studies have demonstrated tumeric's hepato-protective property from a variety of hepatotoxic agents, including carbon tetrachloride (CCl_4) (Deshpande, Gadre and Raste, 1998; Park, Jeon, Ko, Kim and Sohn, 2000), Paracetamol (acetaminophen) (Donatus, Sardjoko and Vermeulen, 1990) and *Aspergillus* aflatoxin (Soni, Rajan and Kuttan, 1992). Hepato -protective effect of tumeric is due to its antioxidant properties and its ability to reduce the formation of pro-inflammatory cytokines. According to Soni *et al*., (1992), tumeric and curcumin also reversed biliary hyperplasia, fatty changes and necrosis caused by a aflatoxin production.

Also *in vitro* and *in vivo* animal studies have shown anti-carcinogenic property of turmeric and its flavonoid component curcumin against colon (Chauhan, 2002, Reddy and Rao, 2002), breast cancer (Ramachandran, Fonsera and Jhabuala, 2002; Somasundaran, Edmund and Moore, 2002; Shao, Shen and Liu, 2002) and prostate cancer (Dorai, Cao, Dorai, Buttyan and Katz, 2001; Hour, Chen and Huang, 2002) and melanoma (Bush, Cheung and Li, 2001). *In vitro* studies utilizing human cell lines, rats and mices have shown that curcumin's ability to inhibit three phases of carcinogenesis: tumor promotion (Kawamori, Lubet and Steele, 1999), angiogenesis (Thaloor, Singh, Sidhu, Prasad, Kleinman and Maheshwari, 1998), and turmor growth (Limtrakul, Lipigorngoson, Namwong, Apisarijaku and Dunn, 1997). In studies, carried out by Hanif, Oiog, Shilf and Rigas, (1997) and Dorai *et al.*, (2001) on colon and prostate cancer, the result revealed that curcumin inhibited cell proliferation and tumor growth. Tumeric and

curcumin are also capable of suppressing the activity of several common mutagens and carcinogens in a variety of cell types in both *in vitro* and *in vivo* studies (Mehta and Moon, 1991; Azuine and Bhide, 1992 and Boone, Steele and Kellof, 1992). The anti-carcinogenic properties of tumeric and curcumin are attributed to its antioxidant and free-radical scavenging properties, and its ability to increase glutathione levels (Pizorrno and Murray, 1999).

C. longa extract and its essential oil inhibit the growth of various bacteria, fungi and parasites. For instance a study of chicks infected with the caecal parasite *Eimera maxima* demonstrated that diets supplemented with 1 % tumeric resulted in decrease in small intestinal lesion scores and also improved weight gain (Allen, Danfort and Augustine, 1998). Also study carried out by Apisariykul *et al., (*1995) guinea pigs were infected with pathogenic molds, and result obtained showed that topical application of tumeric oil affects the yeast isolates. According to Rasmussen, Chistensen, Kvist and Karazami, (2000), curcumin has moderate inhibition against *P. falciparum* and *Leishmania major* organisms.

Tumeric's protects the cardiovascular system by lowering cholesterol and triacylglcerol levels, reducing susptibility of LDL (low density lipoprotein) to lipid peroxidation (Ramirez-Tortosa, Mesa and Aguilera, 1999) and inhibiting platelet aggregation. The result revealed that at higher doses lipid peroxidation of LDL was not reduced.

Several research work, have shown that components of *Curcuma longa* exert several protective effects on the gastrointestinal tract. Sodium curcuminate inhibited intestinal spasm and P-tolymethylcarbinol, a turmeric constituent, raised gastrin, secretin, bicarbonate and pancreatic enzyme secretion (Ammon and Wahl, 1991). Tumeric has inhibit ulcer formation caused by stress, alcohol, indomethacin, pyloric ligation and reserpine, significant increasing gastric wall mucus in rats subjected to these gastrointestinal challenges (Rafatulla, Taria and Alyahya, 1990).

Prucksunand, Indrasukhsri, Leethochawalit and Hungspregs, (2001) conducted an open phase 2 trial on endoscopically-diagnosed gastric ulcer patients. The patients were given 600 mg powdered turmeric five times a day. The result showed that after four weeks, 48 percent of patients were healed.

Plate 2.1: A *Euphorbia serrata* plant

Plate 2.2: A *Mimosa pudica* plant

a **b** **c**

Plates 2. 3 **a.** *Curcum longa* plant **b**. *Curcum longa* rhizomes and **c.** ground turmeric

2. 4 PHYTOCHEMICALS

Phytochemical is of Greek origin "Phyto" meaning plant. Therefore phytochemicals are secondary metabolities occurring in plants or are the plant chemicals. Phytochemicals, natural bioactive compounds occurring in plants, leaves, bark, stem, roots and their flowers that gives protection and defense from various diseases. Plants medicinal value lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. The bases of modern drugs are formed from these natural compounds (Edegoa, Okwu and Mbaebie, 2005; Akimo-Laudn, Ibukun, Afor, Obuotor and Farombi, 2007; Rout, Chouldhary, Kas, Das and Jain, 2009). Phytochemicals include flavonoids, alkaloids, saponins, anthocyanins, anthroquinones, carotenoids, glycosides, hydroxycinnanic acids, isoflavones, lignans, tannins, terpenoids, phytosterols, phenolic acid, organosulfides etc. Phytochemicals, though not nutritive but are needed by plants for purpose such as disease and pathogen defense, and provide color, aroma, flavor and other organoleptic properties.

Scientists estimate that there may be as many as 10,000 different phytochemicals. Phytochemical are grouped into two: primary and secondary constituents, depending on their metabolic function in plant. Chlorophyll, amino acid, proteins and sugars are included in primary components while secondary components include alkaloids, terpenoids and phenolic acids (Krishnaiah, Sarbatly and Bono, 2007). These phytochemicals are important in both plants, human and animal health. In plants, they function to attract beneficial, and repel harmful organisms, serve as photo-protectants and also respond to environmental changes. In man, they work with nutrients and fibre to make an integral part of defense against various diseases conditions of man. They display different biological, complementary and over lapping activities such as anti-inflammatory, antioxidant, modulation of detoxification enzymes, immune system stimulation, antibacterial, modulate steroid metabolism, antiviral, antifungal, anti-helminthic effects

.

Different types of phytochemicals are presented in Figure 2.1 below.

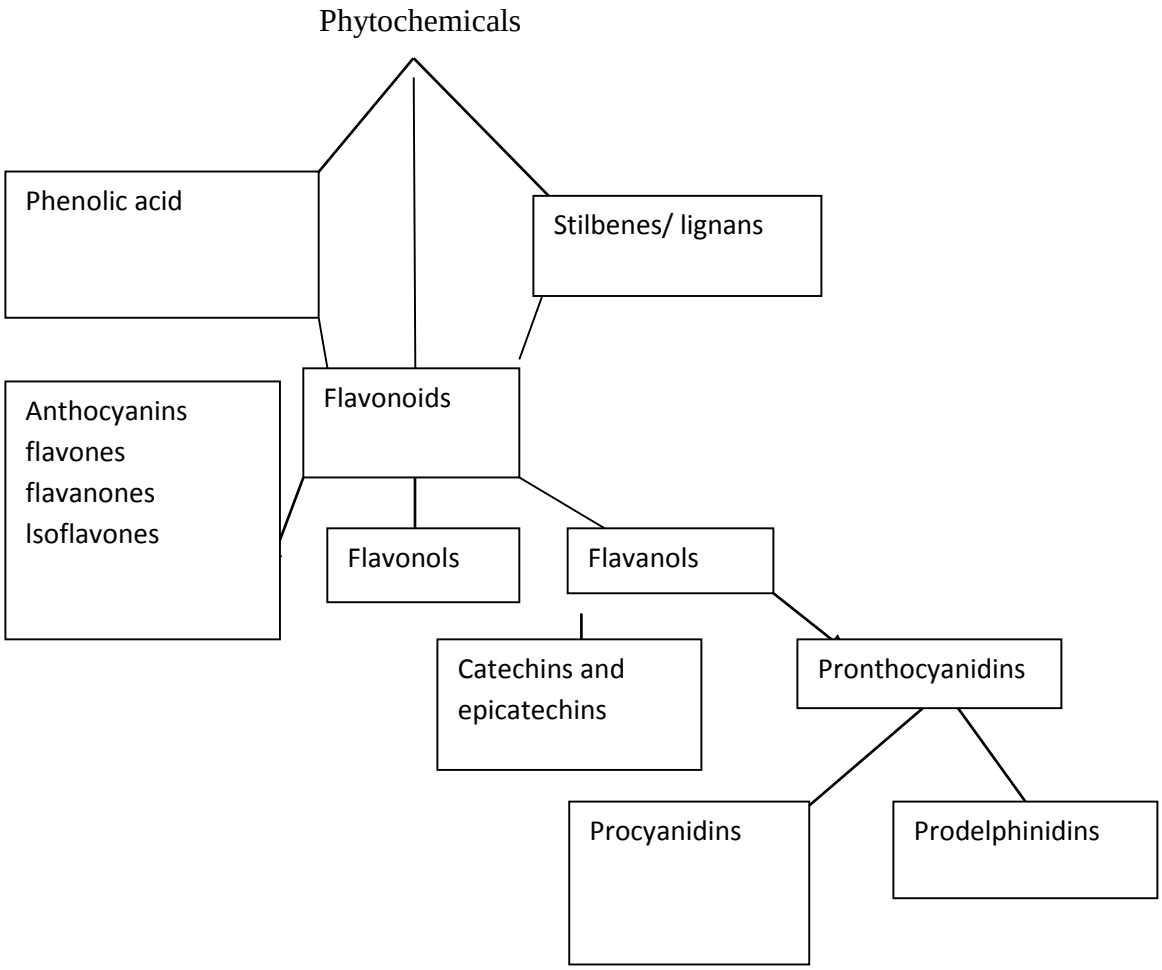


Figure 2.1: Types of phytochemicals

Arts and Holliman, (2005)

2.5 EFFECTS OF PHYTOCHEMICALS

Phytochemicals play a diverse role in human health including antioxidant activity, antimicrobial, anti-inflammatory, hormonal activity, alteration of biotransformation enzyme activity, etc.

2.5.1 Antioxidant Activity

Antioxidant protect cells against the damaging effects of reactive oxygen species, like singlet oxygen superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl (OH), nitric oxide (NO) which leads to oxidative stress that result in cellular damage. Oxidation is important in living organisms for production of energy for biological processes. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced due to the oxidation of cell, leading to cell death and tissue damage. These free radicals are responsible for aging and causing various human diseases (Pal, Ganguly, Tahsin and Acharya, 2010). Antioxidants plays a significant role in the prevention and treatment of diseases by removing free radical intermediates and inhibiting other oxidation reactions by being oxidized themselves (Sies, 1997). In recent years, natural antioxidants, from plants have gained increasing interests among consumers and the scientific community because of its efficacy. Reactive oxygen species exert oxidative stress in the cells of human body leaving each cell to face about 10,000 oxidative hits per second (Lata andAhuja, 2003). Epidemiological studies have shown that frequent intake of fruits and vegetables lower the risk of age-related disease such as coronary heart diseases and cancer (Temple, 2000; La Vecchia, Altieri and Tawani, 2001). Recently, there is high demand of natural antioxidants for use as nutraceuticals and as food additives (Tawaha, Alali, Gharaibeh, Mohammad and El- Elimal, 2007; Jayasri, Mathew and Radha, 2009; Kalim, Bhattacharyya, Banerjee and Chattopadhyay, 2010). Reactive oxygen species when gererated overtakes the antioxidant defense of the cells, the biological systems are been attacked by the free radicals and develop degenerative diseases.

According to Braca, Sortino, Politi, Morelli and Mendez, (2002) and Hazra, biswas and mandal (2008) exertion of oxidative stress on human cells by free radicals causes macromolecules like protein and DNA damage resulting in pathological processes. These processes that prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules, or prevent mutations are significant in cancer prevention (Gordon, 1996) and other oxidative diseases. Several studies have shown the antioxidant ability of plants and

their constituents *in vivo* (Shruthi, Roshan, Timilsina and Sunita, *2013;* Alisi, Asiwe, Ene and Alisi, *2018).*

2.5.2 Antimicrobial Activity

Phytochemicals are produced in plants as antimicrobial agents; these compounds have been evaluated for their potential to inhibit pathogens. It has been observed that some microorganisms that showed resistance to certain standard drugs were sensitive to extracts of plants.

2.5.3 Anti-inflammatory Activity

Plants natural products have been evaluated for their ability to inhibit cyclooxygenase (cox) and inflammation pathways. Thus phytochemicals are potent inhibitors of cylcooxygenase (cox) and interfere with inflammatory process.

2.5.4 Phyto-hormones (pheromes) Activity

The phytoestrogens are estrogen compounds found in plants. But phyto-estrogens have weak estrogen-like property. The body natural estrogens replaced by these weak substance and act as anti –estrogen, in this way, phytoestrogens help work against diseases that depend on estrogen for its growth (e.g. breast cancer).

2.5.5 Alteration of biotransformation enzyme activity

Biotransformation enzymes known as xenobiotic or drug metabolizing enzymes play an important role in regulating the toxic, mutagenic and neoplastic effects of chemical carcinogens. The efficacy of plant constituents (phytochemicals) to protect against neoplastic diseases through modulation of biotransformation enzymes is due to the glucosinolate and isothiocyanate constituent of plants. For example, curcumin from C. *longa* up-regulates hepatic glutathione S-transferase (GST) in rodents (Susan and Rao, 1992; Piper, Sighal, Slameh, Torman, Awasthi and Awasthi, 1998).

2.6 PHYTOCHEMICALS AND THEIR ROLES

2.6.1 Alkaloids

Alkaloids are bitter phytochemicals that occur in some plants. Foods high in alkaloids include black tea, coffee and cocoa. Alkaloids have no single classification. They are often classified on the basis of a structural similarity (e.g Indole alkaloids) or a common precursor (e.g purine alkaloids).The use of alkaloids in medicine dates back 5000 years (Goldman, 2001) and this chemical group are used in drugs formulations consumed by humans. They provides the cholinesterase inhibiting treatments prescribed for the cholinergic dysregulation of Alzheimers disease such as physostigmme (Mukherjee, Kumar and Houghton, 2007)**.**

2.6.2 Flavonoids

Flavonoids are prevalent in photosynthesis cell especially in plant, mostly found in nuts, fruits, vegetables, seeds, stems, flowers, tea and wine (Middleton and Chithan, 1993), honey and propolis (Grange and Davey, 1990) and also represent a part of human diet (Harborne and Baxter, 1999). In flowers, the flavoniods provide colours altractive to plant pollinators (Harborne and Willians, 2000), while in leaves they promote physiological survival of the plant, protection from UV-β radiation. Other roles include photosensitization, respiration control, photosynthesis and sex determination (Middletin and Chithan, 1993)

Research studies have reported flavonoids to have many useful properties which include antimicrobial property, anti-inflammatory property, oestrogenic activity, enzyme inhibition (Harborne and Baxter., 1999), antioxidant activity (Narayana, Sripal, Chaluvadi and Krishna, 2001), antiallergic activity (Middleton and Chithan., 1993), cytotoxic, antitumor activity and vascular activity (Harborne and Williams, 2000)

Flavonoids basic structural feature; 2-phenyl-benzo (α) pyrane of flavane nucleus, that consist of 2 benzene rings (A and B) and heterocyclicpyrane ring (C) (Fig 2.2a) The structures of other classes of flavonoids are shown in Figure 2.2b.

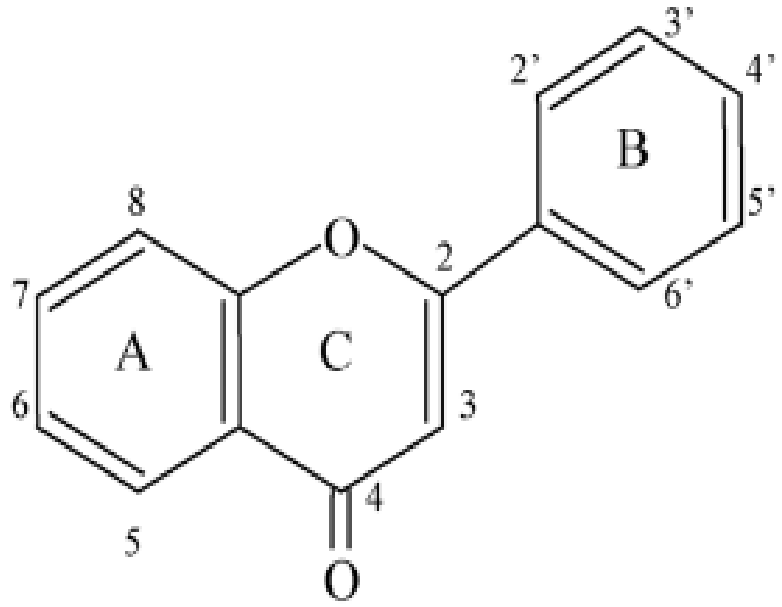
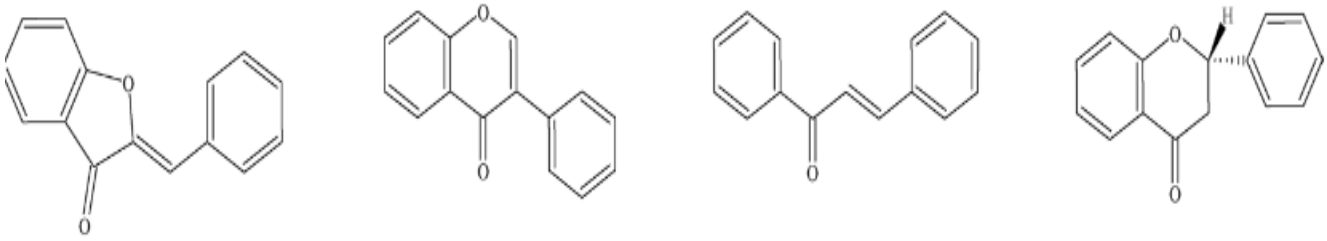
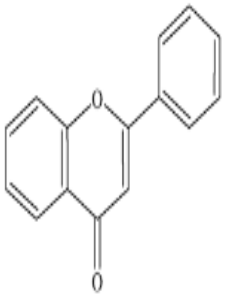


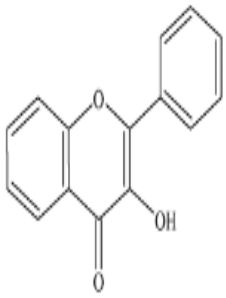
Figure 2.2a: The flavones skeleton structure (Harborne and Baxter., 1999).



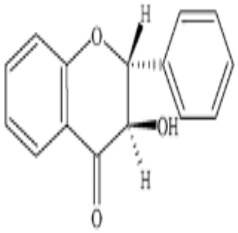
Aurone Isoflavone Chalcone Flavanone



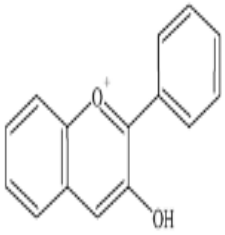
Flavone



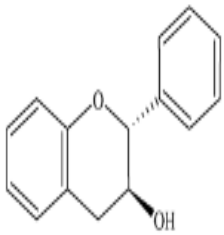
Flavonol



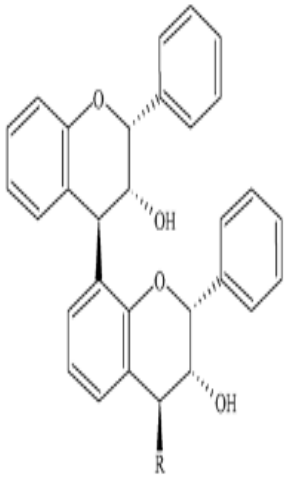
Flavanon-3-ol (also known as 3-hydroxyflavanone or dihydroflavonol)



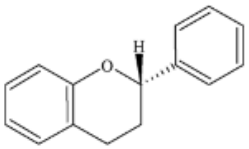
Anthocyanidin



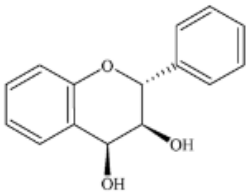
Flavan-3-ol (also known as catechin)



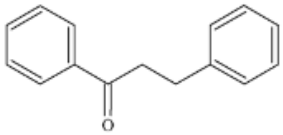
Proanthocyanidin (also known as flavolan or condensed tannin)



Flavan



Flavan-3,4-diol (also known as leucoanthocyanidin)



Dihydrochalcone

Figure 2.2b: The skeletal structure of classes of flavonoids: Aurones (Muziol, Cody and Wojtezak, 2001), Isoflavones, Chalcones (Xu and Lee, 2001), Flavonones (Harborne and Baxter, 1999), Flavanon-3-ols (Harborne and baxter, 1999), Anthcyanidins (Middleton, Kandaswami and Theoharides, 2000), Flavan-3-ols (Harborne and Baxter, 1999)

Proanthocyanidins, (occur as dimmers, trimers, tetramers, and pentamers; R=0, 1, 2 or 3 flavan -3-0l structures, Flavans, Flavan -3-4- diols, Dihydrochalcones (Harbone and Baxter, 1999).

2.6.3 Saponnins

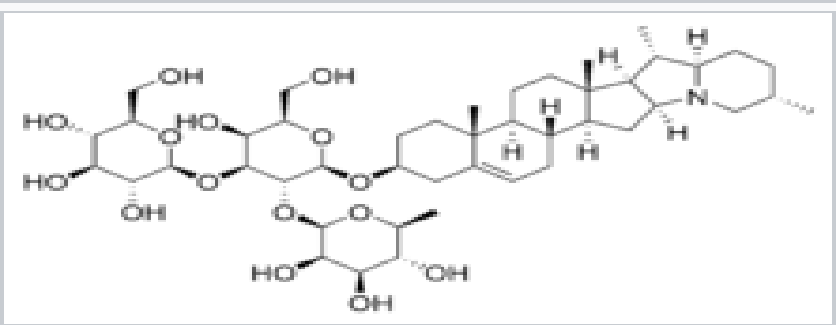


Figure 2.3: Structure of the saponnin: Hostettmann and Marston (1995)

The group of glycosides found in plant species with a characteristic foaming property when mixed with water, forming small stable bubbles are the saponnins. In the digestive system, the saponnins are normally broken down and are toxic when absorbed into the blood stream. They are hydrolysed by acids to give an agylcone (sapogenin), sugar and uronin acids. The saccharide chain linked to the sapogenin core can vary which give another dimension of arrangement which

is monodesmosidic, and bidesmosidic (Hostettmann and Marston, 1995). Plant secondary metabolites are used in the formation of tooth paste, soap, shampoos, cosmetics etc.Saponins are rich in legumes such as chickpeas and soybeans. They remove cholesterol and also effective against colon cancer.

2.6.4 Tannins

According to Hegerman, Zhao and Johnson, (1997), in 1796 tannin was used to denote substances present in plant extracts that were able to mix with protein of animal hides and change them into leather. They are widely distributed in plant and occur in solution in the cell sap, and often vacuoles. Tannins are soluble in water or alcohol a solution that is useful in medicine. It is used to tan and protect leather. Cranberries and pomegranates are bitter because of tannin (polyphenolics). Collagen is strengthened and builds by tannins and vitamin C. Tannins preventing bacteria in the urinary tract from adhering to the walls.

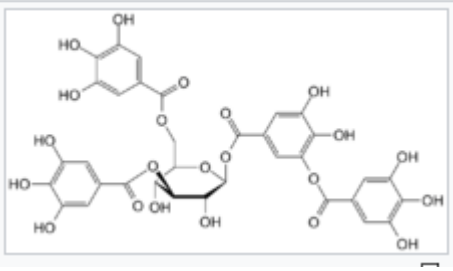


Fig.2.4.Structure of Tannin: Hegerman *et al*. (1997)

Tannins are of two types namely true tannins and pseudotannins. Complex phenolic compounds are true tannins which are classified into hydrolysable (pyrogallol) tannins (Ellagitannins and Gallitannin) and condensed tannins (Catechol and Catechin). Pseudotannin namely Gallic and ellagic acids are simple phenolics. Tannins have a therapeutic value as astringents because they are able to precipitate proteins. According to El-olemyl, Al-Muhtadi and Afifi, (1994), this effect can be used to stop haemorrhage and to treat diarrhea as well as local burn.

2.7 Microorganisms

2.7.1*Wohlfahrtiimonas chitiniclastica*

Wohlfahrtiimonas chitiniclastica an emerging human pathogen identified as the cause of septicemia in humans in South America and Europe.

W. chitiniclastica is a short rod-shaped gamma proteobacterium, Gram- negative bacteria with strong chitinase activity. Septicemia caused by *W. chitiniclastica* has been described in adult humans as originally from wound infections (Almuzara, 2011; Rebaudet, 2009)

Rebaudet, (2009) reported the *W. chitiniclastica* bacteremia infection in homeless women. The first case of fulminant sepsis caused by *W. chitiniclastica* in homeless 70 year old patient was also reported by Almuzara, (2011). *Wohlfahrtia magnifica* fly is believed to play a significant role as mechanical carrier of *W. chitiniclastica*. Toth, (2008) have isolated *W. chitiniclastica* from homogenates of the third-stage larva of the parasitic fly Wohlfahrtia magnifi*ca*. Wound contamination is believed to be the origin of bacteria septicemia. The obligate parasitic fly is most important cause of wound myiasis in warm-blooded vertebrates in East and North Africa, Southeastern Europe, Southern and Asiatic Russia. The larvae are deposited near wound or body opening of animals and humans (Farkas, Hall, Bouzagou and Khallaayoune, 2009). *W.magnifica* flies distribution is progressively expanding because of their broad adaptation capacities and climatic changes.

Lee, (2014) have isolated *W. chitiniclastica* from the larva gut of *Hermetiaillucens*, black soldier fly in South Korea. This report proofs that other obligation parasitic flies may be responsible for the transmission of this bacterium. The first emerging Zoonotic pathogen *W.chitiniclastica* in the United States was reported by Thaiwong, (2014).

2.7.2 *Alcaligenes faecalis*

Alcaligenes faecalis is a Gram-negative rod, non- encapsulated, aerobic non fermentative oxidate-positive (Bizet and Bizet 1997). It is believed to be a normal saprophytic inhabitant of the human intestinal tract. *A. faecalis* are present in the soil, water as well as human intestinal floral and hospital environments. It is the most frequently isolated member of family *Alcaligenaceae* in clinical laboratory and has the ability to produce an alkaline reaction in certain

media. From clinical materials *A. faecalis* has been isolated and recognized as opportunistic pathogen that causes serious infections (Kavunuoglu, Unal and Oguzhan, 2010). It has been reported to cause severe infections like peritonitis and fever clinically resembling typhoid with bacteremia either alone or as a complication of sprue. In new born it causes meningitis and bacteremia in cancer patients (Ashwath and Katner, 2005)

Systemic infection with *Alcaligenes feacalis* is very uncommon. It has been reported to cause sporadic cases of endocarditis, meningitis, chronic otitis, bacteremia, peritonitis, pyelonephritis, endophthalmitis and abscesses (Aisenberg, Rolston and Safdar, 2004; Ashwath and Katner, 2005). *A. feacalis* causes nosocomial infections and often as a result of the contamination of hospital equipments or fluid and have occurred in immune compromised host (Aisenberg *et al*., 2004). Almuzara, Matteo and Cittadini, (2010), reported an out- break of nosocomial-pseudo bacteremia, in a neonatology and pediatrics unit. Skin and Soft Tissue Infection (SSTI' s) caused by *A. feacalis* is very rare. But an investigation carried out by Tena, Femandez and Lago, (2015) in which they conducted a retrospective review of all cases of SSTI's caused by *A. feacalis* that occurred at their University hospital revealed *A. feacalis* should be considered a potential pathogen of SSTI, particularly in patients with vascular diseases or after surgery and that treatment can be difficult in some cases due to high level of resistance to commonly used antibodies.

According to Kavuncuoglu *et al*., (2010), *A. feacalis* has been isolated from a wide range of clinical materials and recognized as an opportunistic pathogen responsible for serious infections and also associated with pancreatic abscess and corneal ulcer (Aisenberg *et al*., 2004; Geong, Min-jung, Eui-yung, Ahn and Cheon, 2009). According to Mantengoli and Rossolini (2005), most strain displays multiple resistances to antimicrobial agents; attributed to the production of extended spectrum-lactamases (ESBLS).

Also Mordi, Yusuf, Onemu, Igeleke and Odjadjare, (2013) in their study on the prevalene of *A. feacalis* in bacteremia, meningitis and wound sepsis in tertiary health care Institutes in Western Nigeria revealed *A. feacalis* capacity to produce extended beta-lactamase enzyme makes it a potential pathogen.

2.7.3 K*lebsiella pneumoniae*

Klebsiella pneunoniae are Gram negative bacteria, rod-shaped, non- motile with a conspicuous polysaccharide capsule. This capsule encases the entire cell surface and provides resistant against many host defense mechanisms. It have become important pathogens in nosocomial infections especially hospital acquired urinary tract infections and chronic obstructive pulmonary disease individuals.

K. pneunoniae have become highly resistant to antibodies. It produces an enzyme known as a carbapenemase (referred to as KPC- producing organisms). Antibiotics called carbapenems will not be used to treat the infection because of the synthesis of carbapenemase. *Klebsiella* species are in the family of Enterobacteriacea. It is a normal body flora, lives inside human intestines where it does not cause disease but if it gets into other areas of the body, it can cause a range of different illness, such as pneumonia, blood stream, wound, surgical site infections, meningitis, urinary tract infections (UTI) etc. *Klebsiella* is a superbug that causes a range of disease, depending on which part of the body is affected .It is a common cause of serious Gram-negative infections in humans like blood stream infections, urinary tract infection and ventilator associated pneumonia (Skogberg, Lyytikainen, Ruutu, Ollgren and Nuorti, 2008). *K. pneumoniae* infections occur in both out break and endemic settings (Khan, Siddiqui, Sultan, Rizviand and Abqari, 2015). Symptoms of *K. pneumoniae* infection differ depending on where the infection is located, and are similar to symptoms of the same diseases caused by other microorganisms. Symptoms include: fever and chills neck stillness, flue-like symptoms, cough, bacteremia and sepsis. One major drug resistance mechanism of *K. pneumoniae* is the production β-lactanase (Pitout, Nordmann and Poirel, 2013).

2.7.4 *Proteus mirabilis*

Proteus mirabilis of the Enterobacteriaceae family is a Gram negative rod and anaerobic bacterium that demonstrates swarming motility from one side of agar surface to the other, passing other species in the media. The main microbiological characteristic of this organism is their robust swarming activity and urease production. It is lactose-nagative, urease-positive, indole -negative and produces hydrogen sulphide (O' Hara, Brenner and Miller, 2000). *P. mirabilis* are widespread in the environment and found mostly in the human intestinal tract

(Armbruster and Mobley, 2012) as part of normal intestinal flora along side with *E. coli* and *Klebsiella* spp, also found in multiple environmental habitats such as hospitals and long term care facilities.

P. mirablis is the most common species identified in clinical specimens, and cause of catheter-associated UTI and community-acquired, cystitis, wound infection, prostatitis, Pyelonephritis, burn infections and occasionally caused respiratory tract infection, eye infection endopthalmitis, chronic suppurative otitis media, meningitis and meningoenecephalitis (Lu ch, Chang, Chuang and Chang, 1999; Stock, 2003). *P. mirabilis* causes bacteremia following catheter-associated urinary tract infection (Melzer and Welch, 2013) and in rare cases has been reported to cause cellulitis endocarditis, empyema, mastoiditis and osteomsyelitis. In rheumatoid arthritis it has been suggested that *P. mirabilis* have a role in its etiology (Wilson, Thakore, Isenberg and Ebringer, 1997). *Proteus mirabilis* causes 90% of Proteus infections and considered as community acquired, and can lead to other health problems: Pneumonia of the lungs, septicemia (generalized infection of the blood) and infection at the level of existence wounds.

Characteristics of *P. mirabilus*

- Use the Urea
- Characteristic swarming motility
- Has fishy odor, due to production of hydrogen sulpfide gas
- Can lead to the formation of struite stones (kidney stone)

Symptoms Include: Urinary tract infection, flank pain, hematuria (blood in urine), dysuria, polyuria (increased urination frequency, urethral discharge (in Men), supra pubic pain, urgency to urinate, back pain, and concentrated urine appearances. *P. mirabilis* can cause symptomatic infections of the urinary track including cystitis and pyelonephritis and can also present cases of asymptomatic bacteremia in elderly and patients with type 2 and 3 diabetes (Mathews and Lancaster, 2011; Papazafiropoulou, Daniil, Sotiropoulos, Balampani, Kokolaki, Bousboulas, Konstantopoulou, Skliros, Petropoulou and Pappas, (2010). These infections can cause bacteremia and progress to potentially life threatening urosepsis and formation of urinary stone (urolithiasis)

2.7.5 *Candida tropicalis*

Fungi are distributed in the environment, associated with animals and humans as commensals, but can be pathogenic when the host immune system is altered (Krasner, 2002). Predisposing factors such as therapeutic application of immunosuppressive drugs, use of broad spectrum antibiotics in various clinical conditions are responsible for an increasing number of immune compromised patients and consequently opportunistic infections globally. Also prolong use of antifungal for prophylaxis in patients is the leading cause of colonization of Non- *Candida albicans Candida* (NCAC) species and increasing resistance to antifungal drugs (Hsueh, Lau, Chuang, Wan, Huang, Shyr, Yan, Yu, and Wu, 2005; Perfect 2004). The incidences of infection caused by *Candida* species (Candidiasis) have risen over the past three decades, because of high number of immune compromised patients, rise in Acquired immune deficiency sydrome (AIDS) epidemic and the extensive use of in-dwelling medical devices.

C. tropicalis which is one of the NCAC species identified as the most widespread pathogenic yeast species of the NCACs. According to Krawczyk, Leibner-Ciszak, Mielech, Nowak, and Kur, (2009) and Antonopoulou, Aoun, Alexopoulos, Baka, Logothetis, Kalambokas, Zannos, Papadias and Grigoriou, *(*2009). *C. tropicalis* is the most common cause of nosocomial Candidaemia in India. *C. tropicalis* is also associated with patients admitted to intensive care units, undergoing prolonged catheterization, receiving broad-spectrum antibiotics or with cancer (Rho, Shin, Song, Park, Kee, Jang, Park, Suh and Ryang, 2004; Kauffman, Vazquez and Sobel 2000; Colombo, Colombo, Guimarães,Silva, Monfardini, Cunha, RadyP, lves, and Rosas, 2007; Nucci and Colombo, 2007). Also *C. tropicalis* display a higher potential for dissemination in neutropenic individuals compared to other NCAC species and *C. albicans* (Colombo *et al*., 2007).

Some studies have documented that *C. tropicalis* was associated with higher mortality than other Non-*Candida albican Candida* species (NCACs) and *C. albicans.* This may be becauses of the virulence factors exhibited by this species such as proteinase secretion, biofilm formation and dimorphism (Negri, Gonçalves, Silva, Henriques, Azeredo and Oliveira, 2010a).

2.7.6 Wickerhamomyces anomalus.

Wickerhamomyces anomalus is non-*Saccharmonyces* yeast that generates ascospore (Ma, Chen, Chen and Chu, 2000). It is also known as *Hansenula anomala, pichia anonmala* or *Candida pelliculosa,* associated with spoilage in processed food and grain products. It is isolated from soil, grains, fruits and warm blooded animals. It is spherical to ellipsoidal budding blastocondia, 2-4 x 2-6 µm in diameter. Pseudohyphae may be present. *W. anomalus* has a widespread prevalence in nature. It is prevalent in plant material, soil, fruits, vegetables, tree exudation, leaves and other organic compounds (Ma *et al.,* 2000; Barchiesi, Tortorano and Di-Francesco, 2015). *W. anomalous* grows in high sugar mediums very well, uses maltose, dextrose, sucrose and galactose and generates ascospore (Ma *et al.,* 2000). It has the capacity to grow on a wide range of carbon sources at low pH under high osmotic pressure. It is propagated in a wide range of environment because of its anaerobic ability (Passoth, Fredlund, Druvefors and Schnurer, 2006). *W. anomalus* contributes to wine aroma as it produces volatile compounds. It produces mycocin killer toxin used as a bio-control agent against other fungi (Naumov, Naumova and Schnurer, 2001; Wang, Li, Jang and Huang, 2009). *W. anomalus* plays a role in wine fermentation as it possesses an active beta-glucosidase (Quatrini, Marineo, Puglia, Restuccia, Caggia, Randazzo, Spagna, Barbagallo, Palmeri and Giudici, 2008; Swangkeaw, Vichitphan, Butzke and Vichtphan, 2009). It has been reported from cases of candidaemia and catheter related infections in humans.

W.anomalus localization in the midget in reproduction systems of the Asian malaria vector *Anopheles stephensi,* suggesting multiple transmission patterns as shown by studies carried out by (Ricci,I Damiani, Scuppa, Mosca, Crotti, Rossi, Rizzi, Capone , Gonella, Ballarini, Chouaia, Sagnon, Esposito, Alma, Mandrioli, Sacchi, Bandi, Daffonchio, and Favia, 2011). *W. anomalus* are found in grape (Cordero-Bueso, Arroyo, Serrano, Tello, Aporta, Vélez, and Valero, 2013), active early in fermentation (Renout, Claisse and Lonvaud-Funel, 2007) and when high levels of acetic acid and ethyl acetate are produced can lead to wine spoilage (Plata, Millan, Mauricio and Ortega, 2003; Rojas, Gil, Pinaga and Manzanares, 2003).

The population of people with suppressed immune system has increase as a result of this advanceds in medical methods, due to the use of central venous catheter, and antimicrobial gent has increased. In recent years, it has been reported that non-*albicans candida* infections have

emerged with increase in the frequency of uncommon pathogenic yeast in nosocomial infections (Hazen, 1995). *W. anomalous* has been rarely reported in epidermics proportion (Pasqualotto, Sukiennik, Severo, De-Amorim and Colombo, 2005). Otağ, Mehmet, Harun, Ali, Ali - Adil and Gurol, (2015) reported *W. anomalous* fungemia cases in pediatric intensive care unit, revealing that hospital acquired infections can develop even from rarely encountered non-*albicans Candida* strains. *W. anomalus* has been reported as the agent in intravenous-drug-use driven candidemia in AIDS patient (Ratcliffe, Davies, Anson, Hales, Beeching and Beadsworth, 2011), meningitis agent in HIV patients (Retcliffe *et al.,* 2011), pancreatitis (Neumeister, Rockemann and Marre, 1992), ventriculitis (Thuler, Faivichenco, Velasco, Martins, Nascimento and Castilho, 1997) and urinary system (Epsinel-ingrott, 2008) infections.

