PHYTOCHEMICAL ANALYSIS AND BIOCIDAL EFFECTS OF ORANGE PEEL AND GARLIC EXTRACT ON CONTAMINATED GRAINS

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REG NO: 20194196898

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULLFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN BIOCHEMISTRY

MAY, 2024

CERTIFICATION

This is to certify that this project titled **"Phytochemical analysis and biocidal effects of orange peel and garlic extract on contaminated grains"** was carried out by **Olaitan, Michael Oluwaseun** with Reg. No: **20194196898** in partial fulfilment for the award of Master of Science (M.Sc.) degree in Biochemistry.

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DEDICATION

To God my Ebenezer.

ACKNOWLEDGEMENTS

My sincere gratitude goes to my supervisors, Prof. C. O. Ujowundu, and Dr. (Mrs.) C. P. Nzebude, for thoroughly supervising this research, for thoroughly reading my write up and making corrections. I would like to specially thank Prof. C. O. Ujowundu for his great mentorship, calmness, understanding and compassionate disposition during the course of this work. Despite his wealth of experience, yet he is very unassuming. Thank you, Sir. Special acknowledgement goes to Prof. Wesley Braid of the Department of Microbiology for his valuable inputs and assistance during the progression of this study. My appreciation also goes to the Head of the Department of Biochemistry, Prof. L. A. Nwaogu for making facilities available in the Department for this work and for fast tracking our postgraduate programme. Also, special thanks to all Biochemistry postgraduate lecturers; Prof. (Mrs) V. A. Onwuliri, Prof. A. C. Ene, Prof. C. O. Ibegbulem, Prof. (Mrs) G. N. Okwu, Prof. C. S. Alisi, Prof. R. N. Nwaoguikpe, Prof. K. M. E. Iheanacho, Prof. (Mrs) A. A. Emejulu, Prof. N. Nwachukwu (of blessed memory), Dr. C. U. Igwe, Dr. (Mrs) C. H. Onuoha and Dr. K. O. Igwe (of blessed memory), Dr (Mrs) F. N. Ujowundu for all the interesting and effective lectures they gave to us. Furthermore, to all other members of staff of Biochemistry Department, FUTO who rendered help in one way or the other. I say a big thank you. I would also like to appreciate the School of Postgraduate Studies, FUTO for the smooth coordination of the postgraduate programme and for ensuring that the programme was fast tracked.

I would like to specially thank my PG classmates (2019/2020) set, especially the class captain, Precious Asonye, for all the support given to me during the course of the lectures and for the series of calls, prayers and encouragement you gave to me when l could not make it. Thank you all. Needless to say, I do not know if there is any way l can actually thank every member of my family for their support.

Words cannot tell how grateful l am to every member of my family for their financial, spiritual, emotional, and all-round unending love care and support. The bond we share as family is just too strong and may God send you helpers always and take every one of you to higher ground in Jesus mighty name. Finally, to all others too numerous to mention, you are not forgotten, thanks a million for being there for me and for being part of my M.Sc. success story, God bless you really good. See you at the top.

TABLE OF CONTENTS

CHAPTER THREE: MATERIALS AND METHODS

CHAPTER FOUR: RESULTS AND DISCUSSION

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

This study aimed to determine the phytochemical composition and antifungal effects of orange-peel and garlic extract on contaminated grains. Standard phytochemical methods were used to test for the presence of bioactive compounds in the extracts. The results of the preliminary phytochemical screening showed that the orange-peel and garlic extract contained various metabolites; polyphenols, flavonoids, alkaloids, saponins, phenols, steroids, antinutrients at varied quantities. *In vitro* antioxidant and free radical scavenging potential of orange-peel and garlic extracts were determined on the basis of their scavenging activity of the DPPH, hydroxyl, hydrogen peroxide, superoxide, nitric oxide free radical, total antioxidant capacity and ferric reducing antioxidant property. The radical scavenging activities exhibited concentration-dependent responses, with garlic and orange-peel extracts demonstrating significant scavenging potentials for various radicals. However, their activities generally plateaued at higher concentrations, suggesting a limit to their scavenging capacities. The extracts exhibited lower scavenging activities compared to ascorbic acid and BHT. Orange-peel extract had the highest FRAP activity at 5 mg/ml, while garlic extract showed a steadier increase in FRAP activity with increasing concentration. Both extracts demonstrated lower TAC concentrations than ascorbic acid. Extracts were assessed for their effectiveness against two fungal species (*Aspergillus flavus and Penicillium notatum*). Garlic extract exhibited potent antifungal properties, inhibiting the growth of both fungi, while orange-peel extract showed copious growth inhibition. The combined effect of the extracts showed scanty growth. The findings from the study suggest that each of these extracts possess antioxidant properties and also highlights their potential as natural agents for fungal control in stored grains.