2.8 Toxicological Assessment of Medicinal Plants

The valuable properties of plants can not be overemphasized, but some of them are known to be toxic as well. Recent studies carried out by Tulay and Nzlem, (2007) indicate that numerous plants are used as food sources, some of them may have mutagenic or genotoxic potential. Recently research studies focused on both toxicity and pharmacology of medicinal plants used by humans. This is very important in order to achieve a safe treatment with plant products (Parra, Yhebra, Sardinas and Buela, 2001). Plants toxicity is from different contaminants or from plant chemical compounds that are part of the plants. Different methods are used for the research of the potential toxicity of the herbal extracts based on the different biological models such as in vivo analysis on laboratory animals. Toxicity tests are regarded an important tool for preliminary evaluation of toxicity (Carballo, Hernandez-Inda, Perez and Garcia-Gravalos, 2002; Mayorga, Perez, Cruz and Caceres, 2010; Veni and Pushpanathan, 2014).

Investigations into toxicity of medicinal plants have been carried out and still need to be studied because of verse group of medicinal plants. To meet the goal of primary health care delivery worldwide, there has been an increasing interest in the use of medicinal herbs (Cowan, 1999). Using of medicinal plant to treat diseases in traditional medicine in Nigeria is not always reliable, guaranted in terms of safety because it is difficult for the traditional healers to monitor or detect delayed effects, adverse effect or arising from long term administration. It is important to evaluate the toxicity of medicinal plant in order to achieve a safe treatment with plant products (Parra *et al.,* 2001). The users often look at the benefits of the medicinal plant and neglect their toxic effects to the various organs.

Nigeria has many plants with diverse medicinal use; but their safety profile cannot be compared to the rate at which they are used. Despite regulatory bodies in Nigeria, some of these medicinal plants have not undergone safety profiling. Hence, this predisposes most of herbal drug users to toxicity. The toxicity can lead to unwanted reactions due to overdose, dependence-addiction, side effect, hypersensitivity, and allergic reaction, sub-chronic and chronic toxic effects. This kind of reaction necessitates toxicity evaluation. One of the mechanisms of toxicity is on the effect on site of action, when the toxicant binds to a targeted receptor with unintended high affinity resulting to unintended reaction (Tanguchi, Armstrong, Green, Golan and Tashjian, 2008). In the body, organs can metabolize non toxic compounds to toxic end product. Idiosyncratic reaction may result from medicinal plant use (Shaw, Graeme, Piere, Elizabeth and Kelvin, 2012). This kind of reaction has no apparent explanation for their occurrence but can be explained based on individual perspective, related to the genetic uniqueness of an individual (Grattagliano, Bonfrate, Diogo, Wang and Wang, 2009). Toxicant or its products can be excreted by the body but if not excreted, it accumulates or the metabolities can cause damage to the cell or DNA, and may also alter the oxidative protective mechanisms leading to cell death through apoptosis or necrosis (Sinha, Das, Pal, and Sil, 2013). According to Aguinaga, Claudiano, Marcusso, Ikefuti, Ortega and Eto, (2014) many medicinal plants have been reported to be toxic to both humans and animals.

According to Senin, (2006) acute toxicity or lethal toxicity of a chemical is the ability of a chemical to cause ill effect "relatively soon" after one administration or a 4 hour exposure of a chemical in air. Relatively soon means a period of minutes, 24 hours or days (up to about 2 weeks) but not longer time (Senin, 2006). LD_{50} means "Lethal Dose 50%", also known as "Median Lethal Dose." The LD_{50} for a particular substance is the amount that cause death in half (i.e. 50%) of a group of some particular animal species, usually rats or mice, through any means of administration. According to Gadanya, Sule and Atiku, (2011), LD_{50} is expressed as the amount of chemical administered in mg/100g (for small animals) or mg/kg (for bigger animals) of the body weight of the test animal. The LD_{50} obtained at the end of a study is expressed in relation to the route of administration of the test substance e.g. LD_{50} (dermal), LD_{50}, (Oral), LD_{50} (intraperitoneal) etc. The most frequently performed lethal study is the oral LD_{50} and the smaller the LD_{50} value, the more toxic the substance. These results obtained are important for drugs, food and accidental domestic poisoning.

CHAPTER III: MATERIALS AND METHOD

3.1 Area of Study

Imo State is in South Eastern Nigeria with Owerri as the capital. The State derives its name from the Imo River. It has twenty – seven Local Government Areas. It lies within latitudes 4^0 45 N and 7^0 15 N and longitudes 6^0 50 E and 7^0 25E. Imo State covers an area of about 5,100 km^2 and has a population of approximately 3.9 million people according to the 2006 census Nigeria. The population is 98% Igbo speaking. The study area is presented in Figure 3.1.

3.2 Reagents and Chemical Used

Methanol (JHD chemical) China, Acetone (JHD chemical) China, petroleum ether (JHD chemical) China, Ethanol (AR chemical) China, Chloroform (AR chemical)China, Fehling's solution A and B, sodium hydroxide (NaOH), distilled water, Wagner's reagent, Dregendroft's reagent, Meyer's reagent, lead acetate, HCl, $FeCl_2$, Acetic anhydride, NH_2OH, Dinitrosallcyclic acid (DNS), iron III chloride, ammonium thiocyanate, Libermann-Burchard reagent.

3.3 Equipment and Instrument Used

Dry oven (DHG-9101-SA) Search Tech Instrument UK, water Bath (TT-6)Techmel & Techmel USA, Electro-thermal incubator DNP Search Tech Instruments China, UV-VIS Spectrophotometer Spectrum Lab 7555 UK, S. Mettler analytical weighing machine FA2104 UK, Centrifuge 80-2 Search Tech Instrument UK, De-Ionizer 50 Search Tech Instrument UK, standard test sieve ASTM Search Tech Instrument UK, Test tube (Pyrex), Petric dish (Pyrex), Glass spreader, Bunsen burner, wire-loop, cotton wool, pipette and pipette filler, conical flask, refrigerator, Hockey stick, spatula, foil paper, slides & cover lips, microscope (B-Bran) Search Tech Instrument UK.

3.4 Collection of Sample Materials.

Fresh samples of *Mimosa pudica, Curcum longa, Euphorbia serrata* were collected from Mbaise, Umuagwo and Orji within Owerri urban. The identification and verification of the plants species were conducted at Green fingers Garden Okigwe Road, Imo State by a Taxonomist, Mr.

Moore. Several field trips were made for the collection of plant materials in order to meet the quantity required, to respect conservation principle and to avoid complete extinction of the species from their natural habitat.

3.5 Source of Test Microoganisms Used in the Study

The source (skin, wound,urine and sputum) clinically preserved isolates were collected before identification into species from stock culture in Medical Microbiology Laboratory, Federal Medical Centre Owerri Imo State.The bacterial culture were maintained at 37^{O} C on Nutrient Agar and the fungi cultures were maintained at 35^{O} C using Sabouraud Dextrose Agar before molecular to investigation determine their species.

3.6 Plant Preparation

The plant materials (leaves of *E.serrata* and *M.pudica*, and rhizomes of *C.longa*) were washed with distilled water and air dried at room temperature for about 2-3 weeks until totally dried. The samples were crushed with electronic blender to be coarse powdered. It was then sieved using 2 mm mesh size to uniform particle. The fine powder was then stored in a cool dry place until use.

3.6.1 Sample Extraction

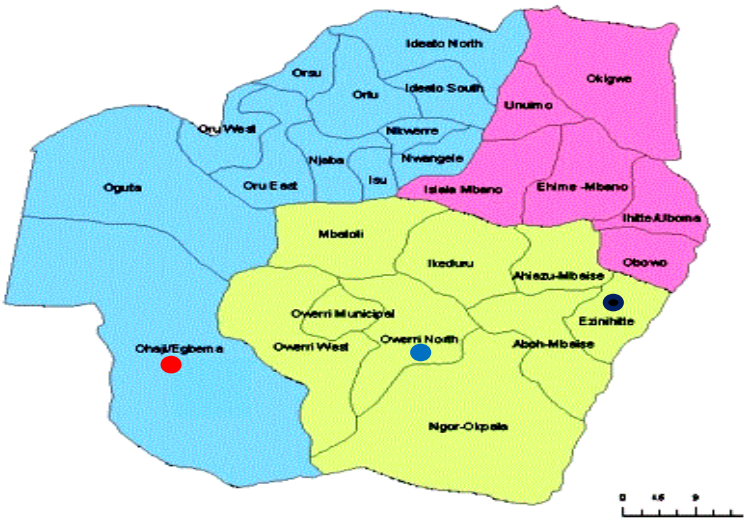
The extraction of active components of the leaves, and rhizome plant materials were done using the Aqueous (cold water), ethanol, and methanol as solvents.

3.6.2 Aqueous Extraction

Fifty (50 g) grams of the samples of each plant (50 g) were extracted with 800 ml distilled water by cold merceration method. The mixture obtained was filtered with muslin cloth and the filtrate was further concentrated using rotary vacuum evaporator at temperature of 50^{0}C. This process of extraction was intended to break and soften the plant cell wall in order to release the soluble phytochemicals. The extracts were stored at 4^{0}C in tight fitted bottle.

3.6.3 Ethanolic Extraction

The sieved fine powder 100 g of each plant sample was extracted with 250 ml ethanol for 48 h by using Soxhlet apparatus at 78^{0}C after allowing for 5 cycles. The extract was evaporated under reduced pressure by using a rotary evaporator and further concentrated in a water bath at 78^{0}C.



Ezinihitte

Owerri North

Ohaji/Egbema

the sampled areas.

Figure 3.1: Map showing the sampled areas.

Chris-Emenyonu and Onweremadu (2011).

3.6.4 Methanolic Extraction

The ground powder (100 g) of each plant sample was extracted with 250 ml methanol for 48 h by using Soxhlet apparatus at 65^{o}C after allowing for 5 cycles. The extract was evaporated under reduced pressure by using a rotary evaporator and further concentrated in a water bath at 65^{0}C after allowing for 5 cycles.The aim of all the extraction is to separate the soluble plant metabolic from the insoluble cellular residue which is left behind. The ground powder samples were placed in a porous bag or the thimble made from cellulose which is placed in the thimble of the soxhlet apparatus. The solvent was heated in the bottom flask, vaporized into the sample thimble, then condensed and dripped back. When the liquid content reached the siphon arm, the content was emptied into the bottom flask again and the process continued for 5 cycles. The concentrate was weighed and kept in the refrigerator.

3.7 Phytochemical Screening

The phytochemical screening of the plant samples for major bioactive constituent like tannins, flavonoids, alkaloids, saponins, phenols, terpenoids, carotenoids, cardiac glycosides, steroids etc was carried out using standard qualitative and quantitative methods.

3.7.1 Qualitative Analysis of Plant Samples

The method used in determination of phytochemical in this research as described by Ejikeme, Ezeonu, and Eboatu (2014) and Harbourne, Boulter and Turner, (1998) were used to carry out the qualitative analysis to ascertain the presence of different phytochemical constituents in the plant samples before proceeding to quantitative analysis as shown below.

3.7.2 Test for Saponins

Principles: Saponin has some characteristics properties that can be used as basis for simple detection:

i. Saponins produce honey comb froth in aqueous media after vigorous shaking and persist on warming.
ii. They are capable of emulsifying olive oil.
iii. Saponins haemolyse red blood cells.

Frothing test

Procedure:

Powdered plant samples, 2.0 g of each was boiled in 20 ml of distilled water in a water bath and filtered. The filterate (10 ml) was mixed with 5 ml of distilled water and shaken energetically for a stable persistent froth to occur. Three drops of olive oil was mixed with the frothing and shaken energetically and observed for the emulsion formation, confirmed the presence of Saponin.

3.7.3 Test for Alkaliods I

Two ml of HCl was added to 5 ml of each extracts, and then 1 ml of Dregendroff's reagent was added. The presence of alkaloid was indicated by the production of a red or orange precipitate.

Test for Alkaliods II

Procedure 5:

Into a test tube, 1.0 ml of each extract was pipetted, 5.0 ml of 2% HCl was also pipetted and was heated in a water bath for 10 minutes, filtered using filter paper (Whatman No1) and the filtrate was used for Wagner's Reagent Test.

Wagner's Reagent Test.

Principle: The principle of Wagner's reagent test is that alkaloids under acidic conditions and at room temperature react with potassium iodide and iodine to give a reddish brown precipitate.

Potassium iodide 3.0 g and 2 g of iodide were weighed, mixed, dissolved in 30 ml distilled water and made up to 100 ml with distilled water.

Procedure:

Pipetted into test tube was 10 ml of filtrate from procedure 5 above, and 1.0 ml of Wager′s reagent was pipetted, mixed properly, and observed for colour change. A reddish brown precipitate indicates the presence of alkaloids. (Ejikeme, *et al*., (2014).

3.7.4 Test for Cardiac Glycosides

Procedure:

Added into a test tube was 10 cm^3 of 50% H_2SO_4, and 1.0 ml of each extract was also added , heated in boiling water for 5 minutes, and 10 $cm^3$of Fehling's solution (5 cm^3 of each solution A and B) was added and boiled. It was oberved for brick red precipitate indicating the presence of cardiac glycosides.

3.7.5 Test for Terpenoids

Procedure:

Into 2 ml of chloroform was added 5 ml of each extract, also 1.0 ml concentrated H_2SO_4 was carefully added which formed a layer**.** Reddish brown colouration of the inner interface was formed, showing the presence of terpenoid.

3.7.6 Test for Steroids

Procedure:

Into 2 ml chloroform was added 0.5 ml of each extract in a test tube, and 1.0 ml concentrated hydrogen sulphide (H_2SO_4) added which formed lower layer. Greenish brown interface colour was observed.

3.7.7 Test for Tannins

Acid Test

The acid test principle is that under acidic condition phlobotannins reacts with dilute HCL to give a red colour precipitate.

Procedure:

To 2.0 ml of 1% HCl was added 3.0 ml of extract and observed for the presence of red precipitate. Red precipitate indicated the presence of phlobotannins.

3.7.8 Test for Flavonoids

Sodium Hydroxide Test

The sodium hydroxide test principle is that under alkaline pH, flavonoids at room temperature forms observable precipitate.

Procedure

Pipetted into the test was 1 ml of extract, 1.0 ml of dilute sodium hydroxide solution was also pipetted and the mixture was observed for colour change. Formation of precipitate indicated presence of flavonoids (Ejikeme *et al*., (2014).

3.7.9 Test for Phenol

Ferric Chloride Test

The principle of this test is that ferric chloride reacts with phenolic nucleus at room temperature to give a greenish brown or black colour.

Procedure:

Pipetted into the test tube was 1.0 ml of extract, 1.0 ml of 10 % FeCl was also pipetted and mixed properly and observed for colour change. Greenish brown or black coloration indicated the presence of phenolic nucleus

3.8 Quantitative Screening of the Plants Samples

3.8.1 Estimation of Saponin

From each plant sample, 5.0 g was measured into 20% acetic acid ethanol and allowed to stand in a water bath for 24 hours at 50^{o}C. This solution was filtered and the extract was concentrated to one-quarter of the original volume using a water bath. Concentrated Ammonium hydroxide (NH_4OH) was added to the extract drop-wise until the precipitate was complete. The solution was allowed to settle, precipitate collected by filtration and weighed. The content of saponin was weighed and calculated in percentage using this formular (Harbourne *et al,* 1998).

Calculation:

% saponin content= (weight of filter paper + residue)-(weight of filter paper) x 100

Weight of sample analysed

3.8.2 Estimation of Flavonoids

Five grams (5.0 g) of each plant samples was extracted with 100ml of 80% aqueous methanol at room temperature. The solution was then filtered and the filtrate evaporated to dryness using a water bath. The sample was then weighed (Harbourne *et al*, 1998). The percentage flavonoid was calculated using this formula.

Calculation:

% flavonoids= (weight of beaker + Residue)-(weight of beaker)x100

Weight of sample analysed.

3.8.3 Estimation of Alkaloids

From each plant samples, 5.0 g of the sample was transferred into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added, covered and allowed to stand for 4 hours at 25^0C. This solution was filtered using Whatman No.1, filtrate was concentrated to one - quarter of the original volume using a water bath. Concentrated NH_4OH was added to the extract drop-wise, the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). Pre-weighed filter paper was used to filter the solution. The residue on the filter paper is the alkaloid, and was dried in the oven at 80^oC. The percentage alkaloid content was calculated (Harbourne *et al*, 1998).

Calculation:

% weight of alkaloid= (Weight of filter paper +residue)- (weight of filter paper) X100

Weight of sample analyzed

3.8.4 Estimation of Tannin by Titration

Follins-Dennis titrating method as described by Uyotismita and Arindan (2015) was used to estimate tannin. Five grams of ground plant sample in a conical flask was added 100ml of petroleum ether and covered for 24 hours. The solution was filtered and allowed to stand for 15 minutes, so that the petroleum ether will evaporate. It was soaked for 4 hours in 100 ml of acetic acid in ethanol for re-extraction. It was filtered and the filterate collected.

To the filterate, 25 ml of NH_4OH was added to precipitate the alkaloids. The alkaloid was heated with hot plate to remove some of the NH_4OH still in solution, the volume was measured, 5 ml was taken and 20 ml of ethanol was added to it. This was titrated with 0.1 M of Sodium hydroxide (NaoH) using an indicator (phenolphthalein) until pink end point was observed.

Tannin content was calculated in %

($C_IV_I = C_2\ V_2$) molarity

Calculation:

Tannic acid $C_IV_I = C_2\ V_2$

Where C_1=concentrated of Tannic acid

C_2=concentrated of Base = 0.1 M

V_1= volume of Tannic = 5ml

V_2= Titre value of sample = titre = 56.20

Tannic acid $C_1 = \frac{C_2\ V_2}{V_1}$

% of tannic acid content $= \frac{C_1 \times 100}{\text{Weight of sample analyzed}}$

3.8.5 Estimation of Cardiac Glycosides

Cardiac glycosides estimation method as described by Osagie (1998) was adopted. A militre (1ml) of 2% solution of 3, 5 dinitrosallcyclic acid (DNS) in methanol was added to 1 ml of the extract and 1 ml of 5% aqueous sodium hydroxide. It was boiled for 2 minutes until a precipitate (brick- red) was observed. The sample was filtered; the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50^0 C. The weight of the filter paper with residue was recorded. The cardiac glycoside was calculated in percentage.

Calculation:

$$\% \text{ cardiac glycoside} = \frac{(\text{weight of filter paper + residue}) - (\text{weight of filter paper}) \times 100}{\text{Weight of sample analyzed}}$$

3.8.6 Estimation of Phytate

The phytate content in the samples was estimated using the method as described by Unuofin, Otunola and Afulayan, (2017). Each of the processed samples 2 g was weighed into three 250 ml conical flasks. Then each sample was soaked for hours in 100 ml of 2% concentrated hydrochloric acid (HCl). The samples were filtered and 50 ml of each filtrate placed in 250 ml beaker and 100 ml (distilled water) was added to each sample. About 10 ml solution of 0.3% ammonium thiocyanate (indicator) was added and titrated with standard Fe_3cl solution which contained 0.00195 g (Fe) per ml. The phytic acid percentage was calculated using the formula:

$$\text{Phytic acid \%} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{2}$$

3.8.7 Estimation of Terpenoid

Into 2.0 ml of chloroform was added 5.0 ml of each extract and was transferred from assay tube to colorimetric cuvette containing methanol 95% (V/V), used as blank to read the absorbance at 538 nm. Measured 200 ml of previously prepared linalool solution in methanol was added to 1.5ml chloroform for the standard curve and serial dilution was done (dilution level 100 mg/200

µl to 1 mg/ 200 µl linalool concentrations). For the serial dilution, total volume of 200 µl was made up by dilution of 95% (V/V) methanol.

3.8.8 Estimation of Phenol

The estimation of phenols was done using spectrophotometric method. The plant sample (2 g) was heated with 50 ml ethanol for 50 minutes. From the boiled sample, 5 ml was pipetted into 50 ml volumetric flask and 10 ml (distilled water) added. Also 2 ml of (NH_40H) solution and concentrated pentanol (5 ml) were added to the mixture. The sample was made up to the mark and left for 30 minutes for colour development and the wavelength measured at 505 nm.

3.8.9 Estimation of Steroids

The steroid content was estimated spectrophotometrically using the method adopted by Owiredu, Teye and Quaye, (2013). Chloroform was added to 1.0 ml of extract to make the volume up to 5.0 ml. Liberman-Burchard reagent (concentrated Sulpuric acid (0.5 ml) in 10 ml of acidic anhydride) was added and mixed. The tubes were covered with black paper and kept under dark for 15 minutes. A green colour complex formed was measured spectrophotometrically at 640 nm. To construct a caliberation curve, cholesterol was used as standard.

3.9 Sterilization of Materials Used

All glass wares were sterilized using hot-air oven at 160°C for 2 hours. Media were sterilized in an autoclave for 15 minutes at 121°C (Muller Hinton agar and Sabouraud Dextrose Agar). Glass spreader was sterilized after dipping into absolute ethanol by passing through flame while wire-loops and straight wires were sterilized by heating to red-hot.

3.10 Isolation of Test Organisms

The test organisms were clinically isolated as follows:

Proteus sp isolated from skin.

Wohlfahrtiimons sp isolated from skin.

Acaligenes sp isolated from urine.

Klebsiella sp isolated from stool.

Candida sp isolated from urine.

Wickerhamonyces sp isolated from sputum.

PURE CULTURE TECHNIQUE

The technique as described by Ogbulie, Uwaezuoke and Ogiehor, 1998 was used for the isolation of the microorganisms. The isolation method used to obtain pure culture is streak plate method. In this technique, a sterile wire loop was used to collect a mixed culture was streaked in a pattern over the surface of the solid medium. This manipulation thins out the bacteria / fungi on the agar surface so that individual bacterium/fungus was separated from each other. The cells were suffiently apart that each develops into a discrete colonies. Each colony arises from a single cell and consequently is a pure culture. Then each colony was aseptically transferred into a fresh sterile medium in a petri- dish to obtain a pure culture stock from which identification of the microorganisms were carried out using DNA sequencing.

3.10.1 Identification of Isolates by DNA Sequencing

Bacteria/Fungi Identification

Methods Used

1: Culture on agar slant

2: DNA extraction ZR fungal/bacterial DNA miniprep™50 preps.model d6005

3: PCR amplification and DNA sequencing by Sanger sequencing method

4: Blasting analysis: NCBI blast online

Sequencing: Sanger Sequencing

Primer used: 27F.1 Forward 5'AGRGTTTGATCMTGGCTCAG 3 and 1492R reverse 5'GGTTACCTTGTTACGACTT 3'

Molecular Analysis:

The method used was based on PCR and Sanger Sequencing analysis. DNA Extraction and Bioinformatics analysis of sequences were performed at Molecular Laboratory Services Division of Teddy & Thaddeus Nigeria Company, Akoka Lagos. Sequencing analysis was done at Inqaba Biotechnology Pty South Africa.

3.10.2 DNA Extraction

A twenty- four hours growth of microbial isolates in BHI broth harvested by centrifugation at 14, 000 x g for 10 minutes was used for DNA extraction. in 1 ml of Ultra -pure water the cells were washed three times by centrifuging at 12,000 rpm for 5 min. ZR Fungal/Bacterial DNA MiniPrep™50 Preps Model D6005 (Zymo Research, California, USA) was used for DNA extraction and purification. Bacterial cells 50-100 mg was re-suspended in 200 µl of sterile water. Re-suspended bacterial cells were transferred into a ZR Bashing-Bead™ Lysis Tube and Lysis solution (750 µl) was added to the tube. A bead beater fitted with a 2 ml tube holder assembly was used to secure the bead containing the solution and processed at maximum speed for 5 minutes. A micro-centrifuge was used to centrifuge the ZR Bashing-Bead™ Lysis Tube at 10,000 x g for 1 minute. The supernatant (40 µl) was pipetted into a Zymo-Spin™ IV Spin Filter in a collection tube and centrifuged at 7,000 x g for 1 minute. Fungal/Bacterial DNA binding buffer (1,200 µl) was added into the filtrate in the collection tube. The mixture (800 µl) was transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuged at 10,000 x g for 1 minute.

From the collection tube, the flow through was discarded and the process was repeated to obtain the remaining products. The DNA pre-wash buffers (200 µl) was added into the Zymo-Spin™ IIC Column in a new collection tube and was centrifuged at 10,000 x g for 1 minute. Then Fungal/Bacterial DNA Wash Buffer (500 µl) was added into the Zymo-Spin™ IIC column and centrifuged at 10,000 x g for 1 minute. Into a clean 1.5 ml micro-centrifuge tube the Zymo-Spin™ IIC column was transferred and DNA elution buffer (100 µl) was then added directly to the column matrix, centrifuged at 10,000 x g for 30 seconds to elute the DNA. The resulting ultra-pure DNA (the filterate) was used as a template during the assay and was transported in ice container to the laboratory for sequencing.

3.10.3 Polymerase Chain Rreaction

Amplification of DNA

Using universal degenerate primers 27F.1 Forward 5'AGRGTTTGATCMTGGCTCAG 3 and 1492R reverse 5'GGTTACCTTGTTACGACTT 3' the PCR reation was performed on the extracted DNA (DeAngelis, Cindy, Beller, Brodie, Chakraborty, Desantis, Fortney Hazen, Osman, Singer, Tom, and Andersen, 2011) that amplified the entire 16s Variable region at annealing temperature of 58^{o} C. Each PCR reaction contained the following: 5µl of 10 × Taq buffer, 2mM $MgCl_2$, 1.5 U Super-Therm DNA Polymerase (Southern Cross), 0.25 mM dNTP's, 0.1µM of each primer, 1µl of extracted DNA and Nuclease Free Water (NFW) up to the final reaction volume of 50 µl. The PCR cycle initial denaturation step started at 94°C for 10 minutes, then followed by 30 cycles of denaturation at 94°C for 1 minute, annealing temperature at 58°C for 1 minute and extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes, then cooling to 4°C. In order to verify amplification few microliters of the samples were run on a 1% agarose gel at 90 V for 30 minutes. The entire PCR reaction was loaded onto a 1% agarose gel with band size (approximately 1500 bp) was excised. Using the GeneJET™ gel extraction kit (Fermentas) the DNA was recovered from the gel slices.

Sanger (dideoxy) sequencing Technique was used to perform DNA sequencing. Automated PCR cycle- Sanger Sequencer™ 3730/3730XL DNA Analyzers from Applied Biosystems (Russell, 2002; Metzenberg 2003), was used to determine the nucleotide sequence of the specific microorganism isolated and result was obtained as nucleotides. Resultant nucleotide base pairs from sequence analysis were performed by BLAST analysis by direct blasting on American data base (http://blast.ncbi.nlm.nih.gov). A read was blasted for every set of isolate and the resultant top hits with minimum E-score for every blast result showing species name was used to name the specific organism.

3.10 .4 DNA Sequencing

Table 3.1: DNA Sequencing for Microbial Samples used in the study.

No	Sample Name	Sample Type (Select from drop-down menu)	DNA Type (Select from drop-down menu)	Primer for sequencing (Select universal primers from drop-down menu)
1	AJ2A	DNA	Genomic	16S
2	BS22	DNA	Genomic	16S
3	BS23	DNA	Genomic	16S
4	BS24	DNA	Genomic	16S
5	ZBA	DNA	Genomic	16S
6	ZBB	DNA	Genomic	16S
7	ZBC	DNA	Genomic	16S
8	ZBD	DNA	Genomic	16S
9	BS18	DNA	Genomic	ITS
10	BS25	DNA	Genomic	ITS
11	BS26	DNA	Genomic	ITS
12	ZFA	DNA	Genomic	ITS
13	ZFB	DNA	Genomic	ITS
14	ZFC	DNA	Genomic	ITS

3.11 Media Preparation

3.11.1 Muller Hinton Agar: Muller Hinton agar was used for the growth of bacteria. Medium was prepared by adding 38 g of dehydrated powder using an electric balance into 100 ml of water (distilled water) and was shaken vigorously to dissolve completely.

3.11.2 Sabouraud Dextrose Agar: This medium was used for the growth of fungi. Medium was prepared by adding 62 g of powder using electronic weighing balance into 1 litre of distilled water and shaken vigorously to dissolve completely.

3.11.3 Media Sterilization and Pouring

Media was sterilized by using autoclave at 121°C 15 minutes for Muller Hinton and Sabouraud Dextrose agar respectively. After sterilization, media was poured in pre-sterilized glass petri plates and allowed to dry and solidify.

3.12 Inoculum Preparation

Active cultures were prepared by inoculating the culture into sterile bottles containing nutrient broth for bacteria and Sabouraud's Dextrose Agar slant for fungi and were incubated for 24 hrs at 37°C for bacteria and 72 hrs at 28°C for fungi.

Turbidity of actively growing bacteria suspension was adjusted to match 1.0 MacFarland; the turbidity standard. This turbidity is equivalent to approximately 1. 2 x 10^8 cfu/ml for bacteria, and 2.0 x 10^5 spore /ml for fungi. This suspension was kept for further use.

3.12.1 Standardization of the Inoculum for Bacteria Test

The nutrient broth of each standard organism were prepared and incubated at 37° C for 24 hrs in a shaker incubator. The broth was centrifuged to remove the peptone water, beef extracts of the broth after incubation. The top was decanted, and the supernatant was washed with sterilized water and centrifuge for 3 times. After centrifuging, the washed micro-organism was collected and re-suspended in sterile water. The inoculum was adjusted to turbidity comparable to I MacFarland standards (Gillespie and Hawky, 2006)

3.12.2 Standardization of the Inoculum for Fungi Test

From old fungal culture, the mycelia spore suspension was obtained by lightly scrapping the surface with a spatula using 30 ml of 0.0l% Tween 80 solutions. The suspension was adjusted to an optical density of 0.5 at 530 mm (1ml = 1 x 10^6 spore) (Espinel and Kerkering, 1991).

3.13 Determination of Antibacterial Activities of *E. serrata, M. pudica* and *C. longa* Extracts.

The antibacterial activity of the extracts was determined using well diffusion method of Rath, Lin, Udolph, Cai, Yany and Chai, (2002). The extract of these medicinal plants were used for the study. The extracts namely: Ethanol, Methanol, and Aqueuos extract, were dissolved in 5% dimethyl Sulfoxide (DMSO). A day old culture of each organism was prepared by taking 2 wire loop of the organism from the stock and inoculated onto the sterile nutrient broth of 5ml, each incubated for 24 hours at 37ºC. The overnight culture was diluted to 10^{-2} (by adding 0.1 ml of each organism into 9.9 ml of sterile water) from where 0.2 ml was aseptically spread into prepared solidified sterile Mueller Hinton agar (Hi-media India) petric dishes using spread method. Then four wells (5 mm diameter) were made on each solidified media petric plates with a sterile cork-borer and 0.1 ml of each extract dilution of (50 mg/ml, 37.5 mg/ml, 25 mg/ml) was added into each well on different plates. Chloramphenicol 0.1ml (250 mg) was used as control. The petric dishes were left for 2 hours on the bench to allow the extract diffuse properly into the media and incubated at 37º C for 24 hours. The zones of inhibition were observed and measured using a transparent ruler in millimeters. The readings were taken in duplicates and the average values tabulated.

3.14 Determination of Antifungal Activities of *E. serrata, M. pudica* and *C. longa* Extracts

The extracts from each plant were tested against fungal pathogen *C. tropicalis and W. anomalus* for their antifungal activities using well diffusion method as modified by Ogu, Madagwu, Eboh and Igborbor, (2011). Sabouraud dextrose agar was prepared according to manufacturer's specifications, autoclaved at temperature of 121ºC for 15 mins, supplemented with 0.05% chloramphenicol and dispensed into the petric dishes. Then 1ml of each standardized spore suspension (10^5 spores /ml) was spread on the solidified Sabouraud's dextrose agar plates using spread plate method. Wells (5 mm) were made on the agar surface with sterile cork borer. The

extract 0.05 ml of each concentration (50 mg/ml, 37.5 mg/ml, and 25 mg/ml) was dispensed into the wells and allowed for diffusion of the extracts to take place and standard drug griseofulin was used as control. The plates were incubated 25^0C for 48hours for growth. Antifungal activities of the extracts as well as the control were measured, and the readings taken in duplicates and recorded as mean diameter of the zones of inhibitions.

3.15 Determination of the Antioxidant Activities *E. serrata, M. pudica* and *C. longa* extracts

3.15.1 Determination of the Free Radical 2,2-Diphenyl-1-Picryihydrazyl (DPPH) Scavenging Activity.

The free radical scavenging activity testing was determined using 2, 2- diphenyl-picrylhydrazy (DPPH) based on the method described by Lim and Quah (2007).

Concentrations of 20, 40, 60, 80 µg/ml of the extract were mixed vigorously in 5 ml of DPPH solution (0.1 mM in methanol) and incubated for 30 minutes in the dark at room temperature. Decolourization of DPPH was measured against blank at 517 nm. The percentage inhibition was calculated using the formular and represented as

$$\%\text{inhibition} = \frac{(Abs\ Blank - Abs\ sample)x\ 100}{Abs\ Blank}$$

3.15.2 Determination of Nitric Oxide Radical Scavenging Activity

The activity was measured based on the method described by Panda, Raj, Shrivastava and Prathani, (2009). To 4ml of the extract, having different concentration (20, 40, 60, 80 μg/ml), 1 ml of sodium nitroprusside (SNP) solution (5 mM) was added and incubated at 27^0C for 2 h. An aliquot (2ml) of the incubation solution was removed and diluted with 1.2 ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride). Absorbance of the chromophore was read immediately at 550 nm and compared with standard ascorbic acid.

$$\text{Nitric oxide scavenging activity (\%)} = \frac{abs(control) - Abs(sample)}{Abs\ (control)} x100$$

Where Abs (control): Absorbance of the control and

Abs (sample): absorbance of the extract.

3.15.3 Determination of Hydrogen peroxide (H_2O_2) Radical Scavenging Activity

The ability of the extract to scavenge hydrogen peroxide was determined based on the method adopted by Ngonda (2013). Two mMol/l solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). Different concentrations of the extracts (20, 40, 60, 80 μg/ml) were added to (0.6 ml) hydrogen peroxide solution. After 10 min the absorbance of hydrogen peroxide at 230 nm was determined against a blank solution (containing phosphate buffer without hydrogen peroxide) and compared with the standard (ascorbic acid).

H_2O_2 activity (%) = $\frac{Abs(control)-Abs(sample)}{Abs\ (control)} x100$

Where, Abs (control): absorbance of the control and

Abs (sample): Absorbance of the extracts/standard

3.15.4 Determination of Total Reducing Power

The antioxidant activities were determined using the ferric reducing antioxidant power (FRAP) assay according to the method described by Sofidiya, Odukayo, Familoni and Inya- Agha, (2006).

Concentrations of the extracts (20, 40, 60, 80 µg/ml) in 1.0 ml deionized water was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferrocyanide. Then mixture was incubated at 50^0 C for 20 minutes, latter 2.5 ml of 10% trichloroacetic acid was added and centrifuged for10 minutes at 3000 rpm. Then upper layer of the solution (2.5 ml) was mixed with 0.5 ml of 0.1% $feCl_3$. The absorbance measured at 700 nm. All the tests were performed in duplicate and average plotted and compared with a standard vitamin C.

3.16. Toxicological Tests

The toxicological testing was carried out at the Department of Biochemistry, Micheal Opara University of Agriculture, Umudike Abia State. The animals used were acquired and bred in the

Animal Breeding and Experimentation Facility of the Department of Biochemistry. In clean metal cages, maintained at room temperature with constant photoperiod of 12 h light/dark cycle for two weeks, the rats were housed for acclimatization. Adult male albino rats aged 8-10 weeks old, weighing 130-160 g were used to perform *in vivo* toxicity studies. The animals were fed on standard animal feed (pelleted diet) and water ad libitum.

3.16.1 Determination of the Acute Oral Toxicity

Acute toxicity testing: The acute toxicity profile was carried out and median lethal dose (LD_{50}) was calculated using Lorke's method as described by Aliyu, Yaro, Chedi and Salisu, (2015)

The experiment had two phases: I and 2 respectively.

Phase 1

In this phase twenty-seven animals were used for the three plant extract (*C. longa, M. pudica* and *E. serrata*).

The twenty-seven animals were divided into nine groups of three animals each. Group 1 animals were administered different doses (10, 100 and 500 mg/kg) of *C. longa*, *M. pudica* and *E. serrata* respectively. The animals were observed for 24 hours to monitor their behaviour as well as for mortality.

Phase 2:

This phase involved the use of nine animals, which were divided into 9 groups of one animal each. The animals were given higher doses (1000, 2900 and 5000 mg/kg) of extract of *C. longa, M. pudica* and *E. serrata* extracts were observed for 24 hours for behavioural changes as well as mortality.

Formular:

$L_{D50} = \sqrt{(D_0 \; X \; D_{100}}$ D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

3.16.2 Determination of the Sub-chronic Oral Toxicity

Thirty- five male rats weighing 130 g -160 g were used in this study. The albino rats were divided into seven groups labelled 1, 2, 3, 4, 5, 6, and 7, each group comprising of five rats.

Groups 1 to 6 were the experimental groups and Group 7 was the control group.

Groups 1 and 2 were orally administered (200 mg /kg and 400 mg/kg) body weight doses of ethanol extract of *C. longa* dissolved in 1% DMSO daily for 28 days.

Groups 3 and 4 were were orally administered (200 mg/kg and 400 mg/kg) body weight doses of ethanol extract of *M. pudica* dissolved in 1% DMSO daily for 28 days.

Groups 5 and 6 were orally administered (200 mg/kg and 400 mg/kg) body weight doses of ethanol extract of *E.serrata* dissolved in 1% DMSO daily for 28 days with aid of an orogastric tube.The extracts were administered daily for twenty-eight days during which food and water were also given daily. In each group, the body weight of all the rats were recorded weekly.

3.16.3 Termination of the Experiment

The rats were sacrified by cervical decapitation on the 29^{th} day, and blood was collected by ocular puncture (media cantus) using ordinary capillary tube and cardiac puncture using syringe and needle. A pair of scissors used to open the animal laid on a dissecting board by cutting through vertical mid-line from neck to peritoneum (Osaro, Udomah, Jobbi, Isah, Abdulrahaman, Onuigwe, Egenti, Musa and Erhabo, 2016). The kidney, liver and heart were excised and preserved.

3.16.4 Collection of Blood Sample

Blood samples were collected by Ocular puncture (media cantus) using ordinary capillary tube and cardiac puncture using a 22 gauge needle attached to a 3ml syringe was inserted to the notch at the caudal aspect of the sternum and directed to the heart. The position of the heart was determined by palpating for the heartbeat. To draw blood, the plumber was pulled backward. The blood collected was divided into two portions:

1. For haematological analysis (collected in EDTA tubes containing anticoagulants; Ethylenediaminetetracetic acid).

2. For biochemical analysis (collected in tubes without anticoagulant).

The blood for biochemical test was kept for one hour to allow adequate clotting at room temperature. To obtain the serum, the blood was centrifuged for 10 minutes at 3000 rpm. The serum obtained was stored in Eppendort tubes at -20°C awaiting biochemical analysis.

3.17 Biochemical Analyses

Biochemical parameters determined in this study includes Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), proteins, urea, albumin, lipid peroxidation (malondialdehyde MDA), Superoxide dismutase (SOD), catalase, Gutathonine reductase (GDx), Creatinine, C- Reactive protein (CRP) using appropriate analytical methods.

3.17.1 Determination of Serum Alanine Aminotransferase (ALT) Activity

The determination of alanine aminotransferase in whole blood was done based on the method described by Yakubu, Olawoile, Arowora and Imo, (2017), by using Randox limited commercial kits.

Principle: Alanine aminotransferase (ALT) assay is based on the principle that pyruvate is formed from:

$$\alpha\text{- Oxoglutarate + L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate + pyruvate}$$

ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenlyhydrazine. The colour intensity was measured against the blank at 540 nm.

Procedure: The blank and sample test tubes were set up in duplicates. Into the sample tubes was pipetted 0.1 ml serum. Buffer solution 0.5 ml containing phosphate buffer, L-alanine and α-Oxoglutarate was added. The mixtures were mixed thoroughly and incubated at 37^0C for 30 minutes and pH 7.4. Reagent containing 2, 4-dinitrophenlyhydrazine 0.5 ml was later added to both tubes and 0.1 ml of sample was added to the blank tube. The tubes were mixed thoroughly

and incubated at 25^0C for 20 minutes. Sodium hydroxide (NaOH) 5.0 ml was added to each tube and mixed and the absorbance was read after 5 minutes at 540 nm against the blank.

3.17.2 Determination of Creatinine

Creatinine level was determined using Direct Endpoint according to the method adopted by Agbafor, Nwaka, Dasofunjo and Ugwu, (2017) using Randox commercial kit.

Principle: In alkaline conditions creatinine reacts with picric acid to form a colour complex, which absorbs at 510 nm. The creatinine concentration in the sample is proportional to the rate of the colour formation. The endpoint method, the difference in absorbance measurements after colour formation yields a creatinine value.

Procedure

The test vial, reagent blank, standard, control, and sample test tubes were labelled and 3.0 ml of working reagent was pipetted into test tubes. To each respective tube 0.1ml of sample was transferred, sterile water was added to reagent blank and mixed. All the tubes were placed in heating bath at 37^0C for 15 minutes. The spectrophotometer wavelength was set at 510 nm while the instrument with the reagent blank was set at zero. The absorbance of all the tubes was read and recorded. (Wavelength range: 500-520 nm).

Calculations

The Creatinine value of unknown (sample) is determined by comparing its absorbance change with that of a known standard.

$$\text{Mg/dl} = \frac{A_{sample}}{A_{\text{standard}}} \times \text{Concentration of Standard}$$

3.17.3 Estimation of extent of lipid peroxidation (malondialdehyde)

Estimation of Lipid peroxidation measured spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin, Rosengren, Shertzer and Camejo, (1993).

During lipid degradation formation of such products like malondialdehyde, ethane and pentane occurs. A red or pink coloured complex which in acid solution absorbs at 532 nm was formed when MDA reacts with thiobarbituric acid.

Procedure: The serum (0.1 ml) was mixed with H_2O (0.9ml) in a test tube. Then 0.5 ml of 25% trichloroacetic acid and 0.5 ml of 1% thiobarbituric acid in 0.3% NaOH were also added to the mixture. In a water-bath the mixture was boiled for 40 minutes and then cooled in cold water. Then 0.1 ml of 20% sodium dodecyl sulfate was added to the cooled solution and mixed. Absorbance was taken at 532 nm and 600 nm against a blank.

$$\% \text{ TBARS} = \frac{\text{A532- A600 x 100}}{0.5271 \text{x} 0.1} \quad \text{(mg/dl)}$$

3.17.4 Determination of Aspartate Aminotransferase (AST)

This was done according to the method adopted by Yakubu *et al*., (2017).

Principle: Aspartate Aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine and the colour intensity is measured against the blank at 546 nm.

Procedure: The blank and sample test tubes were set up in the test tube rank in duplicates and serum 0.1 ml was pipetted into the sample tubes and 0.5 ml of reagent (1) was pipette into both sample and blank tubes. The solutions were thoroughly mixed and incubated at 37 ^{0}C for 30 minutes and pH of 7.4. Reagent (2) 0.5 ml (containing 2, 4-dinitrophenylhydrazine) was added into all the test tubes and the sample (0.1 ml) was added into the blank tubes. The tubes were mixed thoroughly and incubated at 25 ^{0} C for 20 minutes and sodium hydroxide (5.0 ml) solution was added to each tube and mixed. The absorbance was read after 5 minutes at 546 nm against the blank.

3.17.5 Determination of Catalase Activity

This was done according to the method adopted by Atawodi (2011).

Principle: The ultra violet absorption of hydrogen peroxide can be easily measured at 240 nm. Hydrogen peroxide (H_2O_2) decomposition by catalase, the absorption decreases with time and catalase activity can be measured from this decrease.

Procedure: Hydrogen peroxide 2.0 ml and phosphate buffer 2.5 ml were added to a beaker. The sample 0.5 ml was also added and mixed then 1.0 ml of the mixture was added to 2.0 ml of dichromate acetic acid reagent. The absorbance was read at 540 nm at one minute interval in duplicates. Catalase activity was calculated using the following equation:

$$\text{Catalytic concentration (unit/L)} = \frac{\log [\text{abs 1}/\text{abs 2}] \times 0.23}{0.00693}$$

3.17.6 Estimation of Glutathione Peroxidase

This was carried out based on the method described by Uroko, Agbafor, Uchenna, Achi, Egba, Nwege-Anyalowu, Chukwuemeka and Ngwu, (2017).

Procedure: Heparinised whole blood 0.05 ml was diluted with diluting reagent (2 ml) and was used for the assay. Diluted sample 0.05 ml was mixed with 1.0 ml of both R1 and R2 reagent respectively. Initial absorbance of both test and blank were read after 1 min and the timer was started simultaneously. Absorbances were read again after 1 and 2 mins intervals at 340 nm. Glutathione peroxidase activity was calculated from the formular below.

U/L of haemolysate = 8412 x ΔA 340 nm/minute.

3.17.7 Estimation of Reduced Glutathione

The estimation of reduced glutathione level was determined by the method adopted by Exner, Wessner, Manhart and Roth, (2000).This method was based on the appearance of yellow colour when 5, 5'- dithio-bis-2-nitrobenzoic is added to compound containing sulphydryl groups and the colour was read at 412 nm.

Procedure: Sample (0.2 ml) was mixed with EDTA solution (1.8 ml) and then precipitating reagent (3.0 ml) was added, mixed thoroughly and centrifuged after 5 minutes. To the filterate (2.0 ml), 0.3 M disodium hydrogen phosphate solution (4.0 ml) and 5, 5'- dithio-bis-2-nitrobenzoic reagent (1.0 ml) were added and the yellow colour observed was read at 412 nm in

spectrophotometer. Set of standard solutions containing 20-100 µg of reduced glutathione was treated similarly. The values were expressed as mg/dl for plasma.

3.17.8 Estimation of Total Serum Proteins

Total Serum Proteins was estimated using the method described by the method adopted by Oyinloye *et al*., (2016)

Principle: Proteins form a stable complex with Cu^{2+} at alkaline pH 7.0.

Procedure: Three test tubes were labelled blank, standard and sample. Serum (0.02 ml) was added to the sample tubes, protein (0.02 ml) was added to the standard test tube and water (0.02 ml) was added to the blank test tube. Protein reagent (1.0 ml) was added to the test tubes, mixed well and allowed 25 minutes at room temperature (20-25^{o} C) for 25 minutes and absorbance was taken at 540 nm.

Total serum Proteins (in g/dl) = $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5$ = g of protein/dl

3.17.9 Determination of Alkaline Phosphatase (ALP)

Alkaline Phosphatase (ALP**)** activity was determined using the method described by the method adopted byYakubu *et al*., (2017)

Principle: Alkaline phosphate reacts with phenolphthalein monophosphate (a colourless substrate), give rise to phosphoric acid and phenolphthalein which turns pink at alkaline pH values.

P-nitrophenylphosphate + H_20 --------→ ALP ------→ PO_4^{2-} + P-nitrophenol (pink at pH=9.8)

Procedure: Two test tubes labelled: blank and sample test tubes were set up in duplicates. Sample (0.05 ml) was pipetted into the sample test tubes, distilled water (0.05 ml) was pipetted into the blank tube and substrate (3.0 ml) was pipetted into each tubes respectively, was mixed thoroughly and the initial absorbance taken at 405 nm. The absorbance of the sample and the blank read again three times at one minute intervals using a stop watch.

Calculation: Activity of ALP (in U/L) = Absorbance of sample x 3300

Absorbance of standard

3.17.10 Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was determined using the method described by Roopashree, Danga, Ran and Narendra, (2008)

Enzymes that catalyze the conversion of two superoxides into hydrogen peroxide and oxygen are SOD.

The benefit here is that hydrogen peroxide is substantially less toxic than superoxide. Erythrocyte superoxide dismutase (SOD) activities serve as antioxidant enzymes.

The principle: Based on the inhibition of nitroblue tetrazolium reduction.

Procedure: Adrenaline (0.01 g) was dissolved in distilled water (17.0 ml), then serum (0.1 ml) and phosphate buffer (0.9 ml) at pH 7.8 was taken in triplicates in 2.5 ml buffer. Adrenaline solution (0.3 ml) was added and mixed inside the cuvette. At 480 nm the absorbance was taken 5 times at 30 seconds interval. Superoxide dismutase activity was determined by the changing rate of absorbance.

3.17 .11 Determination of Urea

Urea level was determined using Urease Berthelot based on the method adopted by Agbafor *et al.,* (2017) as described in Randox commercial kit.

Principle: In the presence of urease urea in serum is hydrolyzed to ammonia. The ammonia is then measured photometrically by Berthelot reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

NH_3 + hypochlorite + phenol → Indophenol (blue compound)

R1. Sodium nitroprusside ($R1_b$) and Urease ($R1_a$) solution

The contents of vial R1a was transferred into bottle R1b and mixed thoroughly.

R2. Phenol

Bottle R2 contents were diluted with 660 ml of sterile water. The bottle was rinsed thoroughly and mixed.

R3.Sodium hypochlorite

Bottle R3 contents were diluted with 750 ml of sterile water and also the bottle was rinsed thoroughly and mixed.

Procedure:

Test tubes were labelled: blank, standard and sample, and pipetted into test tubes as follows:

	Blank	Standard	Sample
Sample	----	----	10 µl
Standard (CAL)	----	10 µl	----
Distilled water	10 µl	----	----
Reagent 1	100 µl	100 µl	100 µl

The above was mixed and incubated at 37^{o}C for 10 minutes

Reagent 2	2.50 ml	2.50 ml	2.50 ml
Reagent 3	2.50 ml	2.50 ml	2.50 ml

The above was immediately mixed and incubated at 37^{o}C for 15 minutes.

The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was read against the blank.

Calculation

$$\text{Urea Concentration} = \frac{A_{sample}}{A_{standard}} \times \text{Standard concentration (mmol/l)}$$

$$\text{Urea Concentration} = \frac{A_{sample}}{A_{standard}} \times \text{Standard concentration (mg/dl)}$$

3.17.12 Determination of Lactate Dehydrogenase (LDH) Activity

Lactate Dehydrogenase (LDH) activity was determined using the method described by the method adopted byYakubu *et al*., (2017)

Principle: The oxidation of lactate to pyruvate in the presence of NAD which is subsequently reduced to NADH is catalyzed by lactate dehydrogenase. The rate of NADH formation measured at 340 nm is directly proportional to serum LDH activity.

Procedure:

Sample reagent 0.025 ml was added, mixed and incubated at 37^{o}C for 1 minute. After 1 minute, the absorbance was read and recorded then tubes were returned to 37^{o}C. The readings were repeated every minute for next two minutes. The average absorbance difference per minute (abs/mins) was calculated.

Caculation: The absorbance Δabs/min. multiplied by 6592. (IU/L).

3.17.13 Determination of C - reactive protein

Determination of C - reactive protein was done using the method described by the method adopted by Oyinloye *et al*., (2016

Principle: Turbilyte-CRP is a turbidimetric immunoassay for the determination of C-reactive protein in human serum and is based on the principle of agglutination reaction.

Procedure: The test specimen was mixed with activation buffer [R1], Turbilyte-CRP latex reagent [R2] and then allowed to react. The formation of an insoluble complex producing

turbidity indicates the presence of C-reactive protein in the test specimen, which is measured at 630 nm. The increase in turbidity is proportional to the concentration of CRP in the specimen.

Pipetted into the cuvetie;

	For calibration	For sample
R1	450 µl	450 µl
R2	50 µl	50 µl
The solution was mixed well and incubated for 5 minutes.		
Calibrator	5 µl	
Sample		5 µl
The solution was mixed well and the absorbance A1 at 10 seconds and A2 at 2 minutes was read respectively.		

Concentration of CRP = $\frac{\text{Absorbance of Sample} \quad X \quad \text{Conc of standard}}{\text{Absorbance of Standard}}$

3.17.14 Determination of Serum Albumin

It was determined using Bromocresol Green according to the method described by Oyinloye *et al*., (2016).

Principle: Based on its binding to 3, 3, 5, 5, tetrabromo-m-cresolsulphonephthelein (bromocresol green, BCG) an indicator. The albumin-BCG-complex absorbs at 578 nm, the absorbance is directly proportional to the concentration of albumin in the sample.

Procedure

Wavelength	Hg 578 nm
Spectrophotometer	630 nm (600-650 nm)
Cuvette	1 cm light path
Incubation temperature	20-25°C
Measurement	Against reagent blank

Pipetted into test tubes:

	Reagent	Standard	Sample
Distilled H_2O	0.01 ml	-	-
Standard (CAL)	-	0.01 ml	-
Serum or plasma	-	-	0.01 ml
BCG reagent (RI)	3.00 ml	3.00 ml	3.00 ml

$$\text{Albumin Conc. (g/l or g/dl)} = \frac{\text{Abs sample} \times \text{Conc. of standard}}{\text{Abs standard}}$$

3.18 Haematological Analyses

The blood collected from all experimental animals for haematology was collected through ocular puncture and cardiac puncture in EDTA - containing tubes. The parameters including Red blood cells (RBC), White blood cells differential count (WBC), Haemoglobin concentration (HB), packed cell volume (PVC) and platelets count were determined using haemocytometer and cyanomethaglobin method.

3.18.1 Determination of Erythrocyte Count by Haemocytometry

The method of Ochei and Kolhatkar (2008) was used. The blood specimen was diluted in the ratio of 1:200 with RBC diluting fluid. Using a counting chamber under high power (x40) objective the cells were counted and number of cells was calculated and reported as the number of red cells of whole blood.

Procedure: Aliquot of blood (0.02 ml) added to 3.98 ml of sodium citrate and mixed well. After 5 minutes, by holding the pipette vertical the first few drops of blood were discarded and the counting chamber was charged with the fluid. Then, it was allowed for 3 minutes to settle. The centre large square with 25 small squares were adjusted by switching to low power (10x) objective to light, then adjusted to high power (40x) objective and red blood cells in the four corner squares and one central square were counted.

Calculation

Total RBC / cu mm = number of cells counted x dilution factor

Area counted x depth of fluid where

(1) Dilution = 1:200

(2) Area counted = 1/5 sq.mm

3.18.2 Determination of Total Leucocyte Count by Haemocytometry

This was determined by haemocytometry following the method described by Ochei and Kolhatkar (2008). The red cells lysis the glacial acetic acid while gentian violet, slightly stains the nuclei of the leucocyte. The diluting fluid was used to dilute the blood sample 1:200 ratio in a white blood cell pipette and the cells were counted using a counting chamber under low power microscope. The number of cells in undiluted blood was reported as the number of white cells of whole blood.

Procedure: An aliquot of blood (0.02 ml) was added to diluting fluid (0.38 ml) (Acetic acid, tinged with gentian violet) and mixed. Well-mixed diluted blood was used to charge the counting chamber (after discarding the first five drops) with the aid of a pipette. Cells were allowed for 3 minutes to settle in a moist chamber. The four corners of the chamber can be seen under a low power (10X) objective and the cells were counted in all the four marked corner squares.

Calculation

Total WBC / cu mm = Number of cells counted x dilution factor

Area counted x depth of fluid

Where:

(1) Dilution = 1:200

(2) Area counted = 1/5 sq.mm

(3) Depth of fluid= 1/10 mm

(4) Number of red cells counted = N x 200

3.18.3 Estimation of Packed Cell Volume (PCV)

It was estimated based on the method described by Ochei and Kolhatkar (2008). With a clean heparinised capillary tube the blood samples were taken, and sealed with plasticine. The filled tubes were placed in the microhaematocrit, centrifuge and spun for 5 minutes at 12,000 g. Spunned tubes were placed into a scale and the PCV was read as a percentage.

$$\text{PCV \%} = \frac{\text{Packed RBC column height} \times 100}{\text{Total blood volume height}}$$

3.18.4 Determination of Haemoglobin (Hb) Concentration

Haemoglobin was determined using cyanomethaglobin method as adopted by Ochei and Kolhatkar (2008).

Principle: The hemoglobin was mixed with Drabkin's solution which contains potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate and ferricyanide form methaemoglobin which cyanide converted to cyanmethaemoglobin which produces a colour which was measured.

Procedure: Whole blood (0.02 ml) was added to Drabkin's solution (5.0 ml) in a test tube (1:250 dilutions). The mixture was allowed to stand for 10 minutes and the absorbance was read colorimetrically at a wavelength of 540 nm while Drabkin's solution was used as blank.

Calculation: Grams of haemoglobin per 100 ml of blood was determined as haemoglobin

3.18.5 Determination of Platelet

The platelet count was done by haemocytometry based on the method described by Ochei and Kolhatkar (2008).

Principle: The diluting fluid haemolyses the red cells leaving the white cells and platelets intact. The platelets are then counted in a counting chamber.

A well-mixed anticoagulated blood was diluted 1:200 using 1% ammonium oxalate solution and then load into a charged Neubauer counting chamber. The cells were counted from the appropriate ruled areas corner squares.

Total number of platelet counted/mm^3 = number of platelets counted x 500

3.19 Histopathological Analyses

The histological examination was carried out as described by Culling (1975). Internal organs (the kidney, liver and heart) of the experimental animals were excised and preserved 10% buffered formalin solution and labelled accordingly for histological examination. The rats fixed tissues were dehydrated with increasing concentrations of ethanol. The tissues were passed through xylene solution in order to clear the ethanol and also facilitate molten paraffin wax infiltration at 55^o C. In a wax block, the tissues were embedded. Using rotary microtome paraffin sections of 6 µm thickness were cut and placed on cleaned glass slides. The sections were stained with staining reagents; hematoxylin and eosin. The stained slides were viewed using a light microscope where photomicrographs of tissue were taken.

3.20 Data Analysis

Data obtained from the study were analyzed using appropriate software (Microsoft Excel was used for calculating means and standard deviations. The software Table curve 2D systat, USA and Sigma plot 10. 0 Systat USA was used for mathematical modelling of data from free radical scavenging activities. The percentage inhibition was calculated relative to control using equation 1. The inhibition data generated were fitted into appropriate non-linear equations (Alisi, Ojiako, Osugwu and Onyeze, 2011). The models used in this study are Logistic dose response abc (Equation 2), Logistic dose response abcd (Equation 3), Weilbull cumulative abcd (Equation 4), Sigmoid models abc (Equation 5), and Sigmoid model abcd (Equation 6) respectively.

The parameters were estimated by iterative minimization of least squares using Levenberg-Marquardt algorithm. The data of % inhibition or scavenging ability, fitted into the equation was used to evaluate the thresholds inhibitory concentrations (I_{C50}) which are the concentrations of the extracts that inhibited or scavenged 50% of the respective radicals. Equation with the highest Pearson correlation (R^2) and appropriateness to data was selected (Alisi *et al*., 2011). Lower fit standard error was also considered as a criterion for acceptance of an equation as an appropriate model.

Equations

$$\%\ Inhibition = \left[\frac{Control_{ABS} - Test_{ABS}}{Control_{ABS}}\right] \times 100 \qquad 1$$

Logistic dose response model

$$y = \frac{\mathrm{a}}{1 + \left(\frac{\mathrm{x}}{b}\right)^{\mathrm{c}}} \qquad 2$$

Where: x is the concentration of the extract, a is the maximum response (of untreated control), b is the IC_{50}, c is parameter determining the relative slope at I_{C50}.

$$y = a + \frac{b}{1 + \left(\frac{\mathrm{x}}{c}\right)^{d}} \qquad 3$$

Where: x is the concentration of the extract, b is the maximum response (of untreated control), c is the IC_{50}, d is parameter determining the relative slope at IC_{50}.

$$y = a\left[1 - exp\left[-\left[\frac{x + c(In\ 2)^{1/d} - b}{c}\right]^{d}\right]\right] \qquad 4$$

Where: x is the concentration of the extract, a is the maximum response (of untreated control), b is the IC_{50}, c is parameter determining the relative slope at IC_{50}.

$$y = \frac{a}{1 + \exp -\frac{x-b}{c}} \qquad 5$$

Where: x is the concentration of the extract, a is the maximum response (of untreated control), b is the IC_{50}, c is parameter determining the relative slope at IC_{50}.

$$y = y_0 + \frac{a}{1 + \exp -\frac{x-b}{c}} \qquad 6$$

Where: x is the concentration of the extract, a is the maximum response (of untreated control), b is the IC_{50}, c is parameter determining the relative slope at IC_{50}, y_0 is a correction term

CHAPTER IV: RESULTS AND DISCUSSION

4.1 Results

4.1.1 Qualitative and Quantitative Phytochemical Result

The result of this study (Table 4.1) shows that the three plants extract contained phytochemicals in variable amounts. The qualitative analysis results demonstrated that, most of them are either high or moderate in alkaloids, saponnins, cardiac glycosides, phytates, phenols, oxalates, steroids, flavonoids and tannins. The three solvent (aqueous, ethanolic and methanolic) extracts demonstrated different levels of phytochemicals extracted. For the aqueous extracts, the higest phytochemical was recorded for oxalate (+++) (*M. pudica*), followed by alkaloids, saponnin, cardiac glucoside, oxalate, flavonoids and tannin (++) (*C. longa* and *E. serrata*). The least was recorded for cardiac glucoside, phytate, phenol, terpenoid and tannin (+)(*E.serrata, M.pudica*); for the ethanolic and methanolic extracts, M.pudica recorded the highest (+++,++) phytochemical while the least was recorded for *E.serrata*. The result have shown the presence of different levels of alkaloids, cardiac glucosides, phytate,oxalate and flavonoids in various extracts using three solvents. Some phytochemicals were undected like phenol was not detected in the leaf extract of *E. serrata*. Saponin, steroid and tannin were not detected in the aqueous leaf extract of *E. serrata*. Steroid and terpenoid were not detected in the methanol extract of *E. serrata*. The result of the phytochemical screening of the leaf extract in aqueous, ethanol and methanol suspension of *M. pudica*, showed the presence of alkaloid, cardic glycosides, phytate, oxalate, flavonoid and tannin in all the solvent used. Saponin and steroid were not detected in the aqueous leaf extract of *M. pudica*, phenol was not detected in both the ethanol and methanol leaf extract of *M. pudica*. Also steroid was not detected in the methanol leaf extract of *M. pudica*. The result of phytochemical screening of the aqueous, ethanol and methanol extract of *C. longa* showed the presence of alkaloid, saponin, cardiac glycoside, phytate oxalate, and tannin. Phenol, terpernoid and steroid were not detected in aqueous and ethanol extract of *C. longa*. Flavonoid was not detected in aqueous extract of *C.longa*. While all the phytochemicals screened were detected in methanol extract of *C.longa* as shown in Table 4.1. Qualitative phytochemical extraction with ethanolic and methanolic solvent were better than aqueous solvent

Figure 4.1 shows the percentage quantitative constituents of each phytochemical extracted from *E. serrata, M.pudica and C. longa.* Result showed that *Euphorbia serrata* extract, recorded tannins highest constituent (35.2%); followed by flavonoid (9.83%) while phytate was the least (2.77%). *Mimosa pudica* extract, recorded flavonoid as highest phytochemical constituent (53.8%); tannins, (24.0%); and phytate as the least (2.52%). The highest phytochemical constituent of *Curcum longa* extract was tannins, (24.2%); flavonoid, (13.18%); and the least was cardiac glycosides (3.3%).

Oxalate was the highest in all the three plants evaluated (5.40, 4.90, 2.40) for *E. serrrata, M. pudica and C. longa* respectively. This was followed by phenol (1.63, 1.23, 1.01) for *C. longa, M. pudica and E. serrrata* while carotene and steroid were the least constituent in all the plants

4.1.2 IDENTIFIED MICROORGANISMS USED IN THE STUDY

Table 4.2 shows the DNA sequences and the Gene bank Ascession numbers of the bacteria and fungi used in the study. Results were obtained from nucleotide sequence analyses; showed microbes molecularly identified as *Wohlfahrtiimonas chitiniclastica, Proteus mirabilis, Alcaligenes faecalis, Klebsiella pneumoniae, Candida tropicalis, and Wickerhamomyces anomalus.* Results from this were for appropriate identification of microbes used for the determination of antibacterial and antifungal activities of these different solvent extracts

Table 4.1: Qualitative phytochemical screening of the leaf of *E. serrata, M. pudica* and rhizome *of C. longa*

Constituent/ Component	Aqueous Extract			Ethanol Extract			Methanol Extract		
	E. Serrata	*M. pudica*	*C. longa*	*E. serrata*	*M. pudica*	*C. longa*	*E. serrata*	*M. pudica*	*C. longa*
Alkaloid	++	++	++	++	+++	+++	++	+++	+++,
Saponnin	ND	ND	++	+++	++	++	++	+++	+++
Cardic glycoside	+	+	++	+	+++	+++	++	++	+++
Phytate	+	+	+	+	+	+	+	+	+
Phenol	ND	+	ND	ND	ND	ND	ND	ND	+
Terpenoid	+	+	ND	ND	++	ND	ND	+++	+
Oxalate	++	+++	++	++	+	++	+	++	++
Steroid	ND	ND	ND	+++	+	ND	ND	ND	+
Flavonoid	++	+	ND	+++	++	+++	++	++	+++
Tannin	ND	+	++	+++	+	++	+	++	+++

Legend: +++ = High, ++ = Moderate, + = Low, ND = Not detected

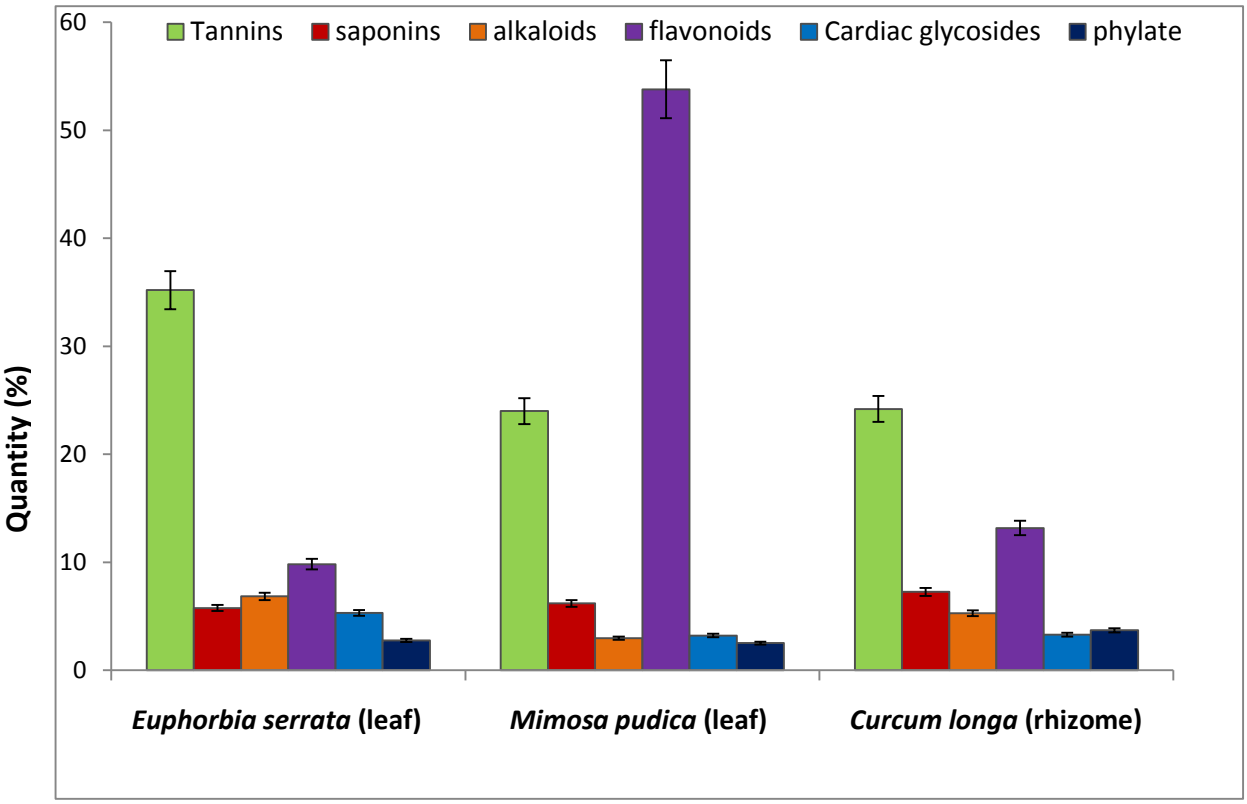


Figure 4.1: The percentage quantitative constituent of phytochemicals of *E. serrata, M. pudica* and *C. longa.*

Table 4.2: DNA Sequence and Gene Bank Ascession Numbers of the Bacteria and Fungi used

S/N	Sample No	Query Length	Sequence Nucleotide obtained (FASTA format)	Similarity/ E-score	Gene Bank Accession Number/	Identity Of Isolate Obtained
1.	ZBA	**788**	**>ZBA_907-R_H10_22** GCTCCAGGCGGTCGATTTACGC GTTAGCTCGGACACGCTCAAGA GCACAACCTCAATCGACATCGT TTACGCGTGGACTACCAGGGTA TCTAATCCTGTTTGCTCCCCACG CTTTCGCACCTGAGCGTCAGTCT TTGTCCAGGGGGCCGCCTTCGC CACCGGTATTCCTCCAATCTCTA CGCATTTCACCGCTACACTGGA ATTCTACCCCCCTCTACAAGACT CTAGCCTGCCAGTTTCGATGCAT TCCCAGGTTGAGCCCGGGGATT TCACATCCGACTTGACAGACCG CCTGCGTGCGTTTACCCCAGAA TTCCATTAACGCTTGCACCCTCC GATTACCGCGGTGCTGGCACGG AGTTAGCCGGTGCTTCTTCTGCG GTAACGTCAATGAGGTATTAAC CTACCCTTCCTCCCGCTGAAAGT ACTTTACAACCCAAGGCCTTCTT CAACACGCGGCATGGCTGCATC AGGCTTGCGCCCATTGTGCAAA TTCCCCACTGCTGCCTCCCGAGG AGTCTGGCCGTGTCTCAGTCCA GTGTGGCTGTCATCCTCTCAACC AGCTAGGATCGTCGCCTAGGTA GCCTTACCCACCTACTAGCTAAT CCCATTGGGCATCGATGCAGGC CGAAGTCCCCCTTTGTCGGACTT ATGCGGTATTAGCACCGTTTCC AGTAGTTATCCCCCTCATCGGC AGATCCCATACATTACTCACCC GTCCGCCGCTCGTCACAAAGCA AGCCTGACCTATTGTGTGTCTCC CCAGCAATCTCCAGGGCCCCCC CCCC	**93%/0.0**	CP016159.1	*Klebsiella pneumoniae*
2.	ZBB	**886**	**>ZBB_907-R_A11_02** AGCGGGTCTTTATCGCGTTAGCT GCGCAATACGGCTCAAGAGCCC		KX417275.1	*Proteus mirabilis*

			ACCGTAAATCGACATCGTTTAC GGTGGACTACCAGGGTATCTAA TCCTGTTTGCTCCCCAGCTTTCG CACCTGAGCGTCAGTTTTGGCC AGGGGGCGCCTTCGCCACGGTA TTCCTCCACATCTCTACGCATTT CACCGCTACACTGGAATTCTAC CCCCCTCTCAACTCTAGCAACC AGTTCAATGCAATTCCCAAGTT AAGCTCGGGGATTTCACATTGA CTTAATTGACCGCCTGCGGCGC TTTACGCCCAGTAATTCCGATA ACGCTTGCACCCTCGATTACCG CGGTGCTGGCACGAGTTAGCCG GGCTTTTCTGCGGGTAACGTCAT TGAAAGGGGATTAACCTATCAC CTTCCTCCCCGCTGAAAGACTTT ACAACCCTAAGGGCTTCTTCAT ACACCGATGGTGGATCAAGGTT GCCCCATTGGGCAAAATTCCCC ACTGGTGCCTCCCAGGATGGGC GGTCTCATCCCAGGGGGTGATC ATCCTCTCAAACCATAAGATCC CCTGGGAGCTTTACCCCCCACT ACTAATCCCATATGGGCTCCAT AGGAAGGGCAAACCCTGTTTTC CAAATTATGGGATTACCACTTC AAATATCCCCCCTTATGAACCA TAACATTATCCCGCCCCTCGAC ATAAAAGTTTTCCTGTTCCGCTC GACTGGATGGTTAGGCCTCCCT AATCTGCCCAGAATTTTAAAAT TTA			
3.	ZBC	**747**	**>ZBC_907-R_B11_05** AGCGGTCACTTCACGCGTTAGC TGCGCAACAGGCTACCGGGCCT CCTCTATGGATTGTTTAGGGGG GAAACCAGGGTATCTAATCCTG GTTGGTCCCCAACCTTTCCCGCC TGACGGGGCTGTGCCAAGGGGG TGCCCTTCCCACGGGGATTCTTC CGATATTCAACCTTTTCCCGGTT CCCCATAAATTCCGATACTCCCT ACCACAACTTAATTACCCAGAT TAGAGGATTCCCCAGGTAAGCC	**6e**	MF099850.1	***Wohlfahrtiimonas chitiniclastica***

			TGGGGCTTTCACATCTAAATTA ATTAACGCCTACCGGCCTTTACC CCCAAAATTTCCAATAAAGTTG GCCCCCCCGGAATTCCCCGGTG GTGGACGGAATAACCGGGGGTT ATTTTTGAGGGAAACTCAACTG TTAAGGGAATTACCTTAACTGC CATTCTCCCTGGAAAGTGCTTTA CAACCCGAAGGCCTTCTCAACA CGCGGGATGCTGGATCAGGCTT CCCCATTGTCAAATATTCCCCCT GGCTGCCCCCGAGAGTCTGGGC CGTGTCTCACCATGGGTGAATC CCCAACTAGTCTGGTGCCTTTAC CTCCAACTATCCATGGCTCCAG AAGGCCAAGGATCCCGCTTTCC CAAAACAAGGGGAATTACACTC TTTTTAGAATATATCCCCCCTAA TAGGGGAAATCCCAGATTATCA CCCCGCCTCGCAGAGAAGAAAT CCGCCCTTCGACTTGCATGTGA AAGCATACCGCTAGCGTAATTG GGGGAAAAAAAA			
4.	ZBD	**881**	>ZBD_907-R_C11_08 GGGCGGGTCACTTCAGCGTTAG CTGCGCACAGTAAGCCCCAACG CTATGCATCGTTTAGGCGTGGA CTACCAGGGTATCTAATCCTGTT TGCTCCCCACGCTTTCGCCTAGC GTCAGTTCCAGGGGCTGCCTTC GCCATGGTGTTCCTCCATATCTA CGATTTCACTGCTACACGGAAT TCACCCCCTCTGACATACTCTAG CTCGGAGTAAAAATGCAGTTCC AGGTTGAGCCCTGGGATTTCAC TCTCTTCCAACCGCCTCCCGCTT TACGCCCAGTAATCCGAAACGC TGCCCCTCGTATTACCGCGGCTG CTGGCACGAATTAGCCGGGGCT TATTCTGCAGGTACCGTCATTAT CATCAGGTATTAGGGGATGCCT TTTCTTCCTGCCAAAAGTGCTTT ACAACCCGAAGGCTTCATCTAC ACCGGGATGGCTGGATCAGGGT	**00/83%**	KX808583. 1	Alcaligenes faecalis

			GCCCCCATTGTCCAAAATTCCCC ACTGCTGCCTCCCGTAGGAGTC TGGGCGTGTCTCAGTCCCAGTG TGGCTGGTCGTCCTCTCAAACC ATACGGATCGTGCCTTGGTGAG CCTTACCCCACCAACTAGCTAA TCCGATATCGGCCGCTCCGATA GTGAGAGGCTAGCATCCCCCTT TCCCCGTAGGGTATGCGGTATT AGCCACTCTTTCAAATCCCCCGC TACTGGAGATCCGATACATTAC TCACCCGTCCCACTCGCCACCA AGAAGATGCTCTCTGCCCCCTCT ACGATTGCATGTGTAAACATCC CGCTAGCGTCAATCTACCAGTTT CCCAATA			
5.	ZFA	**478**	>ZFA_ITS-4_F11_17 AGGTCAAGTTATGAAATAAATT GTGGTGGCCACTAGCAAAATAA GCGTTTTTGATAAACCTAAGTCT TAAAATAAGTTTCCACGTTAAA TTCTTTCAAACAAACCTAGGAT GTCAACACCAAACCCGGGGGTT TGAGGGAAAATGACTCAAACAG GCATGCCCTTTGAATACCAAAG GGGCAATGGCGTCAAAATTCAT GATTCACAATATCTGCAATTCAT ATTACGTATCGCATTTCCTGCGT TCTTCATCATGCAAACCAAAAT CCGTTGTTGAAAGTTTTGACTAT TGAATAATAAATCAAGTTTGAC TGTAAATAAAAAGTTTGGTTTA GTTATAACCTCTGGGGAGGATT GCTCCCGCCACCAAAAAATTTG TTCAATAAAAAACACTGGGGCA TTAACAAATCAGAATGATCCTT CCGCAGGTCCCCTTGAGG	**93%0.0**	KY848365.1	***Candida tropicalis***
6.	ZFB		>ZFB_ITS-4_G11_20 CCCGCCCCCCTCCGCTTCTTGTT ATCTTGCGCCACCTTGTGYGCTA AGCCGCCCTAATATACTTCTTAT CCTGCCTAGCTGATATAACGAG TTGAAGAACCTAACACATGATT TCAAAAGACTGCTTATTACCTA	**83.1/1.5e**	**LC120363.1**	*Wickerhamomyces anomalus*

			CTCTTGCTATACTYTATTTCAAG TTAACCCTTGACCTAGTATCATT CCTTACCAAACCCCACGGCGGG AAAAAAATGACGCTCYGACAGG ATACCCTCTGGAATACCACAGG GTGCAATGTGGGTTCTAGATTCT ATGATTCACGACAATCTTCAATT CTCAATACGACTCGAATTTCGCT GCTGTCTTCATCGTCGGAAACCT CAATCCTCTGTTGAAAGTTTTAA GATTTTGATTTTTGGGTTTTTCA TGACTATTGTTAAAGGYTTTACC ATTAAAAAAATGGGTTAGACCT TTGGAAAAGCCAAGTCACCACC CAAAGAAAGTTCAAAAAAACTA GGACCATGTGTGTAAGGTTATC TCGCGCAATTAAGCGATGGAAG AAATACTATAATGATCCTTCCCT AGGAAGCCTACGGAAG			
7.	ZFC	**572**	>ZFC_ITS-4_H11_23 GCATCTCCTGATTTGAGGTCAA GTTATGAAATAAATTGTGGTGG CCACTAGCAAAATAAGCGTTTT TGGATAACCTAACGCTAAAATA AGTTTCCACGTTAAATTCTTTCA AACAAACCTACGATTGTCACAC CAAACCCGGGGGTTTGAGGGAA AATGACGTCAAAGGCTGCCCTT TGAATACCAAAGGGCGCATGGC GTTCAAAATTCATGATTCACAA TATCTGCAATTCATATTACGATC GCATTTCCTGCGTTCTTCTCATG CAAACCAAAATCCGTTGTTGAA AGTTTTGACTATGAATAATAAA TCAAGTTTGACTGTAAATAAAA ATTGGTTTAGTTATAACCTCTGG GGTAGGATTGTCCCCCACCAAA AAATTTGTTCATAAAAAACACT GGGGCAATTAAGCAAATCAGAA TGATCCTTCCCAGGTCACCCGA AGTGGTAAGGTTTTTCGCCGCA ATTAGCGCTGGCAAAAGAATAC TGGATGATCCTTCCGCAGGTTC ACCTACGGAAG	**91%/0.0**	KY102481.1	***Candida tropicalis***

Phylogenetic Tree of the Microorganisms

Tools > Multiple Sequence Alignment > Clustal Omega

Results for job clustalo-I20170715-134810-0481-62289600-es

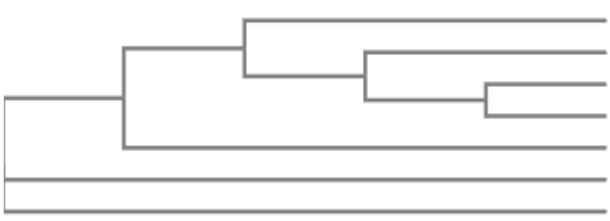
Alignments | Result Summary | Phylogenetic Tree | Submission Details

Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.