Keywords: Antifungal activity**,** Antioxidant, Phytochemicals, garlic extract, orange-peel extract.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Nutrient-rich food items such as cereals and pulses are consumed worldwide, but their chemical composition attracts bacteria and fungi, especially mould. If these microorganisms successfully colonize the food, it can negatively affect its nutritional value and potentially produce dangerous toxins that may result in significant health problems. Therefore, we need to remain aware and vigilant about food safety to prevent such issues from occurring. When grains are stored in conditions with high levels of humidity, fungi that cause storage-related issues can infect them. Many of these fungi that contaminate grains after harvest produce mycotoxins as a secondary metabolite. These mycotoxins are known to be harmful to organisms as they can cause cancer, mutations, and damage to the kidneys and nervous system (Samson, Hoekstra & Frisvad, 2004). Synthetic chemicals are often used to prevent fungal contaminations, but they can be harmful to living cells and the environment. This is important to consider for the sake of our health.

On another note, cereal grains are a great source of energy and nutrients such as carbohydrates, fats, oils, protein, vitamins and minerals. They are also known as "whole grains" in their unprocessed form (Esfandi, Walters & Tsopmo, 2019). Contamination of grains can happen either in the fields or during storage and harvest. Bacteria like *Staphylococcus, Enterococcus, Enterobacter, Pseudomonas, Xanthomonas, Alcaligenes, Flavobacterium, Bacillus* or *Clostridium* are commonly found on grains before harvest. Additionally, typical field fungi such as *Fusarium* and *Alternaria* as well as typical warehouse fungi like *Aspergillus* and *Penicillium* can infest cereal grains and cereals.

When it comes to storing food, particularly cereals, microorganisms are seen as unwanted because they can carry infections that can affect consumers or the next crop of plant. Some

microorganism can even produce toxins like aflatoxin (Juarez-Morales, Hernandez-Cocoletzi, Aguila-Almanza & Terino-Alvide, 2017), which is a biologically active secondary metabolite produced by *Aspergillus flavus*, *Aspergillus parasticus and Aspergillus nomious*. The fungi that produce aflatoxin are present throughout nature, widely distributed and can thrive in different environmental conditions. There have been various reports of different compounds and extracts that contain substances that can inhibit the biosynthesis of aflatoxin. Examples of these compounds and extracts include garlic, clove, cinnamon, and others. Additionally, many herbs, spices and essential oils have antimicrobial properties and are known to prevent the formation of aflatoxins in synthetic media.

Phosphine and methylbromide are the two commonly used fumigants for stored product protection worldwide, with Phosphine are available in metal phosphide preparations, cylinderized formulations and site generators, while methyl bromide comes in cylinders and metal cans.

The use of orange-peel extract as an insect pest management method is gaining popularity, as it has several advantages over synthetic pesticides. Citrus species have been found to have secondary metabolites that exhibit insecticidal activity against various types of beetles and flies. Some of these plants are used as a source of botanical insecticides. The essential oil extracted from orange-peels has been found to have an effect on the growth and feeding habits of insects such as the *Rhyzoportadomonica* rice weevil, *Sitophilus oryzae* and red flour beetle, *Tribolium castanum*, causing them to deter or develop poorly (Tripathi, Prajaoati, Khanuja & Kumar, 2003). Higher plants possess about 3,000 distinct essential oils that have been identified. About 300 of these oils have great economic potential due to their potential use as pesticides as well as their application in the perfume, cosmetic, and pharmaceutical industries (Tripathi *et al.,* 2003). Additionally, garlic extract has demonstrated effective antifungal properties against different types of harmful yeasts.

According to Kutawa, Danladi and Haruna (2018), the antifungal activity of garlic extract was found to be similar to that of a commonly