Download Phylogenetic Tree Data

Branch length: ◉ Cladogram ○ Real



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ZFB_ITS-4_G11_20 0.21432
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ZFC_ITS-4_H11_23 0.08459
ZBD_907-R_C11_08 0.2059
ZBA_907-R_H10_22 0.17332
ZBB_907-R_A11_02 0.19446

Tree Data

4.1.3 Antibacterial and Antifungal Activities of Ethanolic, Methanolic and Aqueous extracts of *E. serrata, M. pudica* and *C. longa Extracts and* control at 50 mg/ml, 37.5 mg/ml and 25 mg/ml

The antibacterial and antifungal activities of ethanolic, methanolic and aqueous extracts of *E. serrata, M. pudica, C. longa* and control (chloramphenicol & griesofulvin) were tested against these Gram negative bacteria (*Wohlfahrtiimons chitiniclastica, Proteus mirabilis, Alcaligenes faecalis* and *Klebsiella pneumoniae*) and fungi (*Candida tropicalis* and *Wickerhamomyces anomalous*) investigated in this study and their potency were assessed by the presence or absence of inhibition zones. The result revealed inhibitions of the microorganisms by the extracts were concentration dependent as higher concentration higher inhibition zones.

Fig 4.2 shows the antibacterial and antifungal activities of ethanolic extracts of *E. serrata, M. pudica, C. longa* and control against the microorganisms at 50 mg/ml revealed that all the extracts demonstrated high inhibitory potency against the tested microorganisms compared to control. *M. pudica* had the highest bacterial inhibition zone of 26 mm against *A.faecalis*, 15.5 mm against *P. mirabilis* and 15 mm against *K. pneumoniae. E.serrata* had 19 mm against *K. pneumoniae*, 11.5 mm against *W. chitinclastica* and 11 mm for *A. faecalis* while *C.longa* had inhibition zone of 13 mm against *W. chitinclastica*, 12 mm against *P. mirabilis* and 9 mm against A. faecalis respectively. C.longa had the highest inhibition zones of 11mm against *W. anomalus* and 8 mm against *C. tropicals* respectively for antifungal activities compared to *E.serrata* and *M.pudica*. The antibacterial activities of the extracts in ascending order *M. pudica> E. serrata> C.longa* while *C.longa > E.serrata >M. pudica* for antifungal activities.

Fig4.3 shows the result of antibacterial and antifungal activities of methanolic extracts of *E. serrata, M. pudica, C. longa* and control (chloramphenicol and griesofulvin) at 50 mg/ml against the microorganisms revealed that the extracts inhibited the microorganism as indicated by their inhibition zones. *C.longa* had the highest inhibition zone of 15.5 mm against *A. faecalis*; followed by *M.pudica11*.5 mm against A. faecalis. Inhibition ability of the extracts were in order *C.longa > M.pudica > E. serrata.. E. serrata* had the highest inhibition zone of 17 mm for *C.tropicalis* and 6.5mm for *W. anomalus* followed by *C.longa*; 10 mm against *C. tropicalis* and 7.5 mm for *A. anomalus* respectively.

In Fig 4.4; results of antibacterial and antifungal activities of aqueous extracts of *E. serrata, M. pudica, C. longa* and control at 50 mg/ml against the microorganisms revealed that the extracts inhibited the microorganisms with varying degree of inhibition zones. The extracts of ethanol and methanol inhibited the microorganisms better compared to aqueous extracts at 50mg/ml.

Fig 4.5 shows result of antibacterial and antifungal activities of ethanol extracts of *E. serrata, M. pudica, C. longa* and control at 37.5 mg/ml against the microorganisms revealed their various inhibition zones. E. serrata, *M. pudica and C. longa* had inhibition zones of 8.5mm against *A. faecali*s, *P. mirabilis and A. faecalis* respectively. *M.pudica* had 8 mm inhibition zone *K. pneumoniae*; E. serrata had 7.5mm against *W. chitinclastica* and *K.pneumoniae*. Antibacterial activities of the extracts followed the order *M. pudica > E. serrata > C. longa.* The extracts of *C.longa* had highest antifungal activity with inhibition zones of 9.5 mm against *C. tropicals.*

Fig 4.6 the results from antibacterial and antifungal activities of methanol extracts of *E. serrata, M. pudica, C. longa* and control at 37.5 mg/ml against the microorganisms investigated in this study revealed that the plant extracts showed antibacterial activity; *E. serrata* having inhibition zone of 12 mm against *A. faecalis* and 10.5 mm against *P. mirabilis*. *E. serrata* had the highest antifungal activity with inhibition zone of 6.5 against *C. tropicalis* and4.5 mm against *W. anomalus* respectively.

Fig 4.7 shows result of antibacterial and antifungal activities of aqueous extracts of *E. serrata, M. pudica, C. longa* and control at 37.5 mg/ml against the microorganisms revealed that *C. longa and E. serrata* extracts demonstrated better antibacterial and antifungal activities compared to *M. pudica* extracts .

Fig 4.8, 4.9 and fig 4.10 shows the antibacterial and antifungal activities of ethanol, methanol and aqueous extracts of *E. serrata, M. pudica, C. longa* and control at 25 mg/ml against the tested microorganisms respectively. The results revealed that various extracts at 25 mg/ml inhibited the microorganisms except aqueous *C.longa* that exhibited no inhibition against *C. tropicalis, A. faecalis* and the fungi *W. anomalus*. Also *M. pudica* exhibited no inhibition against *C.tropicalis*. Ethanol and methanol extracts at 25 mg/ml were better than aqueous extracts.

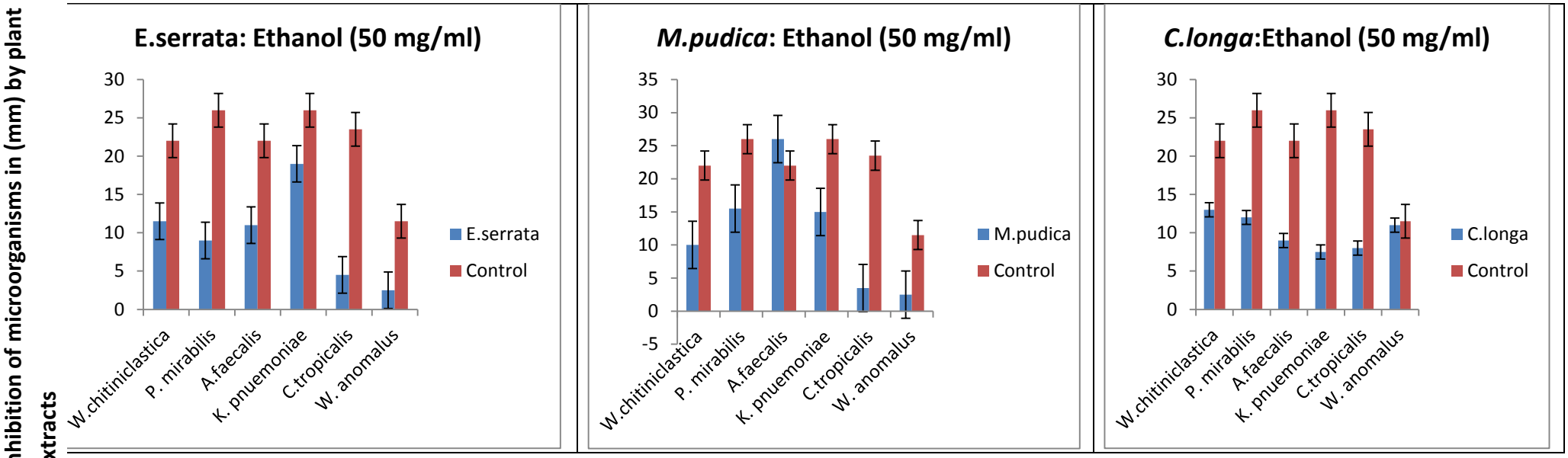

Figure 4.2: Antibacterial and antifungal activities of ethanol extracts of *E. serrata, M. pudica, C. longa* and control at 50 mg/ml.

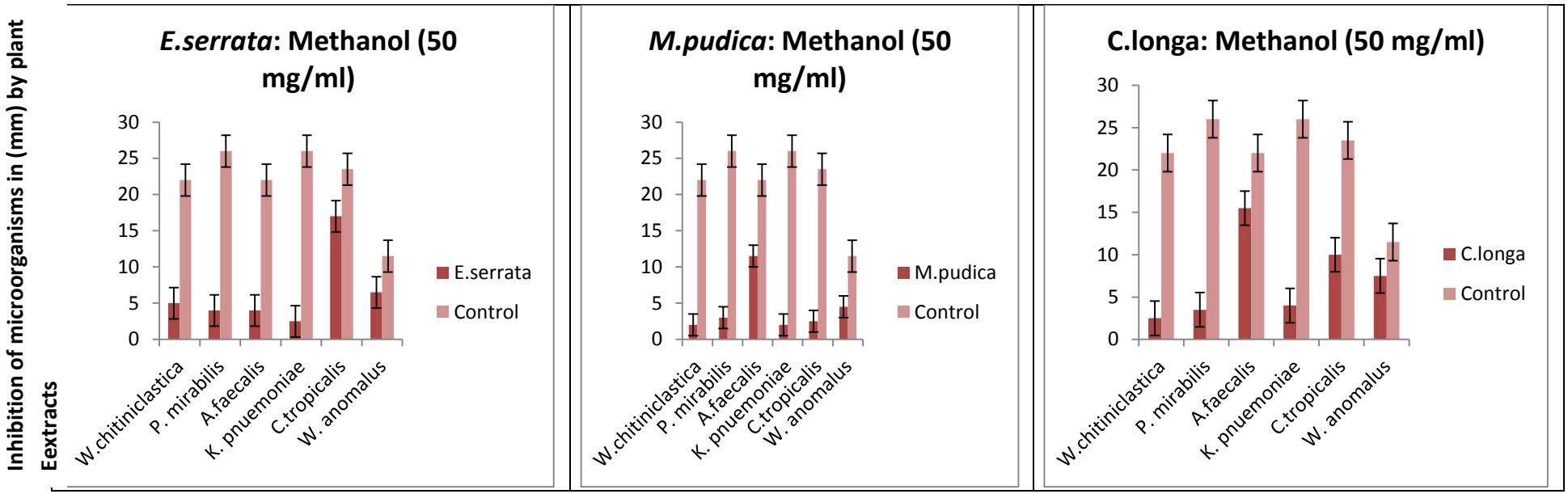

Figure 4.3: Antibacterial and antifungal activities of methanol extracts of *E. serrata, M. pudica, C. longa* and control at 50 mg/ml.

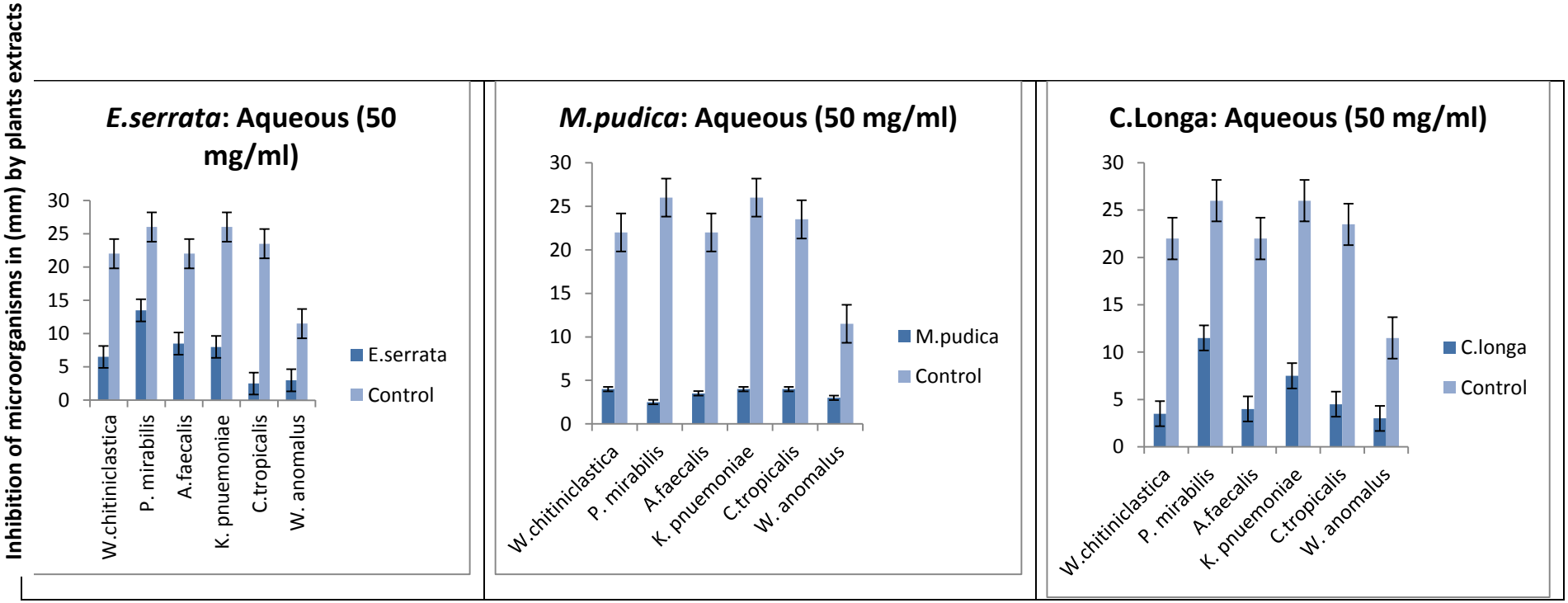

Figure 4.4: Antibacterial and antifungal activities of aqueous extracts of *E. serrata, M. pudica, C. longa* and control at 50 mg/ml

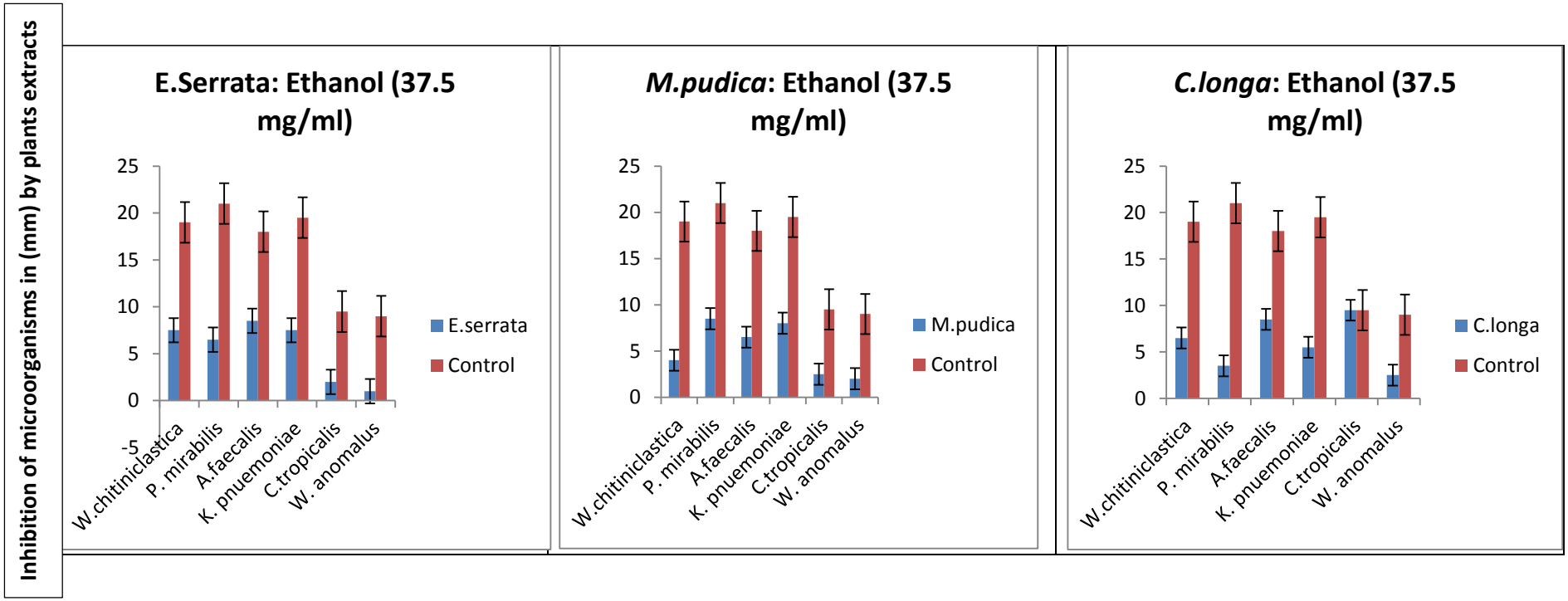

Figure4.5: Anti bacterial and antifungal activities of ethanol extracts of *E. serrata, M. pudica, C. longa* and control at 37.5 mg/ml.

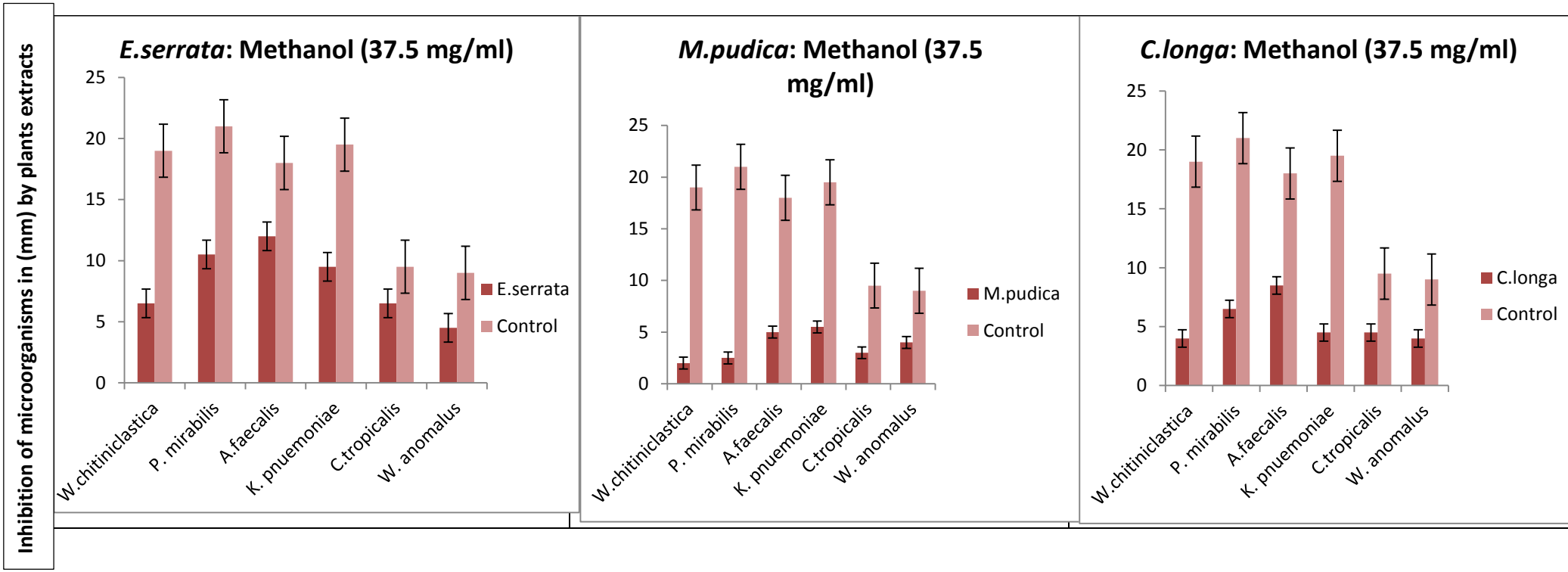

Figure4.6: Antibacterial and antifungal activities of methanol extracts of *E. serrata, M. pudica, C. longa* and control at 37.5 mg/ml.

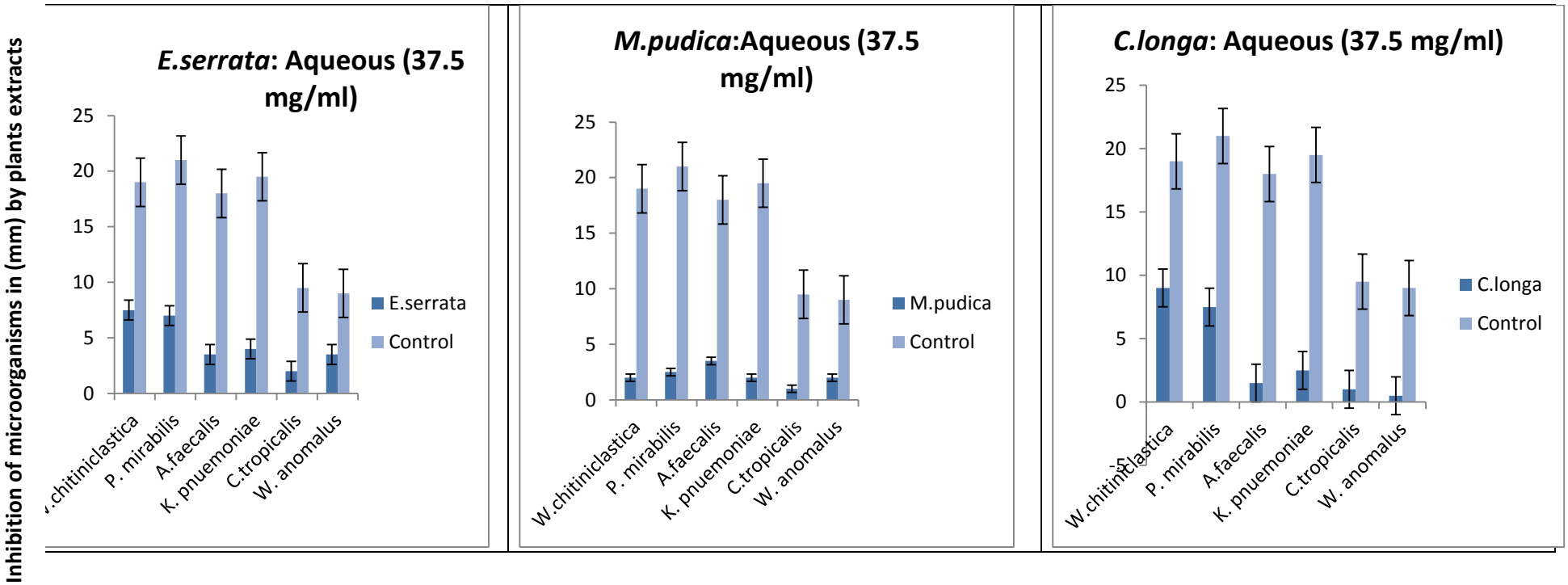

Figure 4.7: Antibacterial and antifungal activities of aqueous extracts of *E. serrata, M. pudica, C. longa* and control at 37.5 mg/ml.

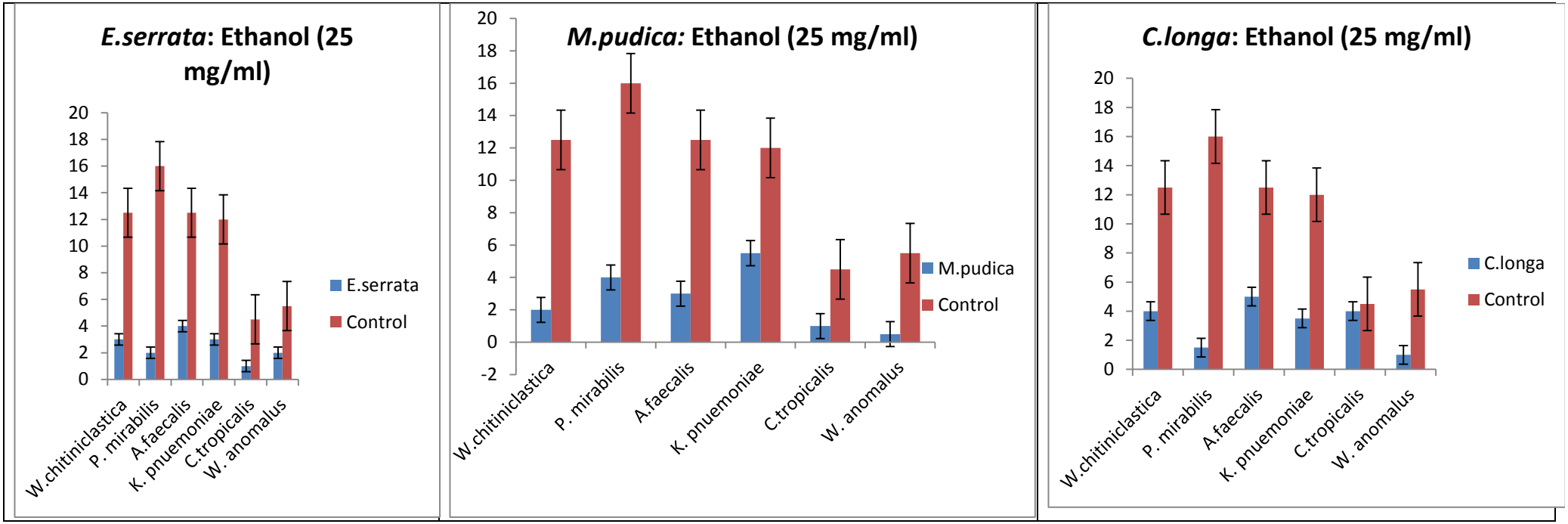

Figure 4.8: Antibacterial and antifungal activities of ethanol extracts of *E. serrata, M. pudica, C. longa* and control at 25 mg/ml.

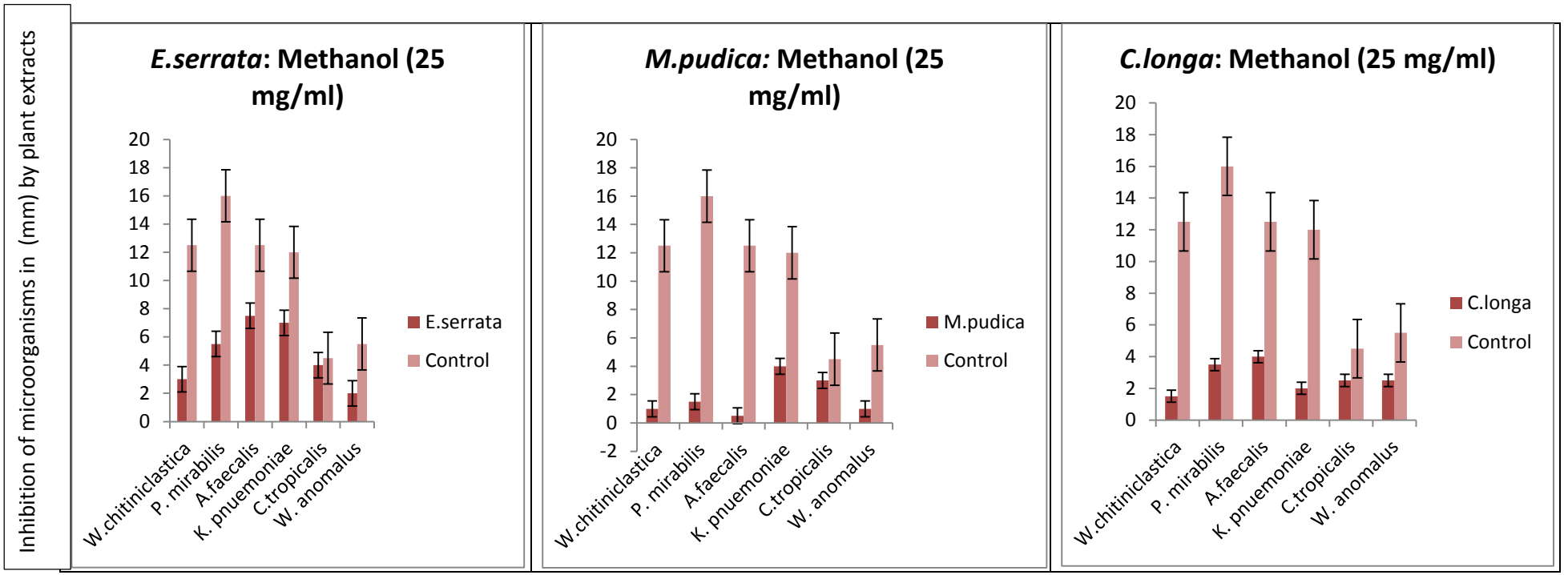

Figure 4.9: Antibacterial and antifungal activities of methanol extracts of *E. serrata, M. pudica, C. longa* and control at 25 mg/ml.

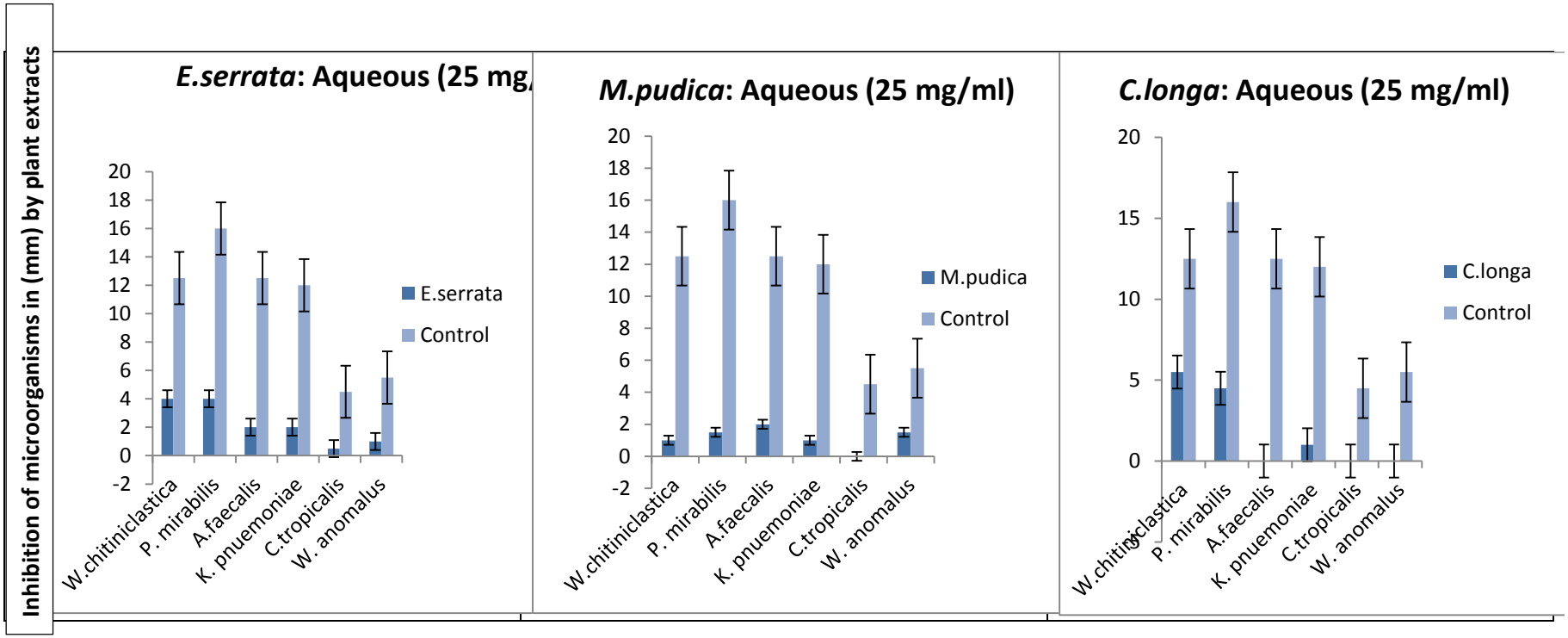

Figure 4.10: Antibacterial and antifungal activities of aqueous extracts of *E. serrata, M. pudica, C. longa* and control at 25 mg/ml.

4.1.4 Antioxidant Activities of *E.serrata, M.pudica* and *C.longa* Extracts.

4.1.4.1 Effects of ethanol and methanol Extracts of *E.serrata, M.pudica, C.longa* and Ascorbic acid on Scavenging of 2,2-Diphenyl-1-Picryhydrazyl (DPPH) Radical.

Figure 4.11 shows that the ethanol extracts of *E. serrata*, *M. pudica* and *C. longa* exhibited high DPPH scavenging activities within the tested doses (20 µg/ml- 80 µg/ml). *C. longa* exhibited the highest DPPH scavenging activity within the highest tested dose (80ug/ml) compared to *E. serrata* and *M. pudica*, and the control. The scavenging activity was concentration dependent, with increasing concentration of extracts. *E. serrata, M. pudica* and *C. longa* extracts scavenging activity for generated free radicals were comparable with the standard -ascorbic acid. The DPPH scavenging activities of the extracts in ascending order were as follows: *C. longa* > *M. pudica*> *E. serrata*. Threshold inhibitory concentration (IC_{50}) obtained for the extracts *E.serrata, M. pudica, C.longa* and ascorbic acid were 28.74µg/ml, 19.64µg/ml, 15.62µg/ml and 15.62µg/ml respectively.

Figure 4.12 shows that the methanol extracts of *E. serrata, M. pudica* and *C. longa* exhibited good scavenging activities within the tested doses (20 µg/ml-80 µg/ml. *E.serrata* and *C.longa* exhibited the highest DPPH scavenging activities within the highest tested dose. The scavenging activity was concentration dependent, with increasing concentration of extracts. The methanol extracts scavenging activity for generated free radicals were comparable with ascorbic acid (standard – antioxidant). The DPPH scavenging activities of the extracts in ascending order were as follows: *E. serrata* > *C. longa* > *M. pudica*. The threshold inhibitory concentration (IC_{50}) obtained for the extract *E.serrata, M.pudica, C.longa* and ascorbic acid were 2.92 µg/ml, 16.10 µg/ml, 12.50 µg/ml and 15.62 µg/ml respectively

Comparative analysis of effects of ethanol and methanol extracts of *E.serrata, M.pudic* and *C.longa* and ascorbic acid on DPPH radical scavenging showed that the DPPH scavenging abilities of ethanol extracts followed a sigmoid abc model while the methanol extracts followed a sigmoid abc and welbull abcd model. Methanol extracts scavenged DPPH radicals better than ethanol extracts as seen in the inhibiyory concentration (IC_{50}) values in Table 4.3.

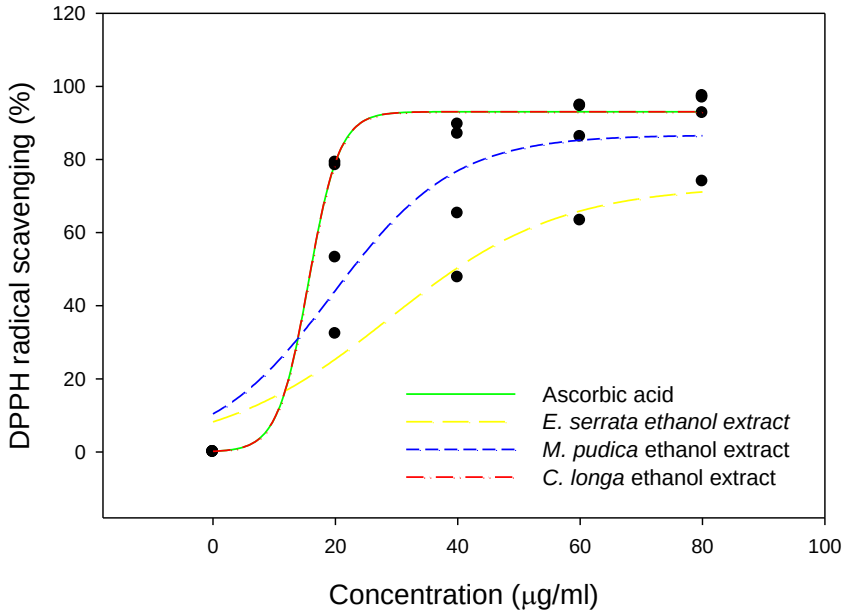

Figure 4.11: Effects of ethanol extracts of *E.serrata, M.pudica, C. longa* and ascorbic acid on scavenging of 2,2-diphenyl-1-picryhydrazyl (DPPH) radic

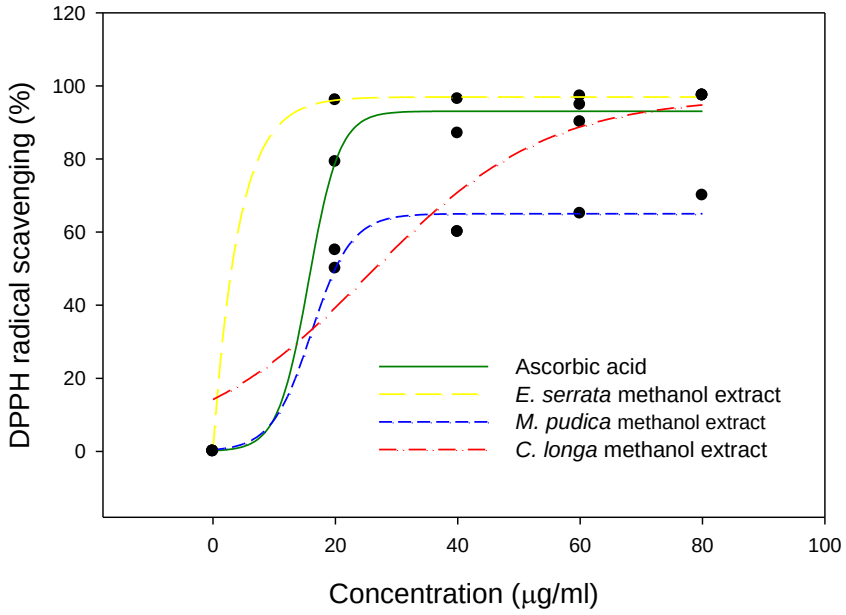

Figure 4.12 Effects of methanol extracts of *E.serrata, M.pudica* and *C.longa* and ascorbic acid on scavenging of 2,2-diphenyl-1-picryhydrazyl (DPPH) radical

Table 4.3 Comparative analyses of effects of ethanol and methanol extracts of *E.serrata, M.pudica* and *C.longa* and ascorbic acid on DPPH radical scavenging.

Threshold Inhibitory concentration (IC_{50}) of DPPH radical scavenging effect of *E. serrata, M. pudica, C. longa ethanolic* and Methanolic extracts and Ascorbic acid standard				
Extraction Solvent	Extracts	Model	R^2	IC_{50}*
	Ascorbic Acid	Sigmoid abc	0.991	15.62
Ethanol	*E. serrata*	Sigmoid abc	0.959	28.74
	M. pudica	Sigmoid abc	0.933	19.64
	C. longa	Sigmoid abc	0.991	15.62
Methanol	*E. serrata*	Webull abcd	0.999	2.92
	M. pudica	Sigmoid abc	0.985	16.10
	C. longa	Sigmoid abc	0.903	12.50

* IC_{50} is the concentration of the extract or standard that scavenged 50% of the generated free radicals.

4.1.4.2 Effects of Ethanol and Methanol Extracts of *E.serrata, M.pudica, C.longa* and Ascorbic acid on Scavenging Nitric Oxide Radical.

The results obtained from this study (Figure 4.13) showed that ethanol extracts of *E. serrata, M. pudica* and *C. longa* inhibited nitrite formation by competing with oxygen to react with NO leading to reduction of nitrite concentration. Inhibitions of nitric oxide by the extracts were comparable to that observed for ascorbic acid. *E, serrata* exerted the highest nitric oxide scavenging activity with inhibitory concentration (IC_{50}) value of 22.67 µg/ml followed by *C. longa* with inhibitory concentration (IC_{50}) value of 48.34 µg/ml and *M. pudica* with inhibitory concentration (IC_{50}) value of 50.02 µg/ml. Ascorbic acid was more effective against nitric oxide radical with inhibitory concentration (IC_{50}) value of 15.46µg/ml. The nitric oxide radical scavenging was concentration dependent.

Result of the study (Figure 4.14) showed that methanol extracts of *E. serrata, M. pudica* and C. longa decreased the amount of nitrite produced from sodium nitroprusside decomposition in vitro. The inhibitions by the extracts were better compared to ascorbic acid. *C. longa* exerted the highest inhibitory concentration (IC_{50}) value of 15.36µg/ml followed by *M. pudica* with inhibitory concentration (IC_{50}) value of 16.17µg/ml and *E. serrata* with inhibitory concentration (IC_{50}) value of 16.55µg/ml as evaluated from the inhibition graph. Nitric oxide radical scavenging abilities of the extracts in ascending order as follows*: M. pudica > E. serrata > C. longa*. The methanol extracts have better nitric oxide scavenging properties than ethanol extracts.

Comparative analysis of effects of ethanol and methanol extracts of *E.serrata, M.pudic* and *C.longa* and ascorbic acid on nitric oxide radical scavenging showed that ethanol extracts abilities to scavenge nitric oxide generated *in vitro* by sodium nitroprusside followed a sigmoid abc model except *C. longa* that followed a logistic abcd dose response model while methanol extracts scavenged nitric oxide radicals following a sigmoid abc model. Methanol extracts scavenged nitric oxide radical better than ethanol extracts indicated by their IC_{50} values as seen in Table 4.4.

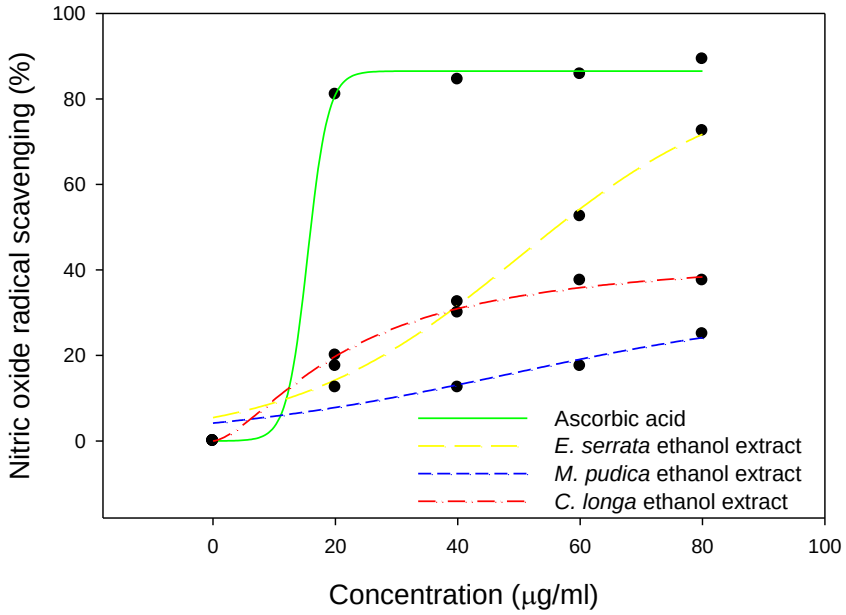

Figure 4.13 Effects of ethanol extracts of *E.serrata, M.pudica, C.longa* and ascorbic acid on scavenging nitric oxide radical.

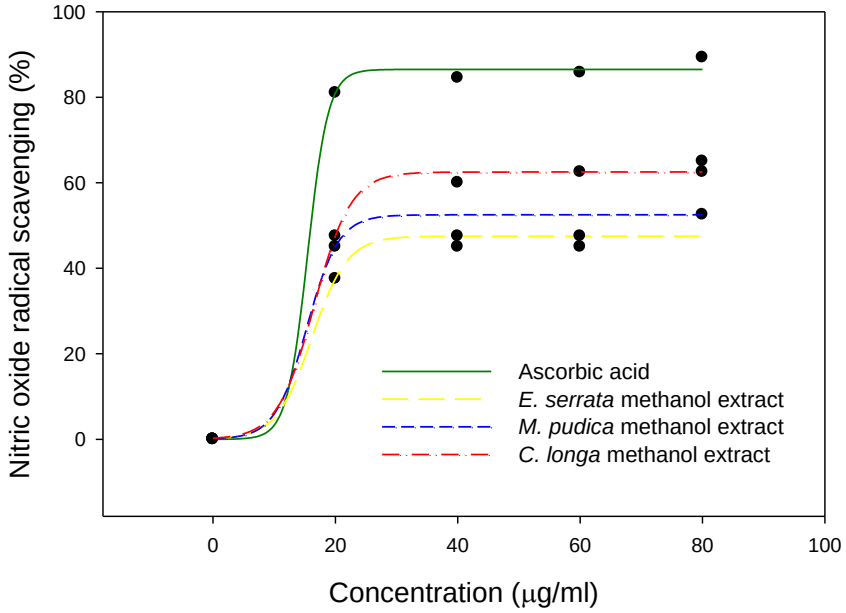

Figure 4.14 Effects of methanol extracts of *E.serrata, M.pudica, C.longa* and ascorbic acid on scavenging nitric oxide radical.

Table 4.4 Comparative analyses of effects of ethanol and methanol extracts of *E.serrata, M.pudica* and *C.longa* and ascorbic acid on scavenging nitric oxide radical

Threshold Inhibitory concentration (IC_{50}) of Nitric oxide radical Scavenging effect of *E. serrata, M. pudica, C. longa ethanolic* and Methanolic extracts and Ascorbic acid standard				
Extraction Solvent	Extracts	Model	R^2	IC_{50}*
	Ascorbic Acid	Sigmoid abc	0.998	15.46
Ethanol	*E. serrata*	Sigmoid abc	0.996	22.67
	M. pudica	Sigmoid abc	0.986	50.02
	C. longa	Logistic abcd	0.871	48.34
Methanol	*E. serrata*	Sigmoid abc	0.996	16.55
	M. pudica	Sigmoid abc	0.978	16.17
	C. longa	Sigmoid abc	0.933	15.36

* IC_{50} is the concentration of the extract or standard that scavenged 50% of the generated radicals.

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4.1.4.3 Effects of Ethanol and Methanol Extracts of *E.serrata, M.pudica, C.longa* and Ascorbic acid on Scavenging Hydrogen Peroxide (H_2O_2) Radical.

Figure 4.15 represents the hydrogen peroxide scavenging abilities of *E. serrata, M. pudica* and *C. longa* ethanol extracts with the standard antioxidant (ascorbic acid). The extracts inhibited H_2O_2 compared with ascorbic acid. The inhibitory concentration (IC_{50}) value of the extracts *E. serrata, M. pudica a*nd *C. longa* were 15.31 µg/ml, 15.26 µg/ml and 15.26 µg/ml as evaluated from the inhibition graph (Figure 4.15). *M. pudica* and *C. longa* had the same inhibitory concentration (IC_{50}) value of (15.26 µg/ml).

The result presented in (Figure 4.16) showed that the methanol extracts of *E.serrata, M.pudica* and *C.longa* inhibited hydrogen peroxide. *E. serrata* exerted the highest inhibition among the extracts with inhibitory concentration of (IC_{50}) value of 15.48 µg/ml followed by *M.pudica* with inhibitory concentration (IC_{50}) value of 24.17 µg/ml and *C. longa* with inhibitory concentration (IC_{50}) value of 35.03 µg/ml. The ethanol extracts scavenging abilities were better than the methanol extracts.

Comparative analysis of effects of ethanol and methanol extracts of *E.serrata, M.pudic* and *C.longa* and ascorbic acid on hydrogen peroxide (H_2O_2) radical scavenging showed that ethanol extracts of *E. serrata, M. pudica a*nd *C. longa* scavenged radicals better than methanol extracts as seen by their IC_{50} values in Table 4.5. Inhibitory concentration (IC_{50}) values of ethanolic *M. pudica* and *C. longa* are the same while IC_{50} values of methanolic extracts of *M. pudica* and *C. longa* are not the same. All the extracts followed a sigmoid model.

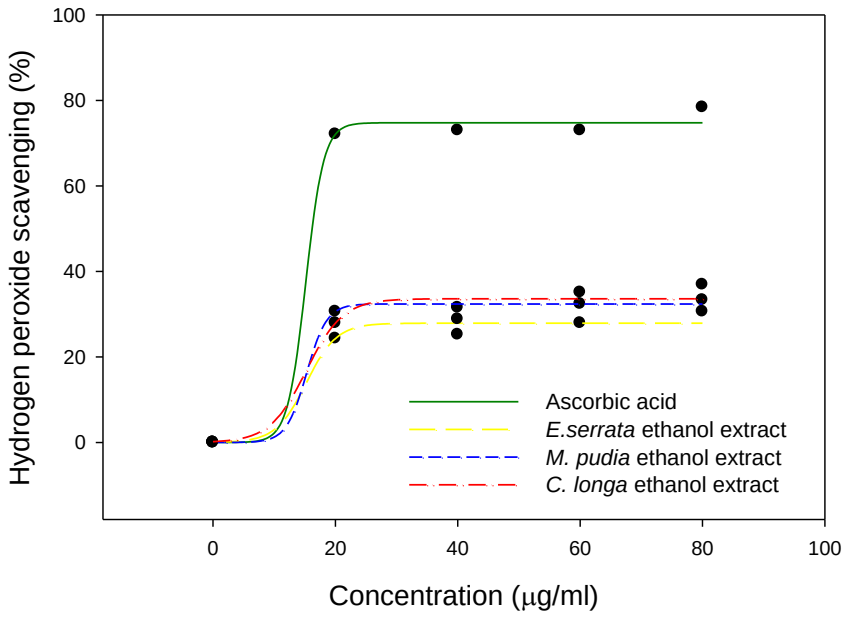

Figure 4.15 Effects of ethanol extracts of *E.serrata, M.pudica, C.longa* and ascorbic acid on scavenging hydrogen peroxide (H_2O_2) radical

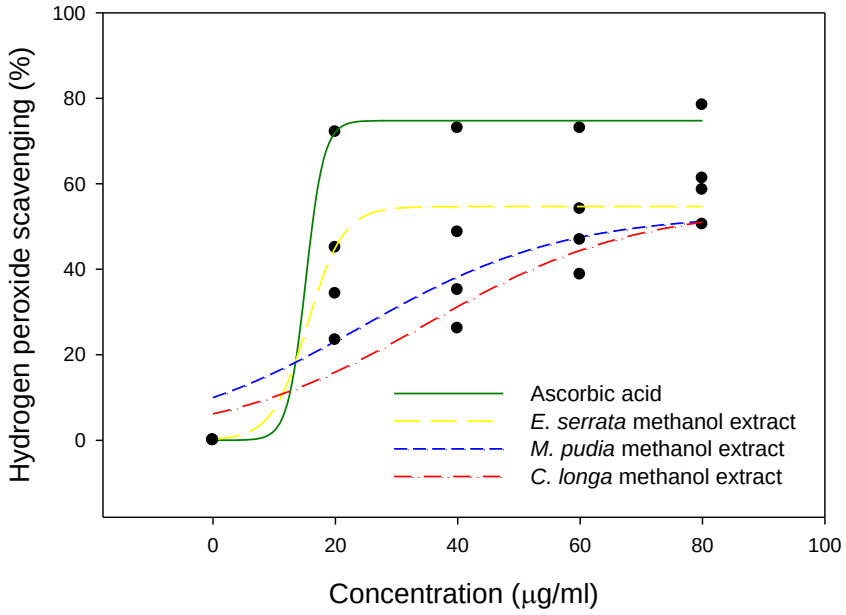

Figure 4.16 Effects of methanol extracts of *E.serrata, M.pudica, C.longa* and ascorbic acid on scavenging hydrogen peroxide (H_2O_2) radical.

Table 4.5 Comparative analyses of effects of ethanol and methanol extracts of *E.serrata, M.pudica* and *C.longa* and ascorbic acid on scavenging hydrogen peroxide (H_2O_2) radical.

Threshold Inhibitory concentration (IC_{50}) of Hydrogen peroxide scavenging effect of *E. serrata, M. pudica, C. longa ethanolic* and Methanolic extracts and Ascorbic acid standard				
Extraction Solvent	Extracts	Model	R^2	IC_{50}*
	Ascorbic Acid	Sigmoid abc	0.996	15.15
Ethanol	*E. serrata*	Sigmoid abc	0.998	15.31
	M. pudica	Sigmoid abc	0.976	15.26
	C. longa	Sigmoid abc	0.959	15.26
Methanol	*E. serrata*	Sigmoid abc	0.966	15.48
	M. pudica	Sigmoid abc	0.796	24.17
	C. longa	Sigmoid abc	0.924	35.03

* IC_{50} is the concentration of the extract or standard that scavenged 50% of the generated radicals

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4.1.4.4 Reducing Power of Ascorbic acid Standard used with Ethanol and Methanol Extracts of *E. serrata, M. pudica* and *C. longa.*

The absorbance of the plants extracts was in a dose dependent manner as increase in concentration increases the absorbance. The $RP_{0.5UA}$ of Ascorbic acid, *E.serrata, M. pudica* and *C. longa* were 12.32 µg/ml, 15.01 µg/ml, 10.01 µg/ml and 9.38 µg/ml respectively for the ethanolic extracts (Figure 4.17). The result showed that *C.longa* had the best Reducing power ability as it was better than the standard. Also *C.longa* and *M.pudica* extracts were better than *E. serrata* extract. The reducing power ability of the extracts was as follows: *C.longa* > *M. pudica* > Ascorbic acid > *E. serrata*. The R^2 values for Ascorbic acid, *E. serrata, M. pudica* and *C. longa* were 0.980, 0.995, 0.999 and 0.999 respectively (Table 4.6).

The $RP_{0.5UA}$ of Ascorbic acid, *E.serrata, M. pudica* and *C. longa* were 12.32 µg/ml, 34.09 µg/ml, 21.77µg/ml and 21.63µg/ml respectively for the methanolic extracts (Figure 4.18). Absorbance of the methanolic extracts was in a dose dependent manner as increase in concentration increases the absorbance. The result showed that ascorbic acid had the best reducing power ability compared to the extracts. *C. longa* and *M. pudica* were also better than E.serrata. The reducing power ability of the plants in ascending order was as follows: Ascorbic acid > C.longa > *M.pudica* > *E.serrata.* The R^2 values for Ascorbic acid, *E.serrata, M. pudica* and *C. longa* were 0.980, 0.999, 0.999 and 0.973 respectively (Table 4.6).
Ethanolic extracts of *C. longa* had the best reducing power compared to the ascorbic acid and the other extracts *(E.serrata, M. pudica)*

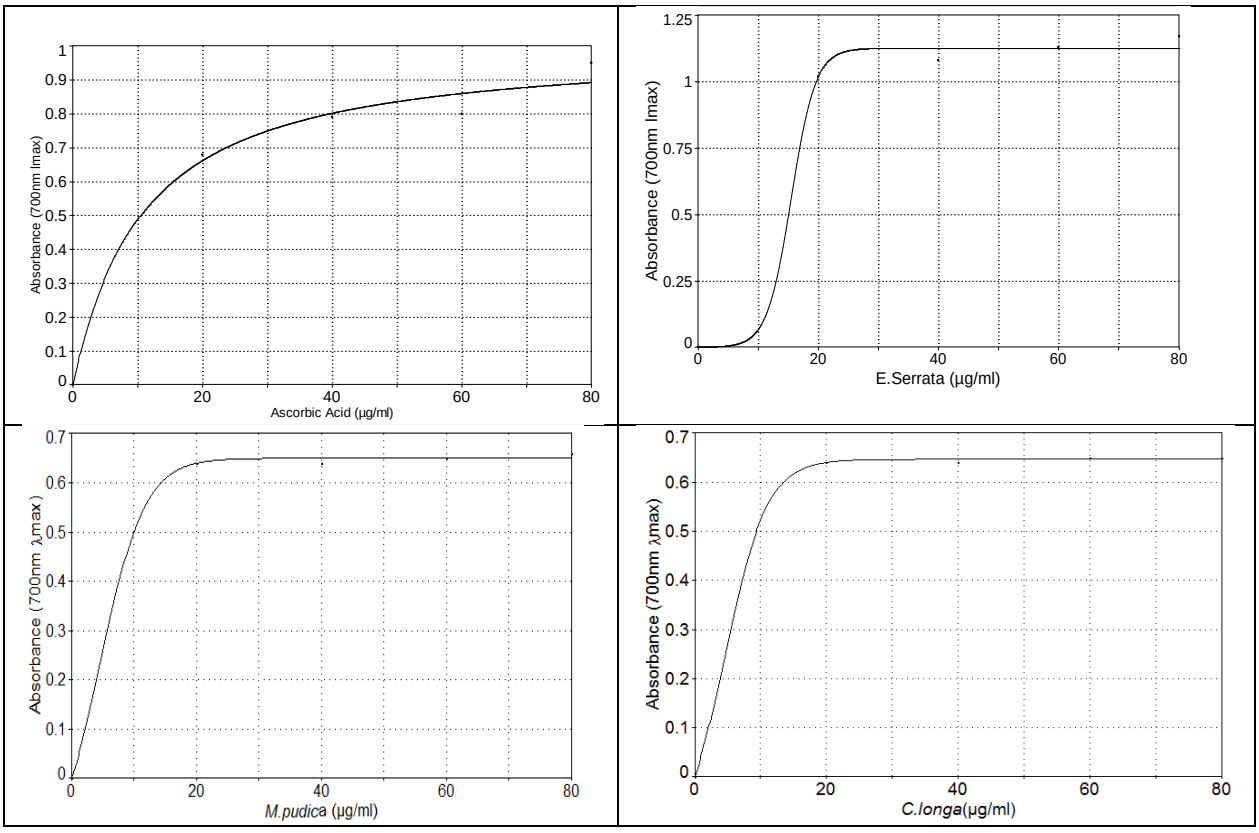

Figure 4.17: Reducing power of Ascorbic acid (standard) and ethanol extracts of *E. serrata, M. pudica* and *C.longa* at 700 nm (RP0.5$_{AU}$ of ascorbic acid *E. serrata, M. pudica* and *C. longa* = 12.32µg/ml, 15.01µg/ml, 10.0 µg/ml and 9.38 µg/ml respectively)

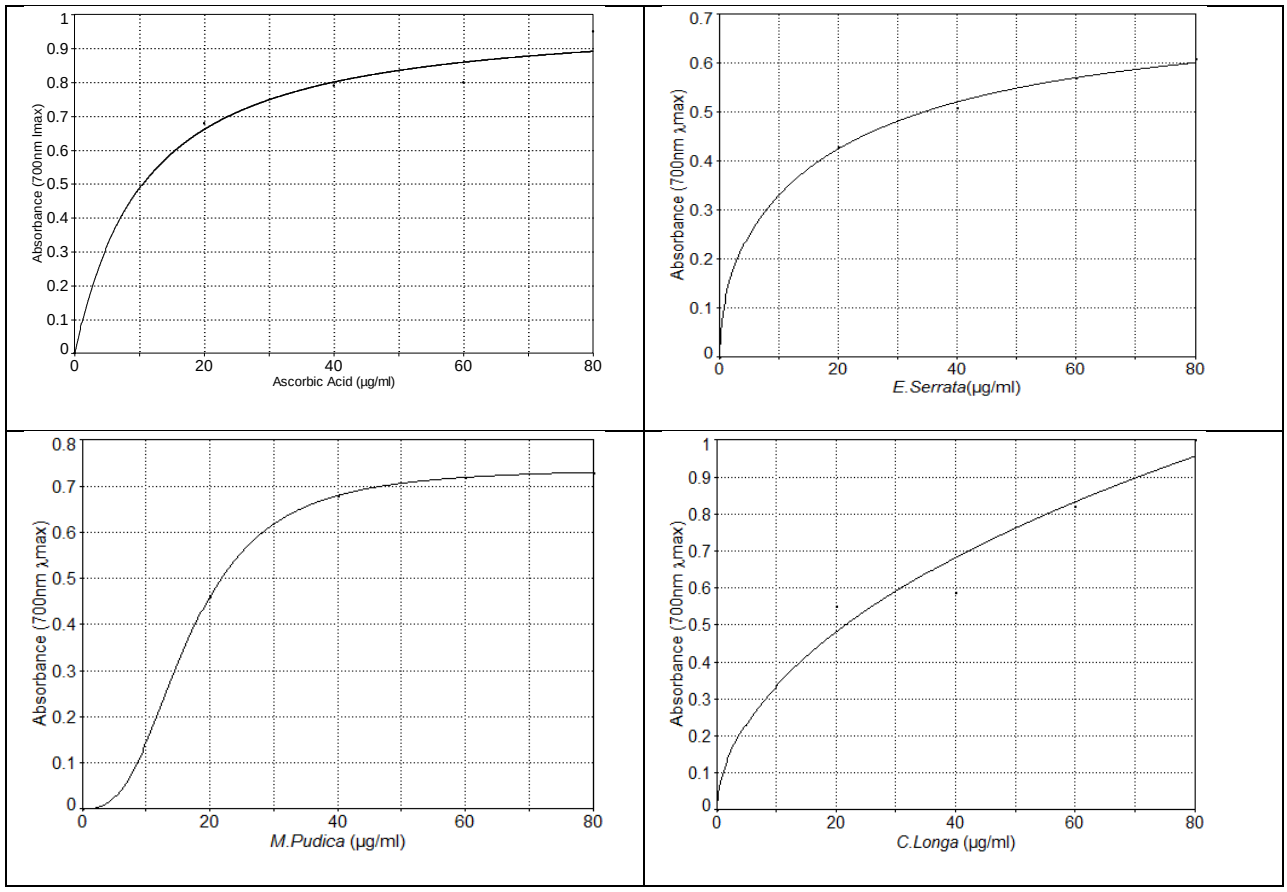

Figure 4.18: Reducing power of Ascorbic acid (standard) and methanol extracts of *E. serrata, M. pudica* and *C.longa* at 700 nm (RP0.5$_{AU}$ of ascorbic acid *E. serrata, M. pudica* and *C. longa* = 12.32 µg/ml, 34.09 µg/ml, 21.77 µg/ml and 21.63 µg/ml respectively).

Table 4.6: Comparative analysis of reducing power of Ascorbic acid standard with ethanol extracts of *E. serrata, M. pudica* and *C.longa.*

Extracts	Mathematical Model		Pearson Correlation(R^2)		Reducing power $RP_{0.5UA}$ (µg/ml)	
	Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol
Ascorbic acid	Sigmoid abcd		0.980	0.980	12.32	12.32
E.serrata			0.995	0.999	15.01	34.09
M. pudica			0.999	0.999	10.01	21.77
C.longa			0.999	0.973	9.38	21.63

$RP_{0.5UA}$ is the concentration of extract able to give 0.5 absorbance reading.

$RP_{0.5UA}$ was evaluated from sigmoid abcd models.

4.1.5 Acute Oral Toxicity

The acute oral toxicity result obtained showed that the ethanolic extract of *E. serrata, M. pudica* and *C. longa* at a dose of 5000 mg/kg had adverse effect on the albino rats when administered 5000 mg/kg body weight of the extract (Table 4.7). The LD_{50} of these plants was therefore estimated to be less than 5000 mg/kg**.** The acute oral toxicity study showed that the ethanol extracts of *E. serrata, M. pudica* and *C. longa* demonstrated mortality when the animals received up to 5000 mg/kg body weight of the extract orally (Table 4.7).

4.1.6 Sub-chronic Oral Toxicity

Daily oral administration of *E. serrata, M. pudica* and *C.longa* extracts for twenty-eight days did not produce any sign or symptom of toxicity in rats, even at the highest dose (400 mg/kg) body weight tested. No mortality or clinical signs of toxicity was seen in any of the experimental animal groups throughout period. It was observed that the experimental rats gained weight when weighted weekly during the study period compared to the control group.

Table 4.7: Acute oral toxicity of ethanol extracts of *C. longa, M. pudica* and *E. serrata*

	C. longa Dosage (mg/kg body weight)	Mortality	*M. pudica* Dosage (mg/kg body weight)	Mortality	*E. serrate* Dosage (mg/kg body weight)	Mortality
PHASE I						
Group I	10	0/3	10	0/3	10	0/3
Group II	100	0/3	100	0/3	100	0/3
Group III	500	0/3	500	0/3	500	0/3
PHASE II						
Group IV	1000	0/3	1000	0/3	1000	0/3
Group V	2900	0/3	2900	0/3	2900	0/3
Group VI	5000	2/3	5000	2/3	5000	2/3

$L_{D50} = \sqrt{(D_0 \; x \; D_{100}}$

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

$LD_{50} = \sqrt{2900 \text{ X } 5000}$

$= \sqrt{14{,}500{,}000}$

$= 3808 mg/kgbw$

4.1.6.1 The Effects of administration of Ethanol Extracts of *E. serrata, M. pudica and C. longa* for 28 days on Body Weights of Albino Rats

In the evaluation of the sub-chronic toxicity effect of the ethanol extract of *E. serrata* on the body weights of albino rats, the result showed that there was significant difference in administered doses (200 mg/kg and 400 mg/kg) mean body weights of rats for 28 days when compared to control group. The administration of (200 mg/kg and 400 mg/kg) doses of the ethanol extract of E. *serrata* for 28 days showed that there was significant increase ($P<0.05$) in weekly weights of Albino rats. However, there was decrease in weekly weights of treated groups of Albino rats when compared to control groups (Figure 4.19).

The effect of ethanol extract of *M. pudica* on the body weights of albino rats administered with 200 mg/kg and 400 mg/kg doses, the result showed that there were significant differences ($p< 0.05$) in the mean body weights compared to control group. (Figure 4.20)

The administration of (200 mg/kg and 400 mg/kg) doses of the ethanol extract of *C. longa* for 28 days showed that there was increased ($P<0.05$) in weekly weights of Albino rats. There was decrease in weekly weights of treated groups of Albino rats when compared to control groups (Figure 4 .21). Also the means of the body weight of the treated groups with ethanol extracted (200 mg/kg and 400 mg/kg) of *E.serrata, M. pudica* and *C. longa* were significantly different from the control mean.

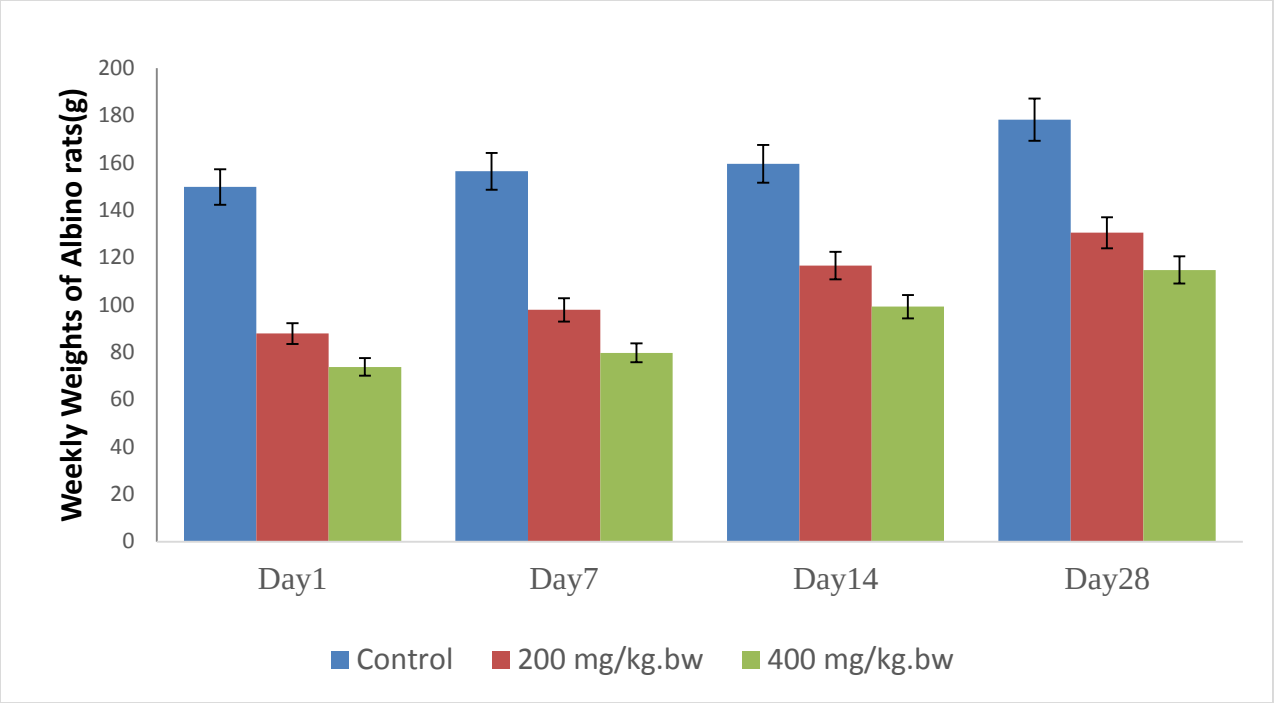

Figure 4.19: The effect of ethanol extracts of *E.serrata on* body weights of albino rats.

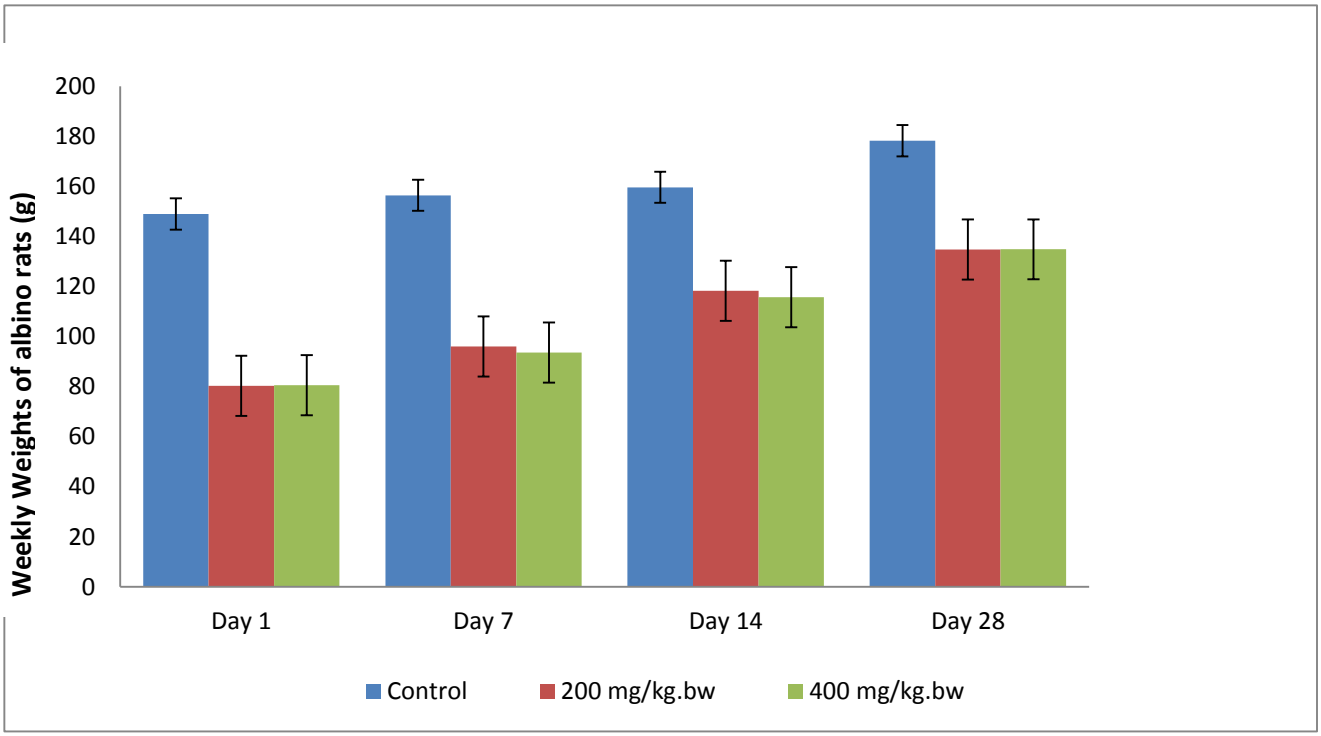

Figure 4.20: The effect of ethanol extracts of *M. pudica* on body weights of albino rats.

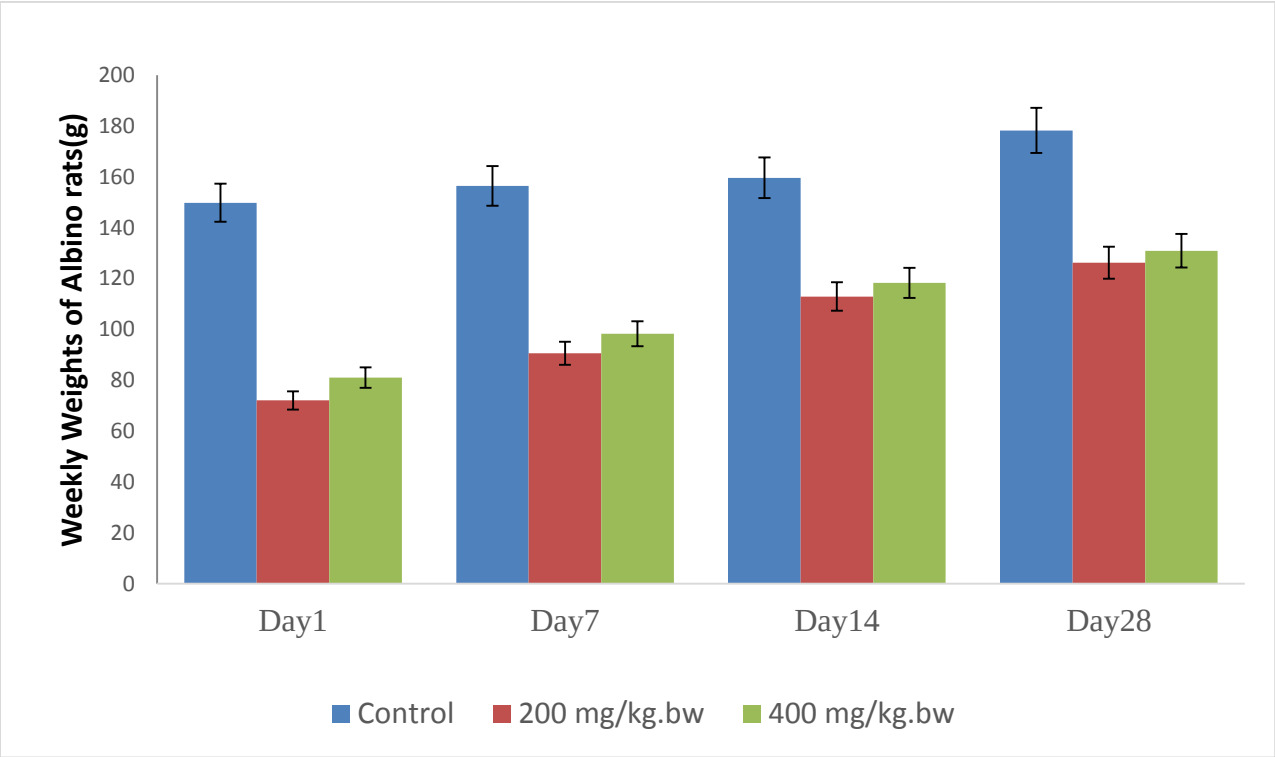

Figure 4.21: The effect of ethanol extracts of *C. longa* on body weights of albino rats.

4.1.6.2 The Effects of Administration of Ethanol Extracts of *E. serrata, M. pudica* and *C. longa* for 28 days on Liver Function Parameters in Albino Rats.

The result of the ethanol extract of *E. serrata*, *M. pudica* and *C. longa* on liver function parameters in albino rats Figure 4.22 showed that there were significant differences in the liver function of treated groups compared to control group.

The total protein (TP), Albumin (ALB), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), urea and creatinine activities significantly increased ($P<0.05$) in a dose-dependent fashion when the rats received doses of 200 mg/kg and 400 mg/kg of the ethanol extract.

Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) activities were higher in experimental groups compared to control group. Alkaline phosphatase (ALP), activity was highest in treated groups compared to control group as 400 mg/kg of *C. longa* gave a higher (18.20 u/l) ALP activity than control (17.80 u/l). ALP activity increases in a dose-dependent manner when treated rats received doses (200 mg/kg and 400 mg/kg) of the ethanol extract of *C. longa* and *E. serrata* except *M. pudica*.

The result also showed that there was a decrease in the total protein (3.8 to 3.6 g/dl) and albumin (2.65 to 2.60 g/dl) activities of the treated groups when 400 mg/kg of *C. longa* extract were administered to the rats

The Lactate dehydrogenase (LDH) activities significantly increased ($p<0.05$) in a dose–dependent fashion compared to the control group. The LDH activities of *C. longa M. pudica* and *E.serrata* were higher than the control (115. 22 u/l) except 200 mg/kg of *C. longa* (114. 99 u/l).

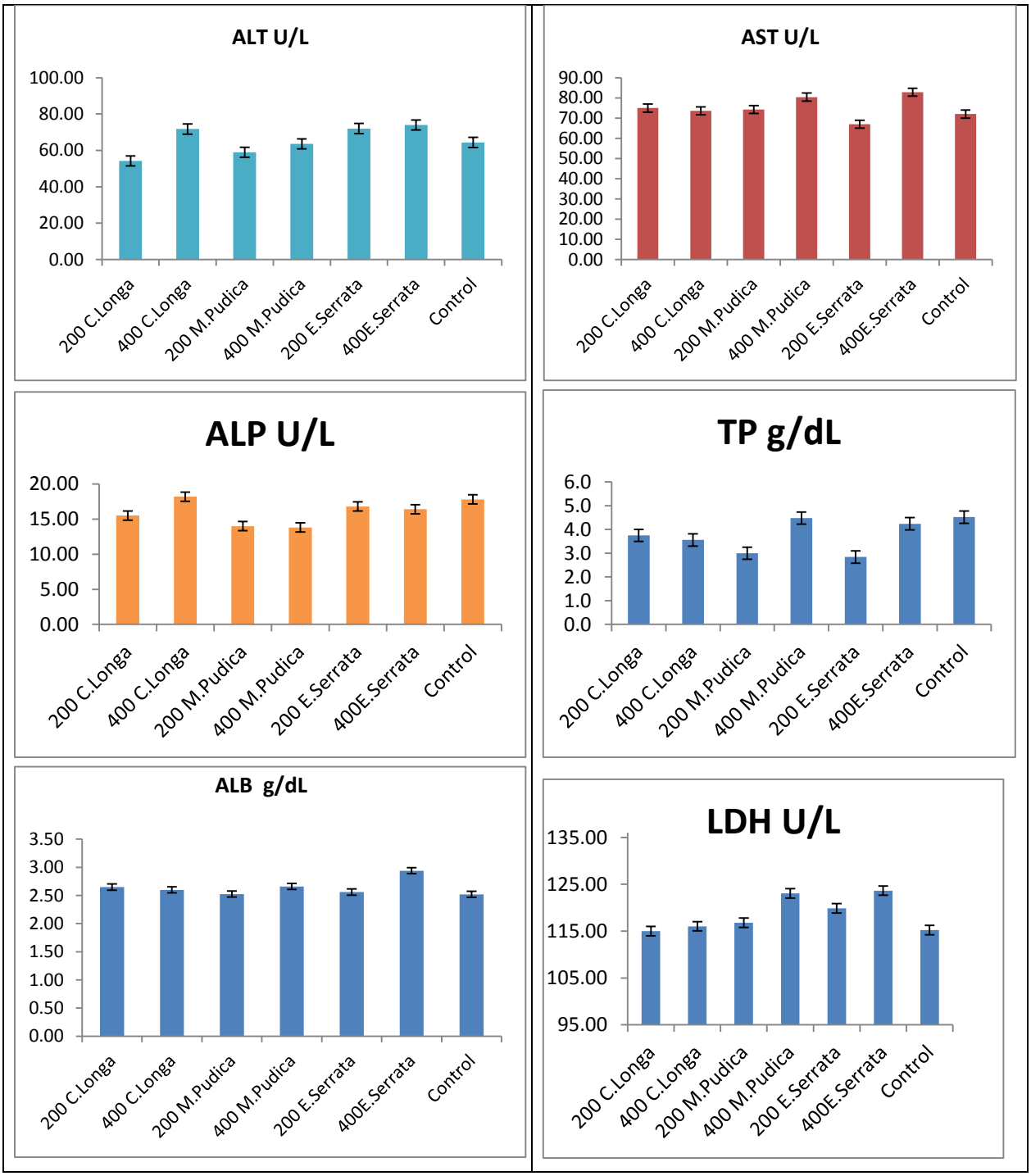

Figure 4.22: The effects of ethanol extracts of *E. serrata, M. pudica* and *C. longa* on liver function parameters in albino rats.

4.1.6.3 The Effects of Administration of Ethanol Extract of *E. serrata, M. pudica* and *C. longa* for 28 days on Renal Function Parameters in Albino Rats.

The result of the ethanol extract of *E. serrata, M. pudica* and *C. longa* on renal function parameters in albino rats (Figure 4.23) showed that there were significant differences in the renal function of treated groups compared to control group. The creatinine and urea activities significantly increased ($P<0.05$) in a dose-dependent fashion when the rats received doses of 200 mg/kg and 400 mg/kg of the ethanolic extract.

Creatinine activities were higher in treated groups with 400 mg/kg doses than in control group. Also the creatinine activities of the extracts at 200 mg/kg of *C.longa, M.pudica* and *E. serrata* had the same value as the control. The urea activity of the treated groups of *E. serrata* and *M. pudica* with 400 mg/kg of extract was higher compared to control.

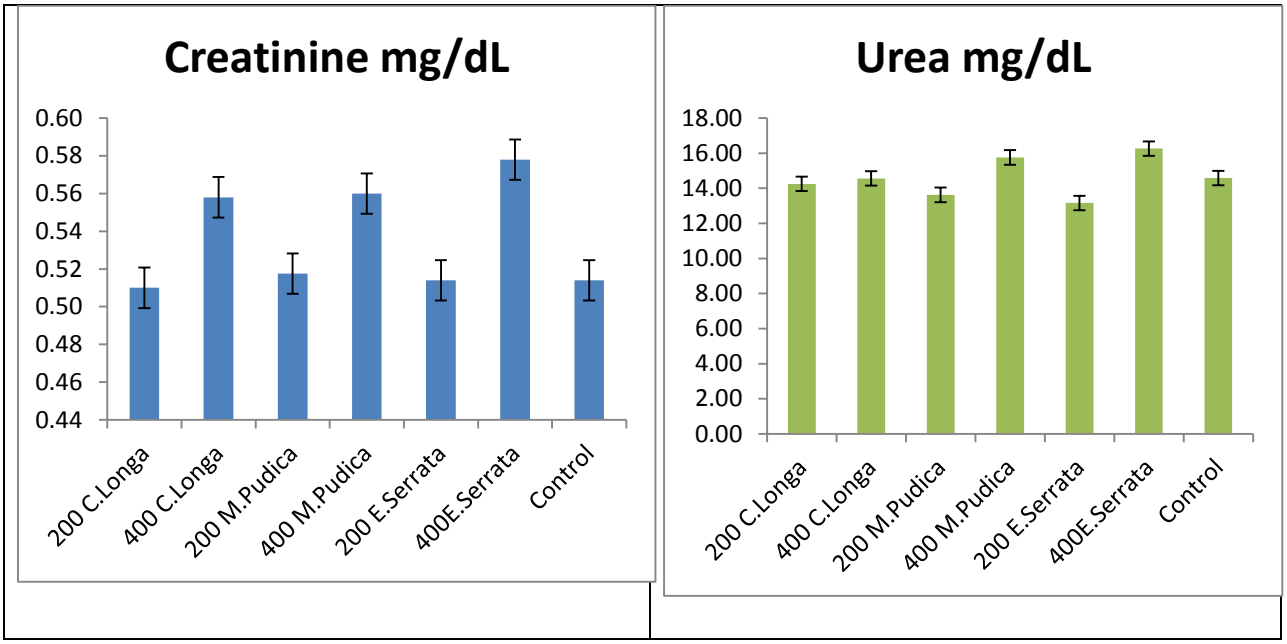

Figure 4.23: The effects of ethanol extracts of *E. serrata, M. pudica* and *C. longa* on renal function parameters in albino rats

4.1.6.4 The Effects of Administration of Ethanol Extract of *E. serrata, M. pudica* and *C. longa* for 28 days on Oxidative Stress Function Parameters in Albino Rats.

The result of the effect of ethanol extract of *C. longa M. pudica* and *E.serrata* on oxidative stress function parameters in albino rats showed that there was no significant difference in oxidative stress function parameters of the rats when compared to the control group.

The catalase (CAT), glutathione peroxidase (GPX), reduced glutathonine (GSH), melondialdehyde (MDA) activities significantly increased ($p<0.05$) in a dose–dependent fashion compared to the control group. Albino rats that received 200 mg/kg of ethanolic *E.serrata* and *M. pudica* extracts had higher superoxide dismutase (SOD) activities than those that received 400 mg/kg dose. SOD activity decrease with increase in concentration of *E. serrata* extract (from 11.01 u/l to 10.82 u/l). The Catalase activity at 400 mg/kg for *M. pudica* and *E.serrata* were higher (4.734 u/l and 4.760 u/l) respectively compared to control (3.253 u/l)(Figure 4.24). GPx activity at 400 mg/kg for *E.serrata* (33.97 u/l) was higher compared to control (32.36 u/l) while GSH and MDA activities were higher in all the extracts *C. longa*, *M. pudica* and *E.serrata* compared to control. Melondialdehyde (MDA) and GSH activities were higher in the treated groups compared to the control.

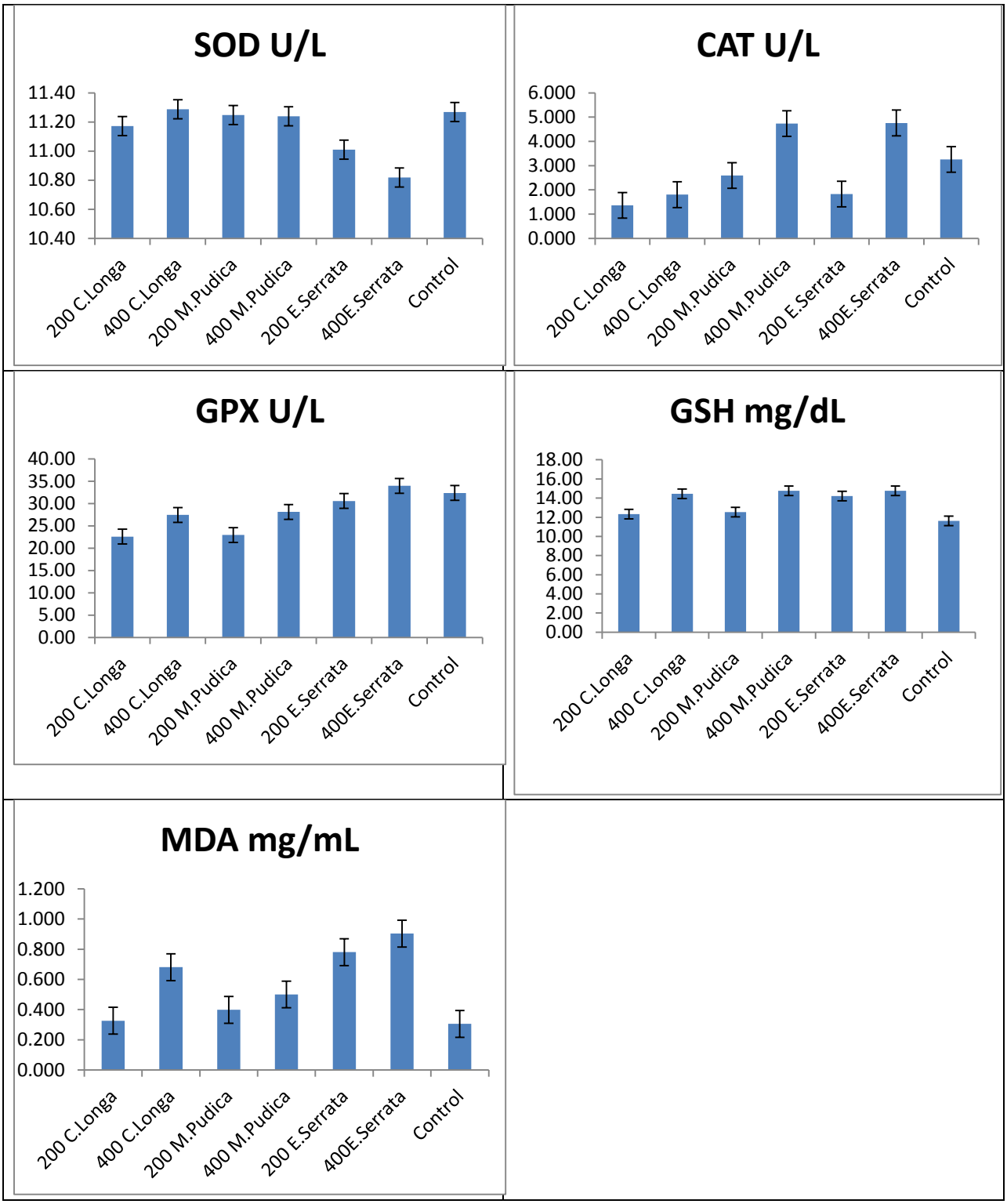

Figure 4.24: The effects of ethanol extract of *E. serrata*, *M. pudica* and *C. longa* on oxidative stress function parameters in albino rats.4.1.6.5 The Effects of Administration of Ethanol Extract of *E. serrata, M. pudica* and *C. longa* for 28 days on C – Reactive Protein.

The result of the effect of ethanol extract of *C. longa, M. pudica* and *E.serrata* on C- reactive protein (Figure 4.25) showed that there was no significant difference in C - reactive protein parameter of the rats when compared to the control group. Albino rats that received 200 mg/kg of ethanolic *E.serrata* and *M. pudica* extracts had higher C - reactive protein (CRP) activities than those that received 400 mg/kg dose. C - reactive protein activities were higher in the treated groups compared to the control. *E. serrata* at 200 mg/kg had the highest C-reactive protein activity of (2.77 mg/dl) but reduced to 2.36 mg/dl with increase in concentration (400 mg/kg).

4.1.6.6: The Effect of Administration of Ethanol Extracts of *E. serrata, M. pudica* and *C. longa* for 28 days on Hematological Profiles in Albino Rats.

Figure 4.26 shows the effect of ethanol extracts of *E. serrata, M. pudica* and *C.longa* on haematological profile in albino rats. The result shows that there was significant difference in hematological profiles in albino rats. The concentration of Hemoglobin (HB), Red blood cell (RBC), PCV and platelets, their concentration increase with increased in concentration of the extracts except *M.pudica* where increase in concentration resulted in decrease in volume for HB and RBC, and PCV % respectively. The platelet value for the control was higher compared to *E. serrata, M. pudica* and *C.longa* extracts. Increase in concentration of the extracts decreased WBC concentration in all the extracts. *C.longa* extracts at 200 mg/kg had the highest WBC (8100 mm^{-3}) compared to control (5760 mm^{-3}). The PCV % of *E. serrata, M. pudica* and *C.longa* at 200 mg/kg and 400 mg/kg were higher compared to control. *E. serrata* at 200 mg/kg had the same PCV % value with the control. *M. pudica* at 200 mg/kg had the highest PCV % (50%) compared to *E. serrata*, *C.longa* and control but decreased to 35% with increase in concentration (400 mg/kg).

The concentrations of HB, RBC, PCV and Platelets significantly increased ($P<0.05$) in a dose dependent fashion for all the extracts except HB, RBC and PCV of *M.pudica* but the concentration of WBC significantly decreased ($P>0.05$) with increase dosage of the extracts (*E. serrata, M. pudica* and *C.longa)* while the PCV for *M.pudica* also decreased with increase in concentration.

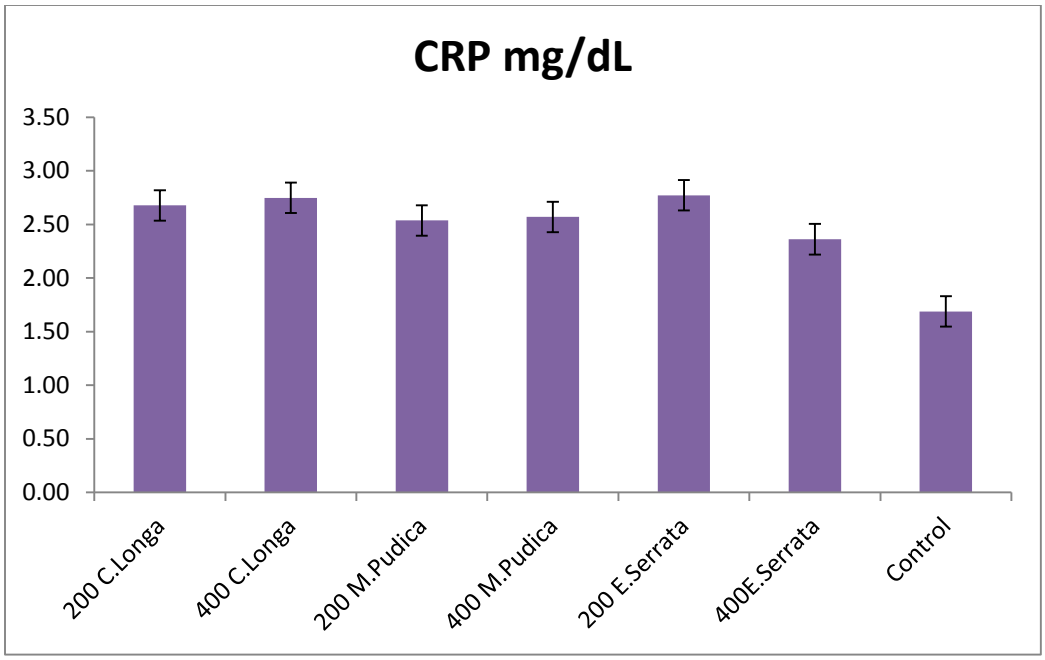

Figure 4.25: The effects of ethanol extracts of *E. serrata, M. pudica* and *C. longa* on C-reactive protein.

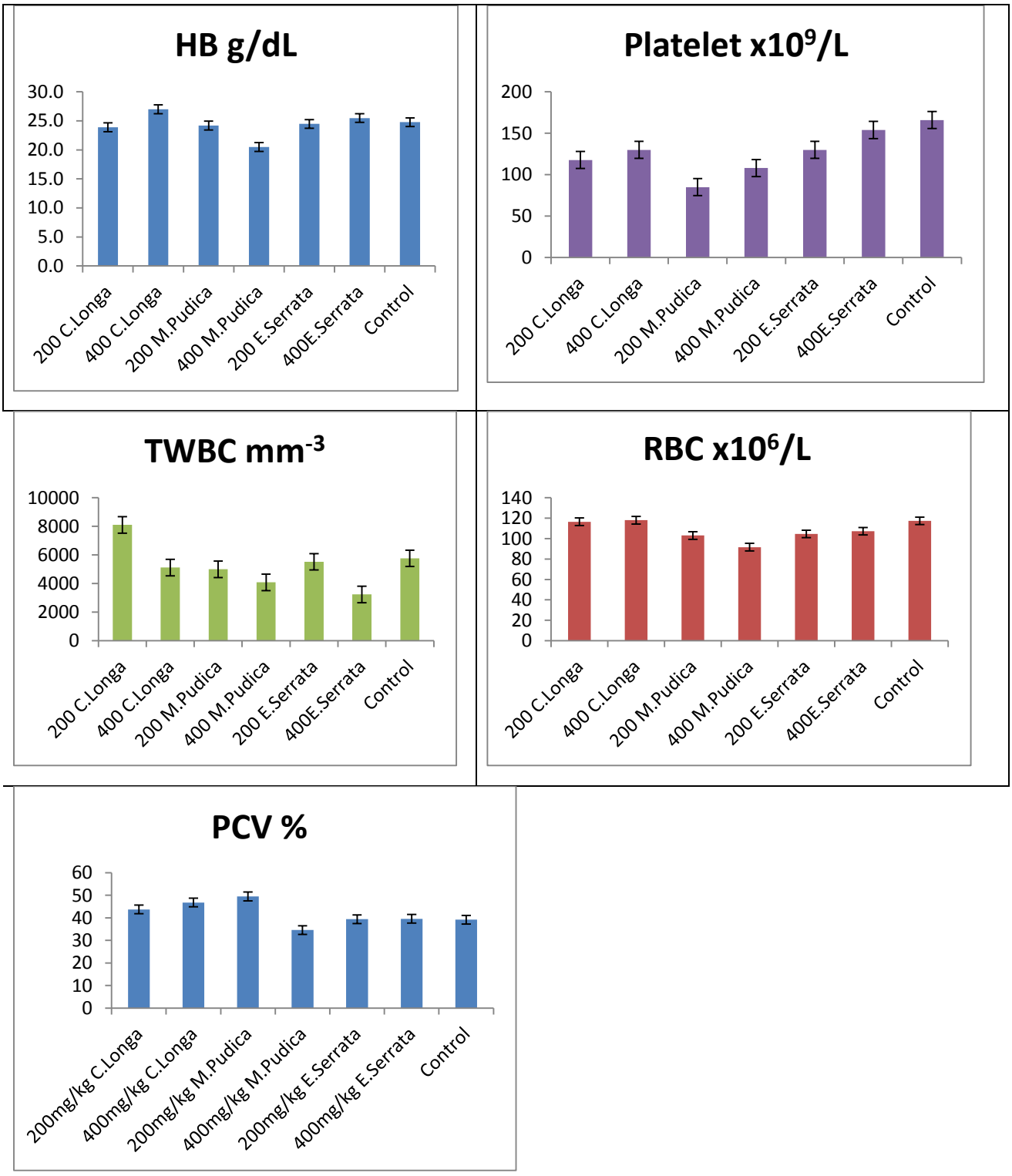

Figure 4.26: The effects of ethanol extracts of *E. serrata, M. pudica* and *C. longa* on hematological profiles in albino rats.

4.1.6.7 Relative Organ Weights of Rats Treated with Ethanol Extracts in Sub-Chronic Toxicity Study.

There was no significant difference ($p>0.05$) in the relative organ weights of each organ recorded in the treatment group compared to the control as shown on Table 4.8. The relative organ weights of the rats that received various doses of the ethanol extract of *E. serrata, M. pudica* and *C. longa* were not significantly different ($p>0.05$) from those of the control group. The groups treated with 200 mg/kg and 400 mg/kg body weight of extracts showed no significant gain in weight of liver, kidney and heart.

4.1.7 Histopathological Result

The results of the histopathological examination of the liver, kidney and heart sections of albino rats administered with ethanol extracts *C. longa , M. pudica* and *E. serrata,* alongside with the control (Group 7a.7b & 7c.) are shown in Figures 4.27, 4.28 and 4.29 below.

4.1.7.1 The effects of (200 mg/kg and 400 mg/kg) of ethanolic extracts of *C. longa* (1a and 2a); *M. pudica* (3a and 4a); *E. serrata* (5a and 6a) on liver histomorphologies in sub – chronic oral toxicity study

Fig 4.27 In groups 1 (1a), 2 (2a), 3 (3a), 5 (5a), 6 (6a) and 7 (7a), the result of liver histopathology showed normal hepatic lobules consisting of normal hepatocytes arranged in radiating interconnecting cords around the central veins. The hepatic cords radiates towards the periphery of the lobules where it meets with the components of the portal triad (hepatic artery, hepatic vein and bile duct).

The result also showed that the liver of animals in this group 4 (4a) showed a mild infiltration of inflammatory cells into the periportal areas.

Table 4.8: Relative organ weights of rats treated with ethanol extracts in sub-chronic toxicity study.

ORGAN	CONTROL	200 mg/kg	400 mg/kg	P-Value
Heart	0.44±0.005[a]	1.57±1.61[b]	1.46±1.49[b]	0.519
Liver	2.965±0.005[a]	11.21±11.53[b]	11.14±11.45[b]	
Kidney	0.73±0.01[a]	2.72±11.53[b]	2.65±2.73[b]	

Data are shown as Mean ± SEM (n=5 for each group)

4.1.7.2 The effects of (200 mg/kg and 400 mg/kg) of ethanolic extracts of *C. longa* (1b and 2b); *M. pudica* (3b and 4b); *E. serrata* (5b and 6b) of kidney histomorphologies in sub – chronic oral toxicity study

Fig 4.28 In groups 1(1b), 2(2b), 4 (4b), 5(5b), and 6(6b) the result of kidney histopathology showed normal renal histo-architectures. Glomeruli in their Bowman's capsules surrounded by normal renal tubules (proximal convoluted tubules, pars recta, distal convoluted tubules and collecting duct) suspended in a highly vascularised connective tissue matrix (renal interstitium).

Group 3 (3b) kidneys, result of kidney histopathology showed mild inflammation and necrosis. Multifocal areas of tubular degeneration and necrosis with infiltration of the renal interstitium by inflammatory mononuclear phagocytes involved mostly the outer medullar.

4.1.7.3 The effects of (200 mg/kg and 400 mg/kg) of ethanolic extracts of *C. longa* (1c and 2c); *M. pudica* (3c and 4c); *E. serrata* (5c and 6c) on heart histomorphologies in sub – chronic oral toxicity study.

Fig 4.29 In groups 1 (1c), 2 (2c), 5(5c), 6 (6c) and 7 (7c), the result of the heart histopathology showed the normal myocardial histo-architecture of laboratory rodents. The result also showed sections with normal epicardial, myocardial and endocardial layers. The myocardium showed normal myocytes arranged in overlapping bundles, surrounded by a rich network of blood vessels and capillaries embedded in a connective tissue matrix. The myocytes contain single centrally located oval to elongated hypochromatic nuclei. Fibroblasts of the connective tissue matrix appear as spindle shaped cells with spindle shaped hyperchromatic nuclei

In groups 3 (3c) and 4 (4c), the result of the heart histopathology showed mild infiltration of inflammatory cells into the highly vascularised myocardial connective tissue matrix.

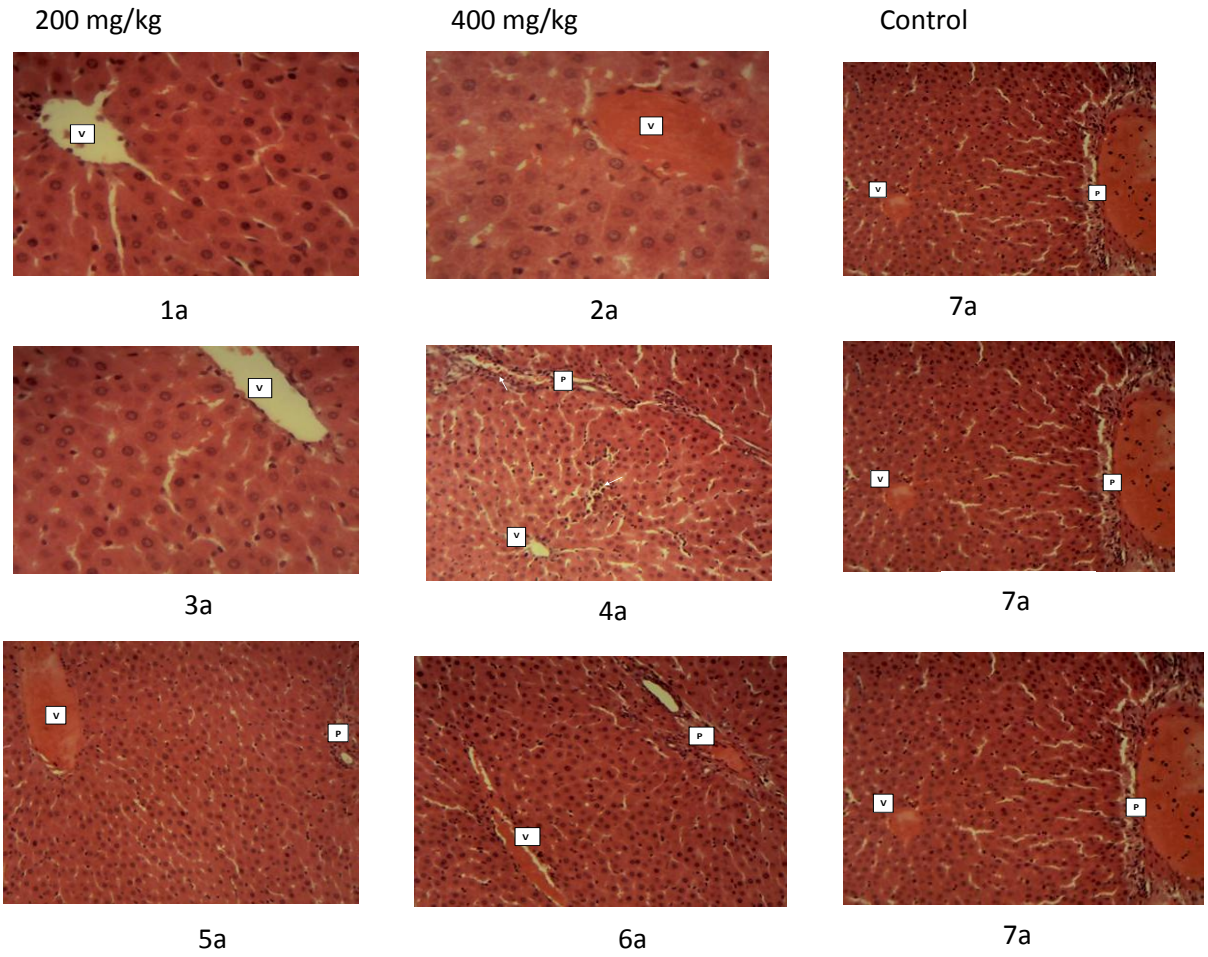

Plate 4.1: The photomicrograph of liver sections of all groups

Legend: Central vein (V), Portal area (P), Inflammatory cell (white arrow), Mag x160

Extracts: *C. longa* (plate:1a and 2a), *M. pudica* (plate:3a and 4a); *E. serrata* (plate: 5a and 6a). Control: (plate:7a.)

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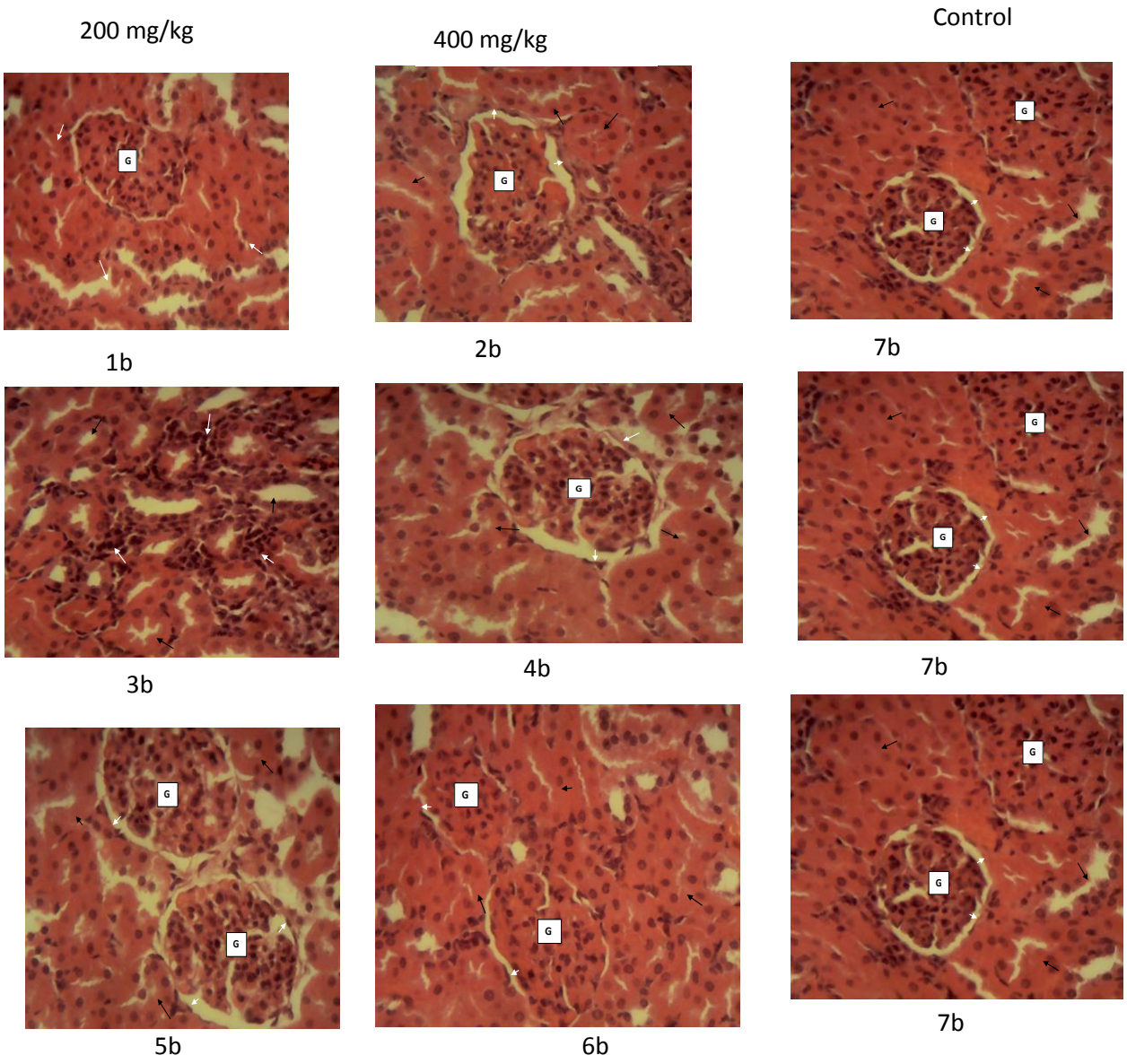

Plate 4.2: The photomicrograph of kidney sections of all groups

Legend: 1b**:** Glomerulus (G); renal tubules (white arrow); 2b, 4b and 6b: Glomerulus (G); Bowman's capsule (white arrow); renal tubules (black arrow); 3b: Renal tubules (black arrow), inflammatory cells in the renal interstitium (white arrow); 5b: Nucleus of cardiomyocyte (white arrow); Pericyte (black arrow). Magnification x400.

Extracts*: C. longa* (plate:1b and 2b); *M. pudica* (plate:3b and 4b); *E. serrata* (plate:5b and 6b). Control (plate: 7b).

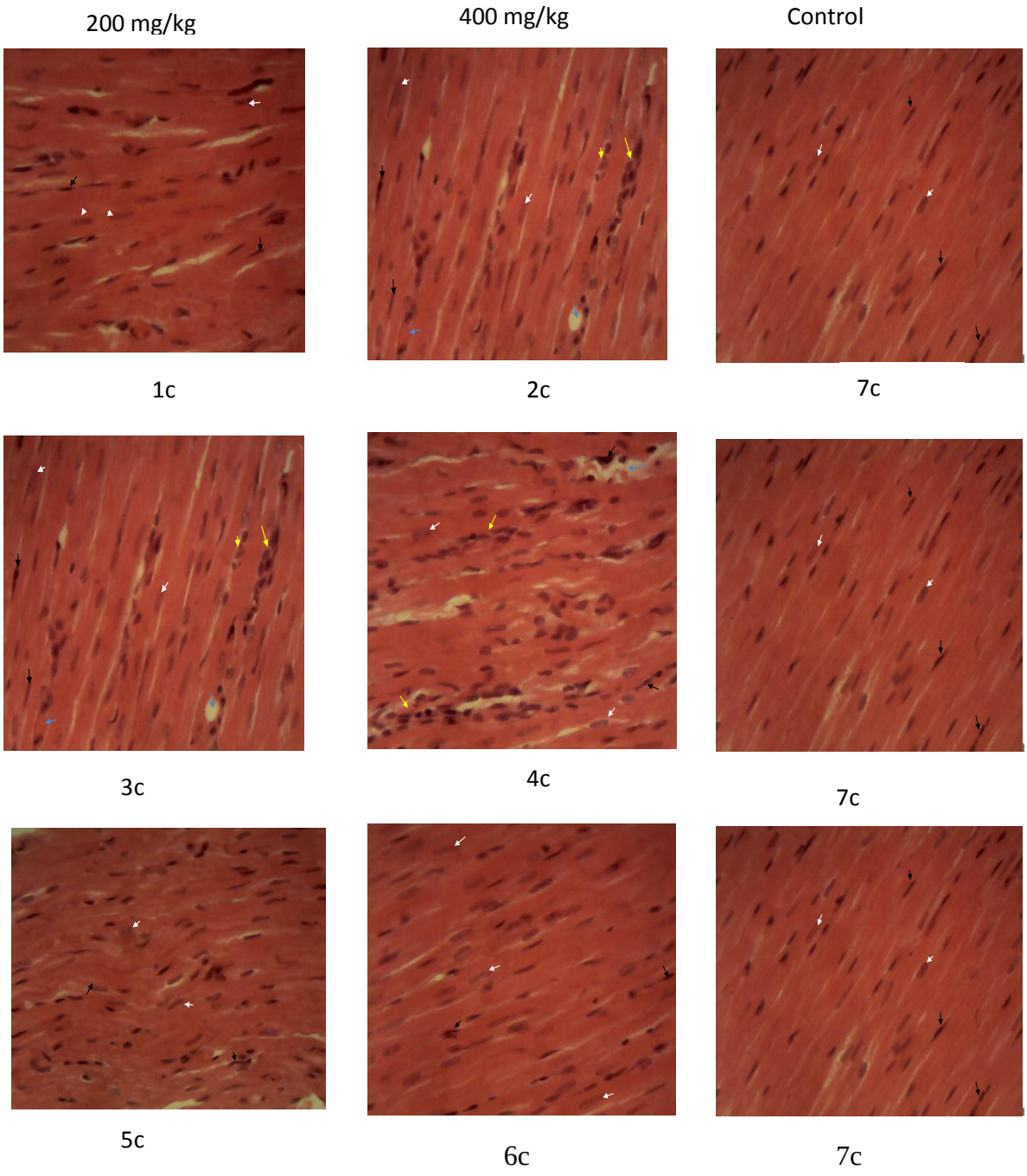

Plate 4.3: The photomicrograph of heart sections of all groups

Legend: Nucleus of cardiomyocyte (white arrow); Pericyte (black arrow), Blood vessel (blue arrow), Inflammatory cells in between myofibres (yellow arrow).

Extracts: *C. longa* (plate:1c and 2c); *M. pudica* (plate:3c and 4c); *E. serrata* (plate:5c and 6c). Control (plate:7c).

4.2 DISCUSSION

Result of the qualitative analysis shows that the plant extracts *E.serrata*, *M. pudica* and *C. longa*,

demonstrated the presence of phytochemical screened to be moderate and high which attest for the plant usage in treatment of ailments.

From the results of quantitative phytochemical screening, there were significant differences ($P<0.05$) in all the phytochemical contents of *E.serrata*, *M. pudica* and *C. longa.* The content of tannins was highest in *E. serrata* (35.2%) compared to *M. pudica* (24%) and *C. longa* (24.2%). Also the content of flavonoid was highest in *M. pudica* (53.8%) compared to *E. Serrata* (9.8%) and phenol contents were high in all the plants extracts (*E. serrata, M. pudica* and *C.longa*). This might be the reason why *E. serrata, M. pudica* and *C.longa* showed much activity in ethanol and methanol solvents against these bacteria. The overall investigation on the phytochemical constituents of aqueous, ethanol and methanol extracts of *E. serrata, M. pudica* and *C. longa* revealed the presence of alkaloid, flavonoid and tannins, oxalate and phenol and others. The phytochemical screening result of this study for *E .serrata* is in line with Dharmananda, 2003 and Tijjani *et al*., (2011) who reported that tannin present in *E. serrata* have various physiological effects like antimicrobial. The potency of medicinal plants is attributed to the action of these potent biological active phytochemical. These phytochemicals are actually produced by plants as secondary metabolites as a defense mechanism to plant diseases. These metabolites have emerged as compounds possessing significant therapeutic agents' potential against human pathogens for example bacteria, fungi and viruses. The phytochemicals found in *M. pudica* in this study is in consonance with the study carried out by Ranjeet *et al*., (2013), Ahmad and Beg, (2001) , Gandhiraja *et al.,* (2009) who reported the presence of bioactive components like alkaloid, tannins, saponnin, etc were in the extracts of *M. pudica* leaves.

The microorganisms were molecularly identified to species level as *Wohlfahrtiimonas chitiniclastica, Proteus mirabilis, Alcaligenes faecalis, Klebsiella pneumoniae, Candida tropicalis, and Wickerhamomyces anomalus.*

The antibacterial and antifungal activities of ethanol, methanol and aqueous extracts of *E. serrate, M.pudica, C.longa* at different concentrations possesses high inhibitory potentials against the tested microorganisms as shown by their inhibition zones.The differences noticed in

the inhibition zones of the crude plant extracts could be linked to the differences in the chemical components of the plants extracts such as alkaloid, tannins, saponins, phenols, flavonoids etc.

In this study, the extracts of *E. serrata, M. pudica* and *C. longa* demonstrated a concentration – dependence antibacterial and antifungal activity with higher concentration of 50 mg/ml showing greater zones of inhibition than lower concentration of 37.5 mg/ml and 25 mg/ml as seen, is similar to observation of Akujobi *et al*., (2004), who reported antimicrobial activity of *Euphorbia* plant inhibiting the growth of various micro-organisms at different concentrations (Osadese and Ukwez, 2004; Perumal *et al*., 2012; Venkatanagaraju and Goli, 2014; Velasco *et al*., 2013).

The antibacterial and antifungal activity of *C. longa* in this study is in line with Negi *et al*., (1999); Eigner and Scholz, (1999); Allen *et al*., (1998); Majeed, BadMaev, Shivkumar and Rajendan, (1995) and Apisariykul *et al*., (1995) who investigated the infestation of guinea pig with dermatophytes and pathogenic mold or yeast, the result obtained showed inhibition of the fungi by the extracts.

The aqueous extract of *C.longa* at 25 mg/ml^{-1} (lowest concentration) exhibited no inhibition against C. *tropicalis, A. faecalis* and *M.pudica*. This may be attributed to the virulence factors demonstrated by this organism like proteinase secretion, the formation of biofilm and characteristic dimorphism. The antimicrobial result in this study is also in consonance with Cowan, (1999) and Draughon, (2004) who studied the antibacterial activity of extracts of *E.serrata* and the result showed the antibacterial activity may be due to the presence of alkaloids, flavonoids and tannins. Similar to observation by Duada *et al*., (2014) and the result revealed that methanol and chloroform extract of *E. serrata* showed antibacterial activity. Also similar to observation of Gandhiraya *et al*., (2009) and they observed that the methanol extract of *M. pudica* leaves exhibited antimicrobial activity against tested micro-organisms (*Aspergillus fumigatus*, *Citrobacter divergens* and *Klebsiella pneumoniae*). In line with this Kokane *et al*., (2009) observed that the methanol extracts of *M. pudica* root showed good wound healing property attributed to its phenolic constituents (tannins). In line with this also, Gandhiraja *et al.*, (2009), observed that methanol and aqueous extracts of *M. pudica* exhibited antifungal activity. Also this result is in consonance with Pawaskar and Kale, (2006) who investigated the antimicrobial activity of *M. pudica* whole plant against some bacterial strains, and the result

showed that the plant can be used to treat infections caused by the microorganisms. Ethanol and methanol extracts demonstrated high inhibitory potentials than aqueous extracts. This may be due to the fact that the solvent did not extract the bioactive constituent of the plant, which may be hydrophilic in nature or contains hydrophilic compounds.

E. serrata, M. pudica and *C. longa* possessed high radical scavenging property. The result of the ethanol extracts of these plants *E. serrata, M. pudica* and *C. longa* showed that the DPPH scavenging activities was concentration dependent with increasing concentration of extracts. *C.longa* was more effective at scavenging DPPH radicals when compared to *E. serrata and M.* pudica as can be seen in their IC_{50} values (15.62 µg/ml, 19.64 µg/ml and 28.74 µg/ml) for *C. longa, M. pudica and E. serrata* respectively for ethanol extracts. The extracts favorably compared to ascorbic acid, a standard free radical scavenger with IC_{50} value of 15.62 µg/ml.Ascorbic acid and C. longa scavenged the DPPH radicals at 80ug/ml of extracts. The DPPH radical scavenging abilities of ethanolic extracts of *E. serrata, M. pudica* and *C.longa* were comparable to ascorbic acid. DPPH radical scavenging abilities of the extracts followed a Sigmoid model except DPPH methanol of *E. serrata* that followed Welbull model. Methanolic extracts of *E. serrata* was more effective at scavenging DPPH radicals when compared to methanolic extracts of *M. pudica* and *C. longa* as can be seen in their IC_{50} values (2.92 µg/ml, 16.10 µg/ml and 12.50 µg/ml) for *E.serrata, M. pudica* and *C.longa* respectively. The methanolic extracts exhibited high DPPH radical scavenging abilities compared to ascorbic acid; with inhibitory concentration (IC_{50}) value of 15.62µg/ml. *E. serrata, M. pudica* and *C. longa* DPPH scavenging abilities were comparable to ascorbic acid. Reduction of DPPH may be related to the high scavenging property by that particular sample (Molyneux, 2004). Both ethanolic and methanolic extracts of *E. serrata, M. pudica* and *C. longa* DPPH scavenging abilities were indicative of a high antioxidant activity which may be attributed to the cumulative and synergistic activities of these plants phytochemicals; these plants are rich in phenolic compounds (flavonoids, tannins) as seen in their quantitative screening. This result is in line with Shruthi *et al.,* 2013; Narayana *et a.,l* 2001 and Alisi *et al*., 2018. From this result, it is suggested that these plant extracts have components within, which are capable of scavenging free radicals through electron donation; therefore the extracts have the potential to prevent the effects of free radicals in biological systems. Methanol extracts gave better DPPH scavenging abilities than ethanol extracts, this could be that methanol as a solvent extracted more phytochemicals than ethanol.

The present study showed that ethanolic and methanolic extracts of *E. serrata, M. pudica* and *C.* longa have the potent nitric oxide scavenging activities. These extracts abilities to scavenge nitric oxide generated in vitro by sodium nitroprusside followed a sigmoid model for both ethanol and methanol extracts except ethanol extract of *C.longa* that followed a logistic dose response model. Nitric oxide radical scavenging for ethanol and methanol extracts was concentration dependent. Ethanol extracts of *E. serrata* was the best nitric oxide radical scavenger with inhibitory concentration of 22.67 ug/ml compared to other extracts. *E. serrata, M. pudica* and *C. longa* extracts nitric oxide scavenging abilities at lower concentrations was not compared to ascorbic acid but at higher concentrations/ doses their marked abilities to scavenge nitric oxide was comparable to ascorbic acid. Methanol extract of *C. longa* exerted better nitric oxide radical scavenging ability with inhibitory concentration (IC_{50}) value of 15.36 µg/ml, than the standard antioxidant (ascorbic acid) with inhibitory concentration (IC_{50}) value of 15.46µg/ml. Ethanolic and methanolic extracts of *E. serrata, M. pudica* and *C.longa* nitric oxide scavenging abilities in this study may be attributed partly to its secondary metabolites like flavonoids ,tannins, saponnins etc contents, which are rich in these plants extracts. This result is in consonance with studies carried out by Alisi and Onyeze (2008), Jagetia *et al.*, (2004), and Madson *et al*., (2000). The result highlight the importance of these extracts in preventing cell damage caused by NO and O_2.

The present study showed that *E. serrata, M. pudica* and *C. longa* ethanolic and methanolic extracts scavenged H_2O_2 effectively compared to ascorbic acid. The ethanolic and methanolic extracts abilities to scavenge H_2O_2 followed a sigmoid model. Inhibitory concentrations (IC_{50}) of values of ethanolic extracts were 15.31µg/ml, 15.26µg/ml and 15.26 µg/ml for *E. serrata, M. pudica* and *C longa* respectively. Methanolic extracts of the plants inhibitory concentration (IC_{50}) values were 15.48 µg/ml, 24.17µg/ml and 35.03 µg/ml for *E. serrata, M. pudica* and *C.longa* respectively. Both ethanolic and methanolic extracts scavenged H_2O_2, and this may be due to the presence of phenol and tannins, thereby neutralizing H_2O_2 to water. Tannins and phenols were qualitatively and quantitatively present in these plants.

The antioxidant properties of *E.serrata, M.pudica* and *C.longa* were affirmed by its strong reducing power. The reducing powers ($RP0.5_{AU}$) of the ethanolic extracts were 15.01µg/ml,10.01µg/ml and 9.38µg/ml for *E.serrata, M.pudica* and *C.longa* respectively. Reducing power ($RP0.5_{AU}$) of the methanolic extracts of *E. serrata, M. pudica* and *C. longa* were 34.09 µg/ml, 21.77 µg/m l and 21.63µg/ml respectively. The extracts possessed high reducing power which is indication of the hydrogen donating ability of the bioactive compounds present in the plant extracts.

Toxicity is an expression of being poisonous, which indicates the state of adverse effects caused by the interaction between the cells and toxicants. In the evaluation and assessment of the toxicity of these medicinal plants, determination of the acute oral toxicity in albino rats is usually an initial step (LD_{50}).

The LD_{50} of the extracts was found to be 3808 mg/kg.bw. *E. serrata* belongs to a family Euphorbiaceae that is known to be toxic. This is in consonance with the study carried out by Rant, Ajmal, Uddin and Roothullah, (2013) who investigated the cytotoxic effect of aerial part of *E. milli* and *E. pulcherima*. The result revealed that the crude alcoholic and ethyl acetate extracts showed excellent cytotoxic activity. The toxicity of *M. pudica* may be because of the mimosine constituent of the plant. Mimosine is a toxic amino acid found mainly in *M. pudica*. Mimosine and its metabolites 3-hydroxy-4- (14)- Pyridone (DHP), are toxic. This may be the reason for *M. pudica* toxicity. This result is in line with the findings of Mauldin and Peter-Kennedy, (2016), who reported that mimosa and its metabolites are toxic. The result of acute oral toxicity of *C. longa* in this study is consonance with the findings of Shanker, Shantha, Ramesh and Murthy, (1980) who reported the safety profile of *C. longa* extracts in animal studies, although susceptibility to hepatotoxicity after ingesting large amounts of *C. longa* extracts have been found in some animal species. This result is also in line with the study carried out by Ireson, Jones, Orr, Coughtrie, Boocock, Williams, Farmer, Steward, Geschen, (2002). They investigated curcumin metabolism as a cancer chemo-preventive agent in human and rat intestine. The result revealed that, there is a difference in curcumin metabolism between humans and rats, a species susceptible to hepatotoxicity with high doses. In this study *E serrata, M. pudica and C. longa* extract at a dose of 5000mg/kg had adverse effect on the tested mice. Result from the acute

toxicity study will help in dose determination in animal studies; help to determine LD_{50} values that provide many indices of potential types of drug mechanism.

The effect of the ethanol extracts of *E. serrata*, *M. pudica* and *C. longa* on the body weight serves as a sensitive indication of the general health status of animals. However, weight gains were observed in all animals administered with 200 mg/kg, 400 mg/kg of *E. serrata, M. pudica* and *C. longa* in weekly weights of Albino rats for 28 days. However there was decrease in weekly weights of treated groups of albino rats when compared to control groups. It can be stated that *E. serrata, M.pudica* and *C. longa* extract had effects on the normal metabolism of animals as corroborated by the significant difference from animals in the control group.The treated groups did not show any significant change in water or food consumption. The weekly gain in body weight in treated groups could be as a result of increment in food and water intake. Loss of appetite is often synonymous with weight loss due to disturbance in the metabolism of protein, fat or carbohydrate. Therefore, the increment in weights observed in treated groups is as a result of the normal food and water intake. This result in this study is in consonance with the result of Duke, (1997); Ezeonwumelu, Julius,, Muhoho, Ajayi, Oyewale and Tanayen, (2011); Kwan, Ibrahim,Yeng, Chen, Subramaniam and Sreenivasan, (2013), who carried out investigations on the acute and sub chronic toxicity study of *Euphorbia hirta* L. methanol extracts in rats. They observed that increase in food and water intake is responsible for the increase in body weight gain. Also the observed increase in weekly body weight of the treated groups could be attributed the nutritional value of *E. serrata*, *M. pudica* and *C. longa* extracts.

The result of the effect of ethanol extract of *E.serrata* and *M. pudica* on liver and renal parameters in albino rats showed that there were significant differences in the liver and renal parameters of the rats compared to control group while *C. longa* effect on liver and renal parameters in albino rats showed that there was no significant differences in the liver and renal function of the rats compared to control group. Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), urea and creatinine activities significantly increased ($P<0.05$) in a dose-dependent fashion when compared to control group. Total protein (TP) activity was higher in control group than in treated groups. The rats that received 200 mg/kg and 400 mg/kg doses had a higher albumin (ALB) and Aspartate aminotransferase activities compared to control group. In

evaluating the toxicity of drugs and plant extracts the assessment of the liver and kidney is a very important index, as both are necessary for the survival of the organism.

Liver and renal kidney parameter indices evaluated in this study were TP, ALB, ALT, AST, ALP, urea and creatinine. The significant increase in the liver and renal function parameters of treated groups with *E. serrata*, is in consonance with findings of Tarkang, Agbor, Arnelle, Yamihe, David and Ngadena, (2012), who carried out an investigation on the acute and chronic toxicity of the aqueous and ethanol leaf extracts of *Carica papaya* Linn in Wister rats. They also observed a dose-dependent increase in AST and suggested that sub-acute administration of *Carica papaya* extracts caused hepatocellular damage. The significant increase in AST with *E. serrata, M. pudica* and *C. longa* suggested that administration of higher doses of this extract may induce the destruction of the liver cells or this may be due to muscle inflammation. Increase in ALT could be attributed to cellular necrosis, suggestive of liver injury. This result is in line with Brautbar and Williams, (2002) who reported elevation in liver enzymes (ALT, AST, ALP) activity in liver diseases or hepatotoxicity. The elevation of ALT could also be attributed to the administration of the crude plants extracts. Also the kidney function indices evaluation for *E. serrata, M. pudica* and *C. longa* correlates with the findings of Muhammad, Hassan, Dangoggo, Hassan and Umar, (2011), who carried out an investigation on the acute and sub-chronic toxicity of kernel extract of *Sclerocanya birrea* in rats. Significant increase in urea and creatinine was observed when experimental rats received higher doses of the kernel extract of *Sclerocanyabirrea* ranging from 3000 to 4000 mg/kg body weight. During deamination, ammonia is removed from the blood by conversion into urea. The increase in urea may be due to the result of high glomerular filtration. Creatinine that is filtered in the glomerular filtrate passes on through the tubular system and is excreted in the urine, not reabsorbed. In this case, creatinine is not excreted in urine rather is reabsorbed.

The result of the effects of ethanol extracts of *E. serrata, M. pudica* and *C. longa* on liver and renal parameters in albino rats in this study is in consonance with animal study carried out by Deshpande *et al.*, (1998) and Park *et al*., (2000), the result demonstrated *C. longa* (tumeric's) hepato-protective effects from various hepatotoxic agents including carbon tetrachloride, acetaminophen (paracetamol) (Donatus *et al* ., 1990) and *Aspergillus* aflatoxin (Soni *et al*., 1992).

The effects of the ethanol extracts of *E. serrata*, *M. pudica* and *C. longa* on oxidative stress and cardiac parameters in albino showed increase in LDH with increase in concentration of these plants extracts. This increase in LDH may be attributed to the different bioactive components of these plants extracts; which may be indicative of some form of tissue damage.

In this study, the effect of C. longa on oxidative and cardiac parameters show that lactate hydrogenase activity was reduced at the dose of 200 mg/kg compared to control but increased with dose of 400 mg/kg compared to control. The result of the ethanol extracts of C. longa on oxidative stress and cardiac parameter in albino rats in this study is in line with Ramirez-Tortosa *et al*., (1999) and Mortellini *et al*., (2000) who evaluated *in vitro* study of the effect of *C. longa* (curcumin) on an inducible stress protein (endothelial heme oxygenase-1) using bovine aortic endothelial cells. The result showed enhanced cellular resistance to oxidative stress by the plant extract.

To determine the extent of the toxicity effect of *E. serrata*, *M. pudica* and *C.longa* extracts on the blood of the albino rats, the evaluation of the hematological parameters can be used and to explain blood related functions of these plant extract or their products. This analysis is very important for risk evaluation as changes in the hematopoietic system have higher predictive value for human toxicity when the data generated are translated from animal studies. The result of the effect of *E. serrata* hematological analysis showed that there was no significant difference on hematological profile in Albino rats. The concentrations of the hematological profile, hemoglobin (HB), white blood cell (WBC), Red blood cell (RBC), packed cell volume (PCV) and platelets, were higher in the control group compared to treated groups could be as a result of the phytochemical constituents of the extracts. White blood cell concentration was higher in 200 mg/kg dose than in 400 mg/kg dose in treated group with *E.serrata* but highest in control group. The result of the ethanol extract of *M. pudica* on hematology profile in albino rats showed that there was no significant difference on hematological profile in albino rats. It was found that the concentrations of the hemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), platelets and packed cell volume (PCV) significantly decreased ($P>0.05$) with increase in dosage. This could be as a result of the different secondary metabolites in the crude plant extracts. The concentration of the parameters was high in control group except in hemoglobin (HB). Result

also showed that white blood cell concentrations was higher in 200 mg/kg dose than in 400 mg/kg dose in treated group with *M. pudica,* but highest in control group. Effect of ethanol extract of C. longa on hematological profile in Albino rats showed that there was no significant difference on the hematological profile. Also the concentrations of WBC, RBC, PCV and Platelets significantly increased ($P<0.05$) in a dose dependent fashion while the concentration of Hb significantly decreased ($P>0.05$) with increase dosage of the extract. The concentrations of white blood cell was higher in 200 mg/kg treated group than in 400 mg/kg treated group and also when compared to the control. The concentration of white blood cells was high in *E.serrata, M. pudica* and *C. longa* when compared to other parameters. Result of sub-chronic oral administration of the ethanol extract of *E.serrata, M. pudica* and *C. longa* on treated and control groups, showed no significant effect ($P>0.05$) in the hematological profile of rats suggesting that *E. serrata, M. pudica* and *C. longa* may not be toxic to the blood system. Significant decrease ($P>0.05$) in hematological profile as dose of extracts increased from 200mg/kg to 400mg/kg may be toxic to the blood system for *E. serrata* and *M. pudica* compared to control, except in C. longa where dose of 200 mg/kg had significant increase in the hematological profile, but significant decrease at 400 mg/kg dose of *C. longa* ethanol extract compared to control group. These increase and decrease in the hematological parameters may be as a result of different bioactive components of the plant extracts working in synergism or antagonism in the hematopoietic system.

The liver, Kidney and heart did not show any significant difference in weight. These insignificant increase and decrease in organ weight could have been as a result of a partial change in size of internal organs. This result is in line with Chunlarattharaphorn, Lertprasertsuke, Ngamjariyawat, Suwanlikhid and Jaijoy, (2007). It may also be as a result of toxicity induced by the extracts.

The microscopic examination of the liver, kidney and heart of rats treated with various doses of *E. serrata,* and *C. longa* extract showed no changes in colour compared to control group rat organs, but the organs of rats treated with various doses of *M. pudica* Group 3 showed changes in colour compared with control. The histological sections of the liver, kidney and heart of experimental rats administered with doses of 200 and 400 mg/kg body weight *E. serrata* and *C.longa* extracts and that of the control group showed that of a normal laboratory animal

The first hand indication of toxicity of chemical or biological substances is the hypertrophy of the organs. No hypertrophy of organs was observed in organs of rats treated with *E. serrata* and *C. longa* except *M. pudica*. The histopathological examination of the kidney and heart in experimental rats that were administered with 200 mg/kg body weight of the ethanol extracts of *M. pudica* revealed mild inflammation and necrosis of the kidney and heart sections respectively. Also the examination of the liver and heart in the experimental rats, that were administered with 400kg/mg body weight of the ethanol extracts of *M. pudica* revealed mild inflammation of inflammatory cells into the periportal areas of the liver and into the highly vascularized myocardial connective tissue matrix of the heart. This result is in consonance with Adedapo, Abatan, Akinloye, Idowu and Olorunsugo, (2003), who reported lymphocytic infiltration in organs to be attributed to the presence of glycosides. The result of this study is consistent with the findings of Builders, Isichie and Aguyi, (2012), who investigated the toxicity of *Parkia biglobosa* stem bark extract in rats and reported that the toxicity of some of the herbal medications might be a result of phytochemical constituents. Also Muhammad *et al*., (2011) reported that large tannin intake may cause kidney and liver damage.

Hepatotoxic chemicals/substances can cause necrosis which occur within distinct zones in the liver, either distributed massively or occur diffusely. Zonal necrosis which is confined to specific zones of the hepatic acinus is produced by many chemicals (Roberts, James and Franklin, 2003). In this study significant increase in serum Aspartate aminotransferase (AST) and Alkaline phosphate (ALP) may be due to hepatic necrosis. However, the liver has the ability to regenerate itself and this makes it able to withstand moderate zonal or diffuse necrosis. Over a period of several days, necrotic cells are removed and replaced with new cells and normal hepatic architecture and function are restored (Robert *et al*., 2003).

CHAPTER V: CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The result of this study has shown that the three plants extract contained phytochemicals in variable amounts. The qualitative analysis results demonstrated that, most of them are either high or moderate in alkaloids, saponnins, cardiac glycosides, phytates, phenols, oxalates, steroids, flavonoids and tannins. The three solvent (aqueous, ethanolic and methanolic) extracts demonstrated different levels of phytochemical extracted. The result have shown the presence of different levels of alkaloids, cardiac glucosides, phytate, oxalate and flavonoids etc in various extracts using three solvents. This study has shown that the percentage quantitative constituents of each phytochemical extracted from *E. serrata, M.pudica and C. longa,* is high.

Results obtained from nucleotide sequence analyses in this study; identified the microbes as *Wohlfahrtiimonas chitiniclastica, Proteus mirabilis, Alcaligenes faecalis, Klebsiella pneumoniae, Candida tropicalis, and Wickerhamomyces anomalus.* This identification has given an appropriate identity of microbes used in this study to specie level for the determination of antibacterial and antifungal activities of these extracts.

This study has shown that crude extracts of E. *serrata, M. pudica* and *C.longa* possess reasonable activity against *Wohlfahrtiimonas chitiniclastica, Proteus mirabilis, Alcaligenes faecalis, Klebsiella pneumoniae,* and have high potential as antibacterial agents. Also these findings provide an insight into the usage of these plants in traditional medicine for treatment of infections caused by these bacteria and fungi. This study has also shown that crude extracts of E. *serrata, M. pudica* and *C.longa* possess reasonable activity against *Candida tropicalis, and Wickerhamomyces anomalus* and have high potential as antifungal agents.

The extracts have a significant antioxidant scavenging activities. Scavenging of DPPH, nitric oxide, H_2O_2 radicals and reducing power ability of the plants extract were concentration dependent.The findings suggest that these plants could be a potential source of natural antioxidant preventing and slowing degenerative diseases.

Mortality from the extracts at a single dose of 5000 mg/kg body weight, as revealed in acute toxicity in this study implies that the extracts could be toxic at elevated or high doses. Sub-

chronic oral toxicity of the extracts 200 mg/kg and 400 mg/kg body weight revealed non-significant difference ($p > 0.05$) on the biochemical parameters (ALT, AST, ALP, LDH, SOD, GSH, TP, GPx, CAT, ALB, Urea and Creatinine).

Haematology parameters (HB, WBC, RBC, PVC) values when compared with the control, showed significant difference ($p < 0.05$). Similar results were also revealed for body weight when compared with control.These findings also revealed that extracts exhibit low chronic toxicity to experimental animals, implying minimal deleterious effect following use.

Examination of the liver, kidney and heart showed no significant morphological changes except with *M. pudica,* where observed pathological changes include fatty degeneration and inflammatory cells (lymphoctyes and macrophages) irrespective of extract dosage, the finding in this study; implies usage with caution.

5.2 RECOMMENDATIONS

These plants are locally sourced since they grow well in Nigeria. However, with caution and dose establishment the extracts of E. *serrata, M. pudica* and *C.longa* can be used in the treatment of ailments caused by *Wohlfahrtiimonas chitiniclastica, Proteus mirabilis, Alcaligenes faecalis, Klebsiella pneumoniae, Candida tropicalis, and Wickerhamomyces anomalus.*

The extracts have good antioxidant activities. Scavenging of DPPH, nitric oxide, H_2O_2 radicals and reducing power ability of the plants extract are comparable to the standard and some are even better than ascorbic acid – the standard antioxidant. This implies that the extracts can be used for treatment of generalized conditions caused by free radicals in the body.

They can be used as inclusions in some health formulations or purified as alternative antimicrobials and antioxidant therapies.

The crude extracts of these plants should be taken with caution, as high dosage can have adverse effects.

There is need to determine the effects of these plants extracts on pregnant animal, their reproductivity to complete the safety profile of these medicinal plants.

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APPENDICES

APPENDIX 1

Quantitative phytochemical constituents of *Mimosa pudica*, *Curcum longa*, *Euphorbia serrata*.

Constituent/ component	*Euphorbia serrata*	*Mimosa pudica*	*Curcum longa*
Tannins %	35.20	24.00	24.20
Saponins %	5.77	6.20	7.26
Alkaloids %	6.85	2.97	5.28
Flavonoids %	9.83	53.80	13.18
Cardiac Glycosides %	5.31	3.22	3.30
Phytate %	2.77	5.52	3.70
Oxalate (µg/l)	5.40	4.86	2.43

APPENDIX 2

DPPH FOR ASCORBIC ACID

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9956	0.9911	0.9823	5.4203

	Coefficient	Std. Error	t	P	VIF
a	93.0603	3.1308	29.7242	0.0011	1.2423
b	2.5179	9.1634	0.2748	0.8093	191.3112<
x0	15.6191	15.9631	0.9785	0.4310	191.2202<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	32246.5537	10748.8512
Residual	2	58.7597	29.3799
Total	5	32305.3134	6461.0627

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	6565.0425	3282.5213	111.7269	0.0089
Residual	2	58.7597	29.3799		
Total	4	6623.8023	1655.9506		

Statistical Tests:

PRESS	86404264.3678
Durbin-Watson Statistic	1.7823 Passed
Normality Test	Passed (P = 0.7531)

K-S Statistic = 0.2805 Significance Level = 0.7531

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9908

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.1879	-0.1879	-0.0347	-1.3728	-4.0433<
3	79.1640	0.0060	0.0011	1.3730	4.0486<
4	93.0545	-6.0745	-1.1207	-1.3726	-4.0313<
5	93.0603	1.7297	0.3191	0.3909	0.2876
6	93.0603	4.3397	0.8006	0.9808	0.9626

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	984.8969<	0.9994	-160.0914
3	979323.6959<	1.0000	5054.4284<
4	0.3141	0.3334	-2.8509
5	0.0255	0.3336	0.2035
6	0.1605	0.3336	0.6811

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.1879	-23.1264	23.5022	-32.7888	33.1646
3	79.1640	55.8423	102.4858	46.1821	112.1460
4	93.0545	79.5886	106.5204	66.1243	119.9847
5	93.0603	79.5896	106.5310	66.1277	119.9929
6	93.0603	79.5896	106.5310	66.1277	119.9929

Fit Equation Description:

```
[Variables]
x = col(10)
y = col(11)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 93.0603}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 2.51791}}
x0 = x50(x,y,.5) "Auto {{previous: 15.6191}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
```

fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 11

DPPH FOR *C.longa* (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Notebook1
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9956	0.9911	0.9823	5.4203

	Coefficient	Std. Error	t	P	VIF
a	93.0603	3.1308	29.7242	0.0011	1.2423
b	2.5179	9.1634	0.2748	0.8093	191.3112<
x0	15.6191	15.9631	0.9785	0.4310	191.2202<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	32246.5537	10748.8512
Residual	2	58.7597	29.3799
Total	5	32305.3134	6461.0627

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	6565.0425	3282.5213	111.7269	0.0089
Residual	2	58.7597	29.3799		
Total	4	6623.8023	1655.9506		

Statistical Tests:

PRESS 86404264.3678

Durbin-Watson Statistic 1.7823 Passed

Normality Test Passed (P = 0.7531)

K-S Statistic = 0.2805 Significance Level = 0.7531

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9908

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	0.1879	-0.1879	-0.0347	-1.3728	-4.0433<
2	79.1640	0.0060	0.0011	1.3730	4.0486<
3	93.0545	-6.0745	-1.1207	-1.3726	-4.0313<
4	93.0603	1.7297	0.3191	0.3909	0.2876
5	93.0603	4.3397	0.8006	0.9808	0.9626

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	984.8969<	0.9994	-160.0914
2	979323.6959<	1.0000	5054.4284<
3	0.3141	0.3334	-2.8509
4	0.0255	0.3336	0.2035
5	0.1605	0.3336	0.6811

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
1	0.1879	-23.1264	23.5022	-32.7888	33.1646
2	79.1640	55.8423	102.4858	46.1821	112.1460
3	93.0545	79.5886	106.5204	66.1243	119.9847
4	93.0603	79.5896	106.5310	66.1277	119.9929
5	93.0603	79.5896	106.5310	66.1277	119.9929

Fit Equation Description:

```
[Variables]
x = col(3)
y = col(4)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
```

b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 93.0603}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 2.51791}}
x0 = x50(x,y,.5) "Auto {{previous: 15.6191}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 11

DPPH FOR E.serrata (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Notebook1
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9792	0.9589	0.9178	8.3031

	Coefficient	Std. Error	t	P	VIF
a	72.9355	9.7648	7.4692	0.0175	3.2910
b	13.9613	5.8949	2.3684	0.1414	1.9036
x0	28.7487	7.1590	4.0158	0.0568	2.3394

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	12657.6696	4219.2232
Residual	2	137.8820	68.9410
Total	5	12795.5516	2559.1103

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	3217.2880	1608.6440	23.3336	0.0411
Residual	2	137.8820	68.9410		
Total	4	3355.1701	838.7925		

Statistical Tests:

PRESS 839.3031

Durbin-Watson Statistic 2.5498 Failed

Normality Test Passed (P = 0.7806)

K-S Statistic = 0.2733 Significance Level = 0.7806

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.8966

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	8.2511	-8.2511	-0.9937	-1.4124	-19.9476<
2	25.4014	6.8986	0.8308	1.3215	2.6239<
3	50.4154	-2.7154	-0.3270	-0.6195	-0.4873
4	65.9079	-2.6079	-0.3141	-0.4076	-0.3010
5	71.1252	2.8348	0.3414	0.7008	0.5705

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
1	0.6784	0.5050	-20.1480
2	0.8905	0.6047	3.2454<
3	0.3311	0.7213	-0.7840
4	0.0379	0.4063	-0.2490
5	0.5259	0.7626	1.0226

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
1	8.2511	-17.1365	33.6386	-35.5761	52.0783
2	25.4014	-2.3797	53.1825	-19.8544	70.6572
3	50.4154	20.0736	80.7571	3.5441	97.2867
4	65.9079	43.1353	88.6805	23.5419	108.2740
5	71.1252	39.9267	102.3237	23.6948	118.5555

Fit Equation Description:
[Variables]

x = col(3)
y = col(5)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 72.9355}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 13.9613}}
x0 = x50(x,y,.5) "Auto {{previous: 28.7487}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 11

DPPH FOR *M.pudica* (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Notebook1
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9657	0.9325	0.8651	13.5309

	Coefficient	Std. Error	t	P	VIF
a	86.7059	10.4090	8.3299	0.0141	1.7893
b	9.8697	5.9361	1.6627	0.2383	1.3602
x0	19.6430	6.3231	3.1065	0.0899	1.4145

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	22731.3944	7577.1315
Residual	2	366.1725	183.0863
Total	5	23097.5669	4619.5134

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	5062.2598	2531.1299	13.8248	0.0675
Residual	2	366.1725	183.0863		
Total	4	5428.4323	1357.1081		

Statistical Tests:

PRESS 3822.2600

Durbin-Watson Statistic 2.7147 Failed

Normality Test Passed (P = 0.8132)

K-S Statistic = 0.2645 Significance Level = 0.8132

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.8166

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	10.4248	-10.4248	-0.7704	-1.4142	-135.2191<
2	44.1368	8.9932	0.6646	1.4083	10.9421<
3	76.9265	-11.7265	-0.8666	-1.3340	-2.8413<
4	85.2770	0.9230	0.0682	0.0879	0.0623
5	86.5148	6.1852	0.4571	0.6768	0.5450

Influence Diagnostics:

Row	Cook's Dist		Leverage	DFFITS
1	1.5794	0.7032	-208.1311	
2	2.3074	0.7773	20.4417<	
3	0.8123	0.5779	-3.3248	
4	0.0017	0.3978	0.0506	
5	0.1820	0.5438	0.5950	

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
1	10.4248	-38.3955	59.2452	-65.5546	86.4042
2	44.1368	-7.1911	95.4648	-33.4776	121.7513

3	76.9265	32.6668	121.1862	3.7940	150.0590
4	85.2770	48.5573	121.9967	16.4454	154.1085
5	86.5148	43.5837	129.4460	14.1786	158.8510

Fit Equation Description:
[Variables]
x = col(3)
y = col(6)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 86.7059}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 9.86971}}
x0 = x50(x,y,.5) "Auto {{previous: 19.643}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 33

DPPH FOR *C.longa* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9504	0.9032	0.8064	16.9391

	Coefficient	Std. Error	t	P	VIF

a	96.9734	19.3165	5.0203	0.0375	3.2710
b	14.4661	9.4665	1.5281	0.2661	1.9163
x0	25.4976	11.0500	2.3075	0.1474	2.2721

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	23654.1023	7884.7008
Residual	2	573.8658	286.9329
Total	5	24227.9681	4845.5936

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	5354.0623	2677.0312	9.3298	0.0968
Residual	2	573.8658	286.9329		
Total	4	5927.9281	1481.9820		

Statistical Tests:

PRESS 4007.9147

Durbin-Watson Statistic 3.0426 Failed

Normality Test Passed (P = 0.9405)

K-S Statistic = 0.2208 Significance Level = 0.9405

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.7376

The power of the performed test (0.7376) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	14.2035	-14.2035	-0.8385	-1.3480	-3.1519<
3	39.3827	15.6173	0.9220	1.4119	17.5977<
4	70.9411	-10.9411	-0.6459	-1.1528	-1.4073
5	88.7967	1.2933	0.0763	0.0977	0.0693
6	94.7832	2.6168	0.1545	0.3017	0.2184

Influence Diagnostics:

Row	Cook's Dist		Leverage	DFFITS
2	0.9597	0.6131	-3.9675	

3	0.8940	0.5736	20.4112<
4	0.9682	0.6861	-2.0805
5	0.0020	0.3894	0.0553
6	0.0854	0.7378	0.3664

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	14.2035	-42.8631	71.2700	-78.3629	106.7698
3	39.3827	-15.8171	94.5825	-52.0446	130.8101
4	70.9411	10.5722	131.3101	-23.6968	165.5791
5	88.7967	43.3172	134.2762	2.8879	174.7055
6	94.7832	32.1781	157.3884	-1.2966	190.8631

Fit Equation Description:
[Variables]
x = col(10)
y = col(14)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 96.9734}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 14.4661}}
x0 = x50(x,y,.5) "Auto {{previous: 25.4976}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 14

DPPH FOR *E.serrata* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Weibull, 4 Parameter
f= if(x<=x0-b*ln(2)^(1/c), 0, a*(1-exp(-(abs(x-x0+b*ln(2)^(1/c))/b)^c)))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
1.0000	0.9999	0.9997	0.7667

	Coefficient	Std. Error	t	P	VIF
a	96.9714	0.5486	176.7620	0.0036	2.0383
b	4.2282	111.3382	0.0380	0.9758	6359781.1543<
c	0.9936	16.8818	0.0589	0.9626	381651.2579<
x0	2.9238	95.2976	0.0307	0.9805	9725515.3849<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	4	37438.9623	9359.7406
Residual	1	0.5879	0.5879
Total	5	37439.5502	7487.9100

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	7488.2583	2496.0861	4246.0633	0.0113
Residual	1	0.5879	0.5879		
Total	4	7488.8461	1872.2115		

Statistical Tests:

PRESS 1.4041E+012

Durbin-Watson Statistic 1.8258 Passed

Normality Test Passed (P = 0.6752)

K-S Statistic = 0.3000 Significance Level = 0.6752

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	3.4502E-005		-3.4502E-005	-4.4999E-005	-1.0426 (+inf)<
3	96.0744	0.0156	0.0204	177.5730<	(+inf)<
4	96.9628	-0.6128	-0.7992	-48.9283<	(+inf)<

5	96.9713	0.1687	0.2200	0.3076	0.0000
6	96.9714	0.4286	0.5590	0.7999	0.0000

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	145909334.3506<	1.0000	(+inf)<
3	596981365498.4307<	1.0000	(+inf)<
4	2242599.4396<	0.9997	(+inf)<
5	0.0226	0.4887	0.0000
6	0.1675	0.5116	0.0000

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	3.4502E-005	-9.7421	9.7421	-13.7774	13.7774
3	96.0744	86.3323	105.8165	82.2970	109.8518
4	96.9628	87.2220	106.7036	83.1863	110.7392
5	96.9713	90.1611	103.7815	85.0849	108.8578
6	96.9714	90.0033	103.9396	84.9938	108.9490

Fit Equation Description:

```
[Variables]
x = col(10)
y = col(12)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y) "Auto {{previous: 96.9714}}
b = xwtr(x,y,.5) "Auto {{previous: 4.22823}}
c = 1 "Auto {{previous: 0.993565}}
x0 = x50(x,y,.5) "Auto {{previous: 2.92383}}
[Equation]
f= if(x<=x0-b*ln(2)^(1/c), 0, a*(1-exp(-(abs(x-x0+b*ln(2)^(1/c))/b)^c)))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
b>0
c>0
[Options]
tolerance=1e-10
stepsize=1
iterations=200
```

Number of Iterations Performed = 28

DPPH FOR *M.pudica* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9922	0.9845	0.9691	4.9897

	Coefficient	Std. Error	t	P	VIF
a	65.0232	2.8902	22.4977	0.0020	1.2042
b	3.2536	5.7953	0.5614	0.6310	25.2008<
x0	16.0979	7.0239	2.2919	0.1490	25.1039<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	15175.2063	5058.4021
Residual	2	49.7937	24.8969
Total	5	15225.0000	3045.0000

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	3170.2063	1585.1031	63.6668	0.0155
Residual	2	49.7937	24.8969		
Total	4	3220.0000	805.0000		

Statistical Tests:

PRESS 1083368.0297

Durbin-Watson Statistic 1.5062 Passed

Normality Test Passed (P = 0.6916)

K-S Statistic = 0.2959 Significance Level = 0.6916

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9752

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.4584	-0.4584	-0.0919	-1.2265	-1.7422
3	49.9639	0.0361	0.0072	1.2259	1.7386
4	64.9813	-4.9813	-0.9983	-1.2239	-1.7273
5	65.0231	-0.0231	-0.0046	-0.0057	-0.0040
6	65.0232	4.9768	0.9974	1.2236	1.7255

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	88.8835<	0.9944	-23.1944
3	14413.3539<	1.0000	294.9127<
4	0.2511	0.3346	-1.2250
5	5.4464E-006	0.3355	-0.0029
6	0.2520	0.3355	1.2261

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.4584	-20.9502	21.8669	-29.8605	30.7773
3	49.9639	28.4955	71.4324	19.6027	80.3252
4	64.9813	52.5620	77.4007	40.1791	89.7836
5	65.0231	52.5882	77.4581	40.2131	89.8332
6	65.0232	52.5876	77.4588	40.2128	89.8336

Fit Equation Description:

```
[Variables]
x = col(10)
y = col(13)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 65.0232}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 3.2536}}
x0 = x50(x,y,.5) "Auto {{previous: 16.0979}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
```

[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10

Nitric Oxide for Ascorbic Acid

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9990	0.9979	0.9958	2.4622

	Coefficient	Std. Error	t	P	VIF
a	86.5000	1.4216	60.8483	0.0003	1.2923
b	1.6897	38.1457	0.0443	0.9687	16133.6648<
x0	15.4553	102.6013	0.1506	0.8941	16133.4665<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	29007.7501	9669.2500
Residual	2	12.1249	6.0625
Total	5	29019.8750	5803.9750

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	5819.7001	2909.8500	479.9786	0.0021
Residual	2	12.1249	6.0625		
Total	4	5831.8250	1457.9563		

Statistical Tests:

PRESS 34560572233.9591

Durbin-Watson Statistic 1.4691 Failed

Normality Test Passed (P = 0.6751)

K-S Statistic = 0.3000 Significance Level = 0.6751

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9996

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.0092	-0.0092	-0.0037	-0.9928	-0.9857
3	81.0000	3.3139E-005	1.3459E-005	1.0081	1.0163
4	86.5000	-2.0000	-0.8123	-0.9948	-0.9897
5	86.5000	-0.7500	-0.3046	-0.3731	-0.2735
6	86.5000	2.7500	1.1169	1.3679	3.8104<

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	23114.9328<	1.0000	-261.4527
3	1900227860.5651<	1.0000	76122.3382<
4	0.1649	0.3333	-0.6999
5	0.0232	0.3333	-0.1934
6	0.3119	0.3333	2.6944<

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.0092	-10.5847	10.6032	-14.9729	14.9914
3	81.0000	70.4060	91.5940	66.0178	95.9822
4	86.5000	80.3835	92.6165	74.2671	98.7329
5	86.5000	80.3835	92.6165	74.2671	98.7330
6	86.5000	80.3835	92.6165	74.2671	98.7330

Fit Equation Description:

```
[Variables]
x = col(24)
y = col(25)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 86.5}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 1.68967}}
x0 = x50(x,y,.5) "Auto {{previous: 15.4553}}
```

[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 15

Nitric Oxide for Ascorbic acid

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9990	0.9979	0.9958	2.4622

	Coefficient	Std. Error	t	P	VIF
a	86.5000	1.4216	60.8483	0.0003	1.2923
b	1.6897	38.1457	0.0443	0.9687	16133.6648<
x0	15.4553	102.6013	0.1506	0.8941	16133.4665<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	29007.7501	9669.2500
Residual	2	12.1249	6.0625
Total	5	29019.8750	5803.9750

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	5819.7001	2909.8500	479.9786	0.0021
Residual	2	12.1249	6.0625		
Total	4	5831.8250	1457.9563		

Statistical Tests:

PRESS 34560572233.9591

Durbin-Watson Statistic 1.4691 Failed

Normality Test Passed (P = 0.6751)

K-S Statistic = 0.3000 Significance Level = 0.6751

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9996

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.0092	-0.0092	-0.0037	-0.9928	-0.9857
3	81.0000	3.3139E-005	1.3459E-005	1.0081	1.0163
4	86.5000	-2.0000	-0.8123	-0.9948	-0.9897
5	86.5000	-0.7500	-0.3046	-0.3731	-0.2735
6	86.5000	2.7500	1.1169	1.3679	3.8104<

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	23114.9328<	1.0000	-261.4527
3	1900227860.5651<	1.0000	76122.3382<
4	0.1649	0.3333	-0.6999
5	0.0232	0.3333	-0.1934
6	0.3119	0.3333	2.6944<

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.0092	-10.5847	10.6032	-14.9729	14.9914
3	81.0000	70.4060	91.5940	66.0178	95.9822
4	86.5000	80.3835	92.6165	74.2671	98.7329
5	86.5000	80.3835	92.6165	74.2671	98.7330
6	86.5000	80.3835	92.6165	74.2671	98.7330

Fit Equation Description:

```
[Variables]
x = col(17)
y = col(18)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
```

sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 86.5}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 1.68967}}
x0 = x50(x,y,.5) "Auto {{previous: 15.4553}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 15

Nitric Oxide for *C. longa* (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Logistic, 4 Parameter
f=if(x<=0,if(b<0,y0,y0+a), if(b>0, y0+a/(1+abs(x/x0)^b),
y0+a*abs((x/x0))^(abs(b))/(1+(abs(x/x0))^(abs(b)))))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9978	0.9956	0.9822	2.0953

	Coefficient	Std. Error	t	P	VIF
a	43.8311	9.5833	4.5737	0.1370	44.7326<
b	-1.5447	0.8387	-1.8418	0.3167	16.8649<
x0	22.6664	6.9353	3.2683	0.1890	13.5333<
y0	0.0000	1.4816	0.0000	1.0000	1.0000

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	4	4108.1098	1027.0275
Residual	1	4.3902	4.3902

Total	5	4112.5000	822.5000

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	983.1098	327.7033	74.6448	0.0848
Residual	1	4.3902	4.3902		
Total	4	987.5000	246.8750		

Statistical Tests:

PRESS 554.5789

Durbin-Watson Statistic 3.2761 Failed

Normality Test Passed (P = 0.9298)

K-S Statistic = 0.2257 Significance Level = 0.9298

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9978

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.0000	0.0000	0.0000	0.0000	0.0000
3	19.8038	0.1962	0.0937	1.0000	(+inf)<
4	30.9570	-0.9570	-0.4567	-1.0000	(+inf)<
5	35.8594	1.6406	0.7830	1.0000	0.0000
6	38.3626	-0.8626	-0.4117	-1.0000	0.0000

Influence Diagnostics:

Row	Cook's Dist		Leverage	DFFITS
2	0.0000	0.5000	0.0000	
3	28.2496<	0.9912	(+inf)<	
4	0.9484	0.7914	(+inf)<	
5	0.1578	0.3869	0.0000	
6	1.2249	0.8305	0.0000	

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.0000	-18.8253	18.8253	-32.6064	32.6064
3	19.8038	-6.7022	46.3097	-17.7642	57.3717
4	30.9570	7.2732	54.6408	-4.6759	66.5899
5	35.8594	19.2999	52.4188	4.5065	67.2122
6	38.3626	14.1006	62.6246	2.3428	74.3825

Fit Equation Description:
[Variables]
x = col(17)
y = col(21)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y) "Auto {{previous: 43.8311}}
b = if(-xwtr(x,y,.5)/20<-2, -2, if(-xwtr(x,y,.5)/20>2, 2, -xwtr(x,y,.5)/20)) "Auto {{previous: -1.5447}}
x0 = x50(x,y,.5) "Auto {{previous: 22.6664}}
y0 = min(y) "Auto {{previous: 0}}
[Equation]
f=if(x<=0,if(b<0,y0,y0+a), if(b>0, y0+a/(1+abs(x/x0)^b),
y0+a*abs((x/x0))^(abs(b))/(1+(abs(x/x0))^(abs(b)))))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=.01
iterations=200

Number of Iterations Performed = 15

Nitric Oxide for *E.serrata* (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9931	0.9862	0.9725	4.7289

	Coefficient	Std. Error	t	P	VIF
a	86.0219	16.6997	5.1511	0.0357	15.7244<
b	18.5870	5.2054	3.5708	0.0703	4.0710<
x0	50.0181	9.6609	5.1774	0.0353	13.4293<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	9330.2752	3110.0917
Residual	2	44.7248	22.3624
Total	5	9375.0000	1875.0000

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	3205.2752	1602.6376	71.6667	0.0138
Residual	2	44.7248	22.3624		
Total	4	3250.0000	812.5000		

Statistical Tests:

PRESS 483.2665

Durbin-Watson Statistic 2.1182 Passed

Normality Test Passed (P = 0.9705)

K-S Statistic = 0.2033 Significance Level = 0.9705

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9796

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	5.4628	-5.4628	-1.1552	-1.4142	-255.6744<
3	14.2706	3.2294	0.6829	0.9877	0.9759
4	31.6925	0.8075	0.1708	0.2431	0.1745
5	54.2904	-1.7904	-0.3786	-0.6700	-0.5380
6	71.7281	0.7719	0.1632	0.7957	0.6806

Influence Diagnostics:

Row	Cook's Dist		Leverage	DFFITS
2	0.3324	0.3327	-180.5479	
3	0.3551	0.5220	1.0198	
4	0.0202	0.5066	0.1768	
5	0.3191	0.6807	-0.7856	
6	4.8044<	0.9579	3.2473<	

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U

2	5.4628	-6.2739	17.1996	-18.0264	28.9520
3	14.2706	-0.4295	28.9707	-10.8308	39.3721
4	31.6925	17.2100	46.1750	6.7179	56.6672
5	54.2904	37.5030	71.0777	27.9122	80.6685
6	71.7281	51.8140	91.6422	43.2578	100.1984

Fit Equation Description:
[Variables]
x = col(17)
y = col(19)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 86.0219}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 18.587}}
x0 = x50(x,y,.5) "Auto {{previous: 50.0181}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10

Nitric Oxide for *M.pudica* (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9333	0.8710	0.7420	4.6314

	Coefficient	Std. Error	t	P	VIF
a	31.2790	24.8400	1.2592	0.3350	35.3072<
b	25.8579	24.2313	1.0671	0.3977	6.1298<
x0	48.3423	49.8496	0.9698	0.4345	33.1106<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	1200.8505	400.2835
Residual	2	42.8995	21.4498
Total	5	1243.7500	248.7500

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	289.6005	144.8002	6.7507	0.1290
Residual	2	42.8995	21.4498		
Total	4	332.5000	83.1250		

Statistical Tests:

PRESS 371.2571

Durbin-Watson Statistic 2.6389 Failed

Normality Test Passed (P = 0.9746)

K-S Statistic = 0.2001 Significance Level = 0.9746

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.6629

The power of the performed test (0.6629) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	4.1787	-4.1787	-0.9023	-1.3929	-5.6944<
3	7.8346	4.6654	1.0073	1.3317	2.7969<
4	13.1384	-0.6384	-0.1378	-0.1941	-0.1386
5	19.1064	-1.6064	-0.3469	-0.5204	-0.3957
6	24.1730	0.8270	0.1786	0.7299	0.6026

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	0.8946	0.5804	-6.6973
3	0.4419	0.4278	2.4182<
4	0.0124	0.4959	-0.1375
5	0.1129	0.5557	-0.4426
6	2.7894	0.9402	2.3881<

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	4.1787	-11.0028	19.3602	-20.8727	29.2301
3	7.8346	-5.1987	20.8680	-15.9764	31.6456
4	13.1384	-0.8949	27.1716	-11.2343	37.5110
5	19.1064	4.2511	33.9618	-5.7487	43.9615
6	24.1730	4.8513	43.4948	-3.5835	51.9296

Fit Equation Description:

```
[Variables]
x = col(17)
y = col(20)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 31.279}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 25.8579}}
x0 = x50(x,y,.5) "Auto {{previous: 48.3423}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200
```

Number of Iterations Performed = 14

Nitric Oxide *C. longa* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9979	0.9958	0.9915	2.4937

	Coefficient	Std. Error	t	P	VIF
a	62.5112	1.4438	43.2962	0.0005	1.1988
b	3.0002	4.4850	0.6689	0.5724	62.6955<
x0	16.5469	5.1788	3.1951	0.0856	62.4337<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	13975.0625	4658.3542
Residual	2	12.4375	6.2188
Total	5	13987.5000	2797.5000

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	2930.0625	1465.0312	235.5823	0.0042
Residual	2	12.4375	6.2188		
Total	4	2942.5000	735.6250		

Statistical Tests:

PRESS 726424.8924

Durbin-Watson Statistic 1.5018 Passed

Normality Test Passed (P = 0.6851)

K-S Statistic = 0.2975 Significance Level = 0.6851

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9980

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.

2	0.2506	-0.2506	-0.1005	-1.2253	-1.7351
3	47.4890	0.0110	0.0044	1.2250	1.7335
4	62.4860	-2.4860	-0.9969	-1.2237	-1.7262
5	62.5111	-0.0111	-0.0045	-0.0055	-0.0039
6	62.5112	2.4888	0.9980	1.2240	1.7281

Influence Diagnostics:

Row	Cook's Dist		Leverage	DFFITS
2	73.9195<	0.9933	-21.0880	
3	38860.8249<	1.0000	483.1673<	
4	0.2530	0.3363	-1.2289	
5	5.0451E-006		0.3352	-0.0028
6	0.2518	0.3352	1.2271	

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.2506	-10.4430	10.9441	-14.8980	15.3992
3	47.4890	36.7594	58.2187	32.3150	62.6631
4	62.4860	56.2633	68.7088	50.0824	74.8896
5	62.5111	56.2992	68.7231	50.1129	74.9094
6	62.5112	56.2990	68.7234	50.1129	74.9095

Fit Equation Description:

```
[Variables]
x = col(24)
y = col(28)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 62.5112}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 3.00015}}
x0 = x50(x,y,.5) "Auto {{previous: 16.5469}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200
```

Number of Iterations Performed = 10

Nitric Oxide for *E. serrata* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9891	0.9784	0.9567	4.3283

	Coefficient	Std. Error	t	P	VIF
a	47.5064	2.5032	18.9781	0.0028	1.2116
b	2.9008	10.1708	0.2852	0.8023	72.0866<
x0	16.1706	13.4682	1.2007	0.3528	71.9112<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	8175.0319	2725.0106
Residual	2	37.4681	18.7340
Total	5	8212.5000	1642.5000

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	1695.0319	847.5160	45.2394	0.0216
Residual	2	37.4681	18.7340		
Total	4	1732.5000	433.1250		

Statistical Tests:

PRESS 1338768.9938

Durbin-Watson Statistic 1.6693 Passed

Normality Test Passed (P = 0.6794)

K-S Statistic = 0.2989 Significance Level = 0.6794

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9576

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.1795	-0.1795	-0.0415	-0.7084	-0.5787
3	37.4919	0.0081	0.0019	0.7079	0.5782
4	47.4936	-2.4936	-0.5761	-0.7062	-0.5764
5	47.5064	-2.5064	-0.5791	-0.7098	-0.5803
6	47.5064	4.9936	1.1537	1.4142	1189.8544<

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	48.6104<	0.9966	-9.8659
3	23770.1628<	1.0000	218.1223<
4	0.0836	0.3345	-0.4086
5	0.0844	0.3345	-0.4114
6	0.3351	0.3345	843.5233<

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.1795	-18.4116	18.7707	-26.1349	26.4940
3	37.4919	18.8688	56.1149	11.1549	63.8289
4	47.4936	36.7229	58.2642	25.9802	69.0070
5	47.5064	36.7361	58.2768	25.9931	69.0197
6	47.5064	36.7359	58.2769	25.9931	69.0198

Fit Equation Description:

```
[Variables]
x = col(24)
y = col(26)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 47.5064}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 2.90084}}
x0 = x50(x,y,.5) "Auto {{previous: 16.1706}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
```

[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10

Nitric Oxide for *M.pudica* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9660	0.9331	0.8662	8.6597

	Coefficient	Std. Error	t	P	VIF
a	52.5030	5.0021	10.4963	0.0090	1.2459
b	2.5927	20.8344	0.1244	0.9123	114.9004<
x0	15.3581	37.3857	0.4108	0.7211	114.8768<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	10293.7695	3431.2565
Residual	2	149.9805	74.9903
Total	5	10443.7500	2088.7500

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	2092.5195	1046.2597	13.9519	0.0669
Residual	2	149.9805	74.9903		
Total	4	2242.5000	560.6250		

Statistical Tests:

PRESS 38257634.0765

Durbin-Watson Statistic 1.6674 Passed

Normality Test Passed (P = 0.6767)

K-S Statistic = 0.2996 Significance Level = 0.6767

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.8182

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.1401	-0.1401	-0.0162	-0.7079	-0.5783
3	44.9939	0.0061	0.0007	0.7082	0.5785
4	52.4991	-4.9991	-0.5773	-0.7070	-0.5772
5	52.5030	-5.0030	-0.5777	-0.7078	-0.5781
6	52.5030	9.9970	1.1544	1.4142	1709.6718<

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	319.7591<	0.9995	-25.2987
3	169734.3932<	1.0000	582.9350<
4	0.0833	0.3332	-0.4080
5	0.0836	0.3337	-0.4090
6	0.3338	0.3337	1209.7883<

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.1401	-37.1098	37.3900	-52.5461	52.8263
3	44.9939	7.7343	82.2535	-7.6992	97.6870
4	52.4991	30.9909	74.0073	9.4772	95.5210
5	52.5030	30.9809	74.0252	9.4742	95.5319
6	52.5030	30.9809	74.0252	9.4742	95.5319

Fit Equation Description:

```
[Variables]
x = col(24)
y = col(27)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 52.503}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 2.59267}}
x0 = x50(x,y,.5) "Auto {{previous: 15.3581}}
```

[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10

H_2O_2 Asorbic Acid

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9978	0.9956	0.9912	3.1235

	Coefficient	Std. Error	t	P	VIF
a	74.7733	1.8033	41.4639	0.0006	1.3097
b	1.4757	131.1731	0.0113	0.9920	59265.2468<
x0	15.1549	430.6626	0.0352	0.9751	59265.1931<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	21967.2390	7322.4130
Residual	2	19.5121	9.7560
Total	5	21986.7511	4397.3502

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	4397.8326	2198.9163	225.3905	0.0044
Residual	2	19.5121	9.7560		
Total	4	4417.3447	1104.3362		

Statistical Tests:

PRESS 125865699627.3970

Durbin-Watson Statistic 1.6667 Passed

Normality Test Passed (P = 0.6751)

K-S Statistic = 0.3000 Significance Level = 0.6751

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9978

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.0026	-0.0026	-0.0008	-0.7270	-0.5993
3	72.0700	5.1626E-006	1.6528E-006	0.4333	0.3219
4	74.7733	-1.8033	-0.5774	-0.7071	-0.5773
5	74.7733	-1.8033	-0.5774	-0.7071	-0.5774
6	74.7733	3.6067	1.1547	1.4142	4550.9487<

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	135106.1960<	1.0000	-524.8322
3	4300306025.5263<	1.0000	84372.1712<
4	0.0833	0.3333	-0.4082
5	0.0833	0.3333	-0.4083
6	0.3333	0.3333	3218.0126<

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.0026	-13.4366	13.4418	-19.0033	19.0085
3	72.0700	58.6308	85.5092	53.0641	91.0759
4	74.7733	67.0142	82.5324	59.2551	90.2916
5	74.7733	67.0142	82.5325	59.2551	90.2916
6	74.7733	67.0142	82.5325	59.2551	90.2916

Fit Equation Description:

```
[Variables]
x = col(31)
y = col(32)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
```

b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 74.7733}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 1.47574}}
x0 = x50(x,y,.5) "Auto {{previous: 15.1549}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 15

H_2O_2 Ascorbic Acid

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9978	0.9956	0.9912	3.1235

	Coefficient	Std. Error	t	P	VIF
a	74.7733	1.8033	41.4639	0.0006	1.3097
b	1.4757	131.1731	0.0113	0.9920	59265.2468<
x0	15.1549	430.6626	0.0352	0.9751	59265.1931<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	21967.2390	7322.4130
Residual	2	19.5121	9.7560
Total	5	21986.7511	4397.3502

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	4397.8326	2198.9163	225.3905	0.0044
Residual	2	19.5121	9.7560		
Total	4	4417.3447	1104.3362		

Statistical Tests:

PRESS 125865699627.3970

Durbin-Watson Statistic 1.6667 Passed

Normality Test Passed (P = 0.6751)

K-S Statistic = 0.3000 Significance Level = 0.6751

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9978

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.0026	-0.0026	-0.0008	-0.7270	-0.5993
3	72.0700	5.1626E-006	1.6528E-006	0.4333	0.3219
4	74.7733	-1.8033	-0.5774	-0.7071	-0.5773
5	74.7733	-1.8033	-0.5774	-0.7071	-0.5774
6	74.7733	3.6067	1.1547	1.4142	4550.9487<

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	135106.1960<	1.0000	-524.8322
3	4300306025.5263<	1.0000	84372.1712<
4	0.0833	0.3333	-0.4082
5	0.0833	0.3333	-0.4083
6	0.3333	0.3333	3218.0126<

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.0026	-13.4366	13.4418	-19.0033	19.0085
3	72.0700	58.6308	85.5092	53.0641	91.0759
4	74.7733	67.0142	82.5324	59.2551	90.2916
5	74.7733	67.0142	82.5325	59.2551	90.2916
6	74.7733	67.0142	82.5325	59.2551	90.2916

Fit Equation Description:
[Variables]
x = col(39)

y = col(40)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 74.7733}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 1.47574}}
x0 = x50(x,y,.5) "Auto {{previous: 15.1549}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 15

H_2O_2 *C.longa* (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9795	0.9593	0.9187	4.2508

	Coefficient	Std. Error	t	P	VIF
a	33.6077	2.4574	13.6759	0.0053	1.2325
b	2.9946	9.3828	0.3192	0.7799	31.3065<
x0	15.2592	14.9833	1.0184	0.4156	31.3152<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS

Regression	3	4165.3310	1388.4437
Residual	2	36.1390	18.0695
Total	5	4201.4700	840.2940

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	852.5930	426.2965	23.5920	0.0407
Residual	2	36.1390	18.0695		
Total	4	888.7320	222.1830		

Statistical Tests:

PRESS 3918456.4639

Durbin-Watson Statistic 1.8282 Passed

Normality Test Passed (P = 0.7822)

K-S Statistic = 0.2729 Significance Level = 0.7822

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.8980

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.2046	-0.2046	-0.0481	-1.3828	-4.6663<
3	27.8825	0.0175	0.0041	1.3827	4.6583<
4	33.5991	-4.7991	-1.1290	-1.3822	-4.6161<
5	33.6077	1.4923	0.3511	0.4302	0.3194
6	33.6077	3.2923	0.7745	0.9492	0.9054

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	525.6633<	0.9988	-134.0045
3	71756.4292<	1.0000	1563.1030<
4	0.3176	0.3328	-3.2602
5	0.0310	0.3342	0.2263
6	0.1508	0.3342	0.6415

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.2046	-18.0742	18.4833	-25.6533	26.0624
3	27.8825	9.5927	46.1722	2.0168	53.7481
4	33.5991	23.0478	44.1503	12.4840	54.7141
5	33.6077	23.0343	44.1812	12.4816	54.7339

6	33.6077	23.0342	44.1813	12.4815	54.7340

Fit Equation Description:
[Variables]
x = col(31)
y = col(35)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 33.6077}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 2.99462}}
x0 = x50(x,y,.5) "Auto {{previous: 15.2592}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10

H_2O_2 *E.serrata* (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9879	0.9760	0.9520	2.6997

	Coefficient	Std. Error	t	P	VIF
a	27.9011	1.5592	17.8949	0.0031	1.2536
b	2.4830	13.9895	0.1775	0.8755	156.7971<

x0	15.2611	26.7432	0.5707	0.6258	156.7853<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	2925.7235	975.2412
Residual	2	14.5765	7.2882
Total	5	2940.3000	588.0600

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	592.9235	296.4618	40.6768	0.0240
Residual	2	14.5765	7.2882		
Total	4	607.5000	151.8750		

Statistical Tests:

PRESS 23434292.1411

Durbin-Watson Statistic 1.5009 Passed

Normality Test Passed (P = 0.6770)

K-S Statistic = 0.2995 Significance Level = 0.6770

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9504

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.0596	-0.0596	-0.0221	-1.2251	-1.7342
3	24.2977	0.0023	0.0008	1.2251	1.7340
4	27.8998	-2.6998	-1.0000	-1.2247	-1.7318
5	27.9011	-0.0011	-0.0004	-0.0005	-0.0004
6	27.9011	2.6989	0.9997	1.2246	1.7312

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	1538.9300<	0.9997	-96.1826
3	1070245.1517<	1.0000	2536.1490<
4	0.2499	0.3332	-1.2243
5	4.1111E-008	0.3335	-0.0002
6	0.2502	0.3335	1.2247

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.0596	-11.5542	11.6735	-16.3662	16.4854
3	24.2977	12.6820	35.9135	7.8706	40.7249
4	27.8998	21.1945	34.6051	14.4876	41.3120
5	27.9011	21.1926	34.6096	14.4873	41.3149
6	27.9011	21.1926	34.6096	14.4873	41.3149

Fit Equation Description:
[Variables]
x = col(31)
y = col(33)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 27.9011}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 2.48302}}
x0 = x50(x,y,.5) "Auto {{previous: 15.2611}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 11

H_2O_2 *M.pudica* (ETHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate

0.9990 0.9980 0.9961 0.9000

	Coefficient	Std. Error	t	P	VIF
a	32.4000	0.5196	62.3537	0.0003	1.2973
b	1.6552	39.6036	0.0418	0.9705	16397.6221<
x0	15.3106	112.2064	0.1365	0.9040	16397.5088<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	4085.6400	1361.8800
Residual	2	1.6200	0.8100
Total	5	4087.2600	817.4520

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	819.0720	409.5360	505.6030	0.0020
Residual	2	1.6200	0.8100		
Total	4	820.6920	205.1730		

Statistical Tests:

PRESS 11008264089.5989

Durbin-Watson Statistic 1.5000 Passed

Normality Test Passed (P = 0.6751)

K-S Statistic = 0.3000 Significance Level = 0.6751

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9997

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.0031	-0.0031	-0.0035	-1.2242	-1.7288
3	30.6000	1.1451E-005	1.2723E-005	1.2179	1.6942
4	32.4000	-0.9000	-1.0000	-1.2247	-1.7320
5	32.4000	-7.1975E-006	-7.9973E-006	-9.7946E-006	-6.9259E-006
6	32.4000	0.9000	1.0000	1.2247	1.7320

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS

2	62564.3949<	1.0000	-611.8326	
3	4530114389.8709<	1.0000	162170.3151<	
4	0.2500	0.3333	-1.2247	
5	1.5989E-011	0.3333	-4.8974E-006	
6	0.2500	0.3333	1.2247	

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.0031	-3.8692	3.8755	-5.4732	5.4795
3	30.6000	26.7276	34.4724	25.1236	36.0764
4	32.4000	30.1643	34.6357	27.9286	36.8714
5	32.4000	30.1643	34.6357	27.9286	36.8714
6	32.4000	30.1643	34.6357	27.9286	36.8714

Fit Equation Description:
[Variables]
x = col(31)
y = col(34)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 32.4}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 1.65517}}
x0 = x50(x,y,.5) "Auto {{previous: 15.3106}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 15

H_2O_2 *C.longa* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter

f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9611	0.9238	0.8476	7.9499

	Coefficient	Std. Error	t	P	VIF
a	54.6326	14.0913	3.8770	0.0605	6.1526<
b	17.0049	10.1804	1.6704	0.2368	2.6736
x0	35.0340	13.8550	2.5286	0.1272	4.3808<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	5844.9957	1948.3319
Residual	2	126.4026	63.2013
Total	5	5971.3983	1194.2797

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	1532.0112	766.0056	12.1201	0.0762
Residual	2	126.4026	63.2013		
Total	4	1658.4138	414.6035		

Statistical Tests:

PRESS 643.6143

Durbin-Watson Statistic 3.2511 Failed

Normality Test Passed (P = 0.9405)

K-S Statistic = 0.2208 Significance Level = 0.9405

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.7918

The power of the performed test (0.7918) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.

2	6.1747	-6.1747	-0.7767	-1.0897	-1.2087
3	15.9707	7.4493	0.9370	1.3787	4.3768<
4	31.2768	-5.1468	-0.6474	-1.0702	-1.1577
5	44.4042	2.4458	0.3077	0.4240	0.3143
6	51.0082	-0.5582	-0.0702	-0.1892	-0.1350

Influence Diagnostics:

Row	Cook's Dist		Leverage	DFFITS
2	0.3832	0.4919	-1.1894	
3	0.7380	0.5381	4.7238<	
4	0.6615	0.6341	-1.5239	
5	0.0539	0.4736	0.2981	
6	0.0748	0.8624	-0.3380	

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	6.1747	-17.8162	30.1656	-35.6056	47.9551
3	15.9707	-9.1204	41.0618	-26.4510	58.3923
4	31.2768	4.0393	58.5143	-12.4486	75.0023
5	44.4042	20.8645	67.9439	2.8813	85.9271
6	51.0082	19.2437	82.7726	4.3283	97.6881

Fit Equation Description:

```
[Variables]
x = col(39)
y = col(43)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 54.6326}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 17.0049}}
x0 = x50(x,y,.5) "Auto {{previous: 35.034}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200
```

Number of Iterations Performed = 9

H_2O_2 *E.serrata* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9827	0.9657	0.9315	6.3236

	Coefficient	Std. Error	t	P	VIF
a	54.6629	3.6555	14.9537	0.0044	1.2290
b	2.9337	9.8429	0.2981	0.7938	42.7585<
x0	15.4792	15.2622	1.0142	0.4172	42.7353<

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	10989.6382	3663.2127
Residual	2	79.9760	39.9880
Total	5	11069.6142	2213.9228

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	2253.4382	1126.7191	28.1764	0.0343
Residual	2	79.9760	39.9880		
Total	4	2333.4142	583.3535		

Statistical Tests:

PRESS 7844728.7627

Durbin-Watson Statistic 1.4670 Failed

Normality Test Passed (P = 0.6821)

K-S Statistic = 0.2983 Significance Level = 0.6821

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.9182

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	0.2780	-0.2780	-0.0440	-1.1634	-1.4471
3	45.0206	0.0194	0.0031	1.1631	1.4459
4	54.6501	-6.0001	-0.9488	-1.1619	-1.4412
5	54.6629	-0.6129	-0.0969	-0.1188	-0.0843
6	54.6629	6.5971	1.0432	1.2785	2.1150<

Influence Diagnostics:

Row	Cook's Dist	Leverage	DFFITS
2	315.5868<	0.9986	-38.2703
3	65074.3305<	1.0000	549.2600<
4	0.2248	0.3331	-1.0185
5	0.0024	0.3342	-0.0597
6	0.2735	0.3342	1.4983

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	0.2780	-26.9109	27.4668	-38.1866	38.7426
3	45.0206	17.8124	72.2288	6.5424	83.4989
4	54.6501	38.9466	70.3536	23.2353	86.0649
5	54.6629	38.9348	70.3911	23.2358	86.0901
6	54.6629	38.9346	70.3912	23.2357	86.0901

Fit Equation Description:

```
[Variables]
x = col(39)
y = col(41)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
[Parameters]
a = sup(y) "Auto {{previous: 54.6629}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 2.93374}}
x0 = x50(x,y,.5) "Auto {{previous: 15.4792}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
```

[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 10

H_2O_2 *M.pudica* (METHANOL)

Nonlinear Regression

Data Source: Data 1 in Plots
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8923	0.7963	0.5926	13.4680

	Coefficient	Std. Error	t	P	VIF
a	52.9472	17.5245	3.0213	0.0943	4.2118<
b	16.5680	16.7551	0.9888	0.4270	2.2476
x0	24.1660	19.0771	1.2668	0.3328	2.7422

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	3	6973.8013	2324.6004
Residual	2	362.7724	181.3862
Total	5	7336.5737	1467.3147

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	1418.0235	709.0118	3.9089	0.2037
Residual	2	362.7724	181.3862		
Total	4	1780.7959	445.1990		

Statistical Tests:

PRESS	2837.3633

Durbin-Watson Statistic 2.5798 Failed

Normality Test Passed (P = 0.9243)

K-S Statistic = 0.2281 Significance Level = 0.9243

Constant Variance Test Passed (P = 0.0500)

Power of performed test with alpha = 0.0500: 0.5267

The power of the performed test (0.5267) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
2	9.9902	-9.9902	-0.7418	-1.3415	-2.9963<
3	23.1627	11.0673	0.8217	1.1841	1.5312
4	38.2417	-3.1017	-0.2303	-0.3837	-0.2819
5	47.4864	-8.7464	-0.6494	-0.8281	-0.7223
6	51.1869	7.3731	0.5475	1.1238	1.3091

Influence Diagnostics:

Row	Cook's Dist		Leverage	DFFITS
2	1.3620	0.6942	-4.5149	
3	0.5029	0.5183	1.5884	
4	0.0871	0.6397	-0.3756	
5	0.1431	0.3850	-0.5715	
6	1.3532	0.7627	2.3470<	

95% Confidence:

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	9.9902	-38.2927	58.2731	-65.4366	85.4171
3	23.1627	-18.5578	64.8832	-48.2416	94.5670
4	38.2417	-8.1066	84.5899	-35.9616	112.4450
5	47.4864	11.5315	83.4414	-20.7098	115.6827
6	51.1869	0.5791	101.7948	-25.7489	128.1228

Fit Equation Description:

```
[Variables]
x = col(39)
y = col(42)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
'Automatic Initial Parameter Estimate Functions
sup(q)=if(mean(q)>=0, max(q), min(q))
b1(q,r)=if(sup(r)>0, xwtr(q,r,.5)/4, -xwtr(q,r,.5)/4)
```

[Parameters]
a = sup(y) "Auto {{previous: 52.9472}}
b = if(b1(x,y)=0, 1, b1(x,y)) "Auto {{previous: 16.568}}
x0 = x50(x,y,.5) "Auto {{previous: 24.166}}
[Equation]
f= a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

Number of Iterations Performed = 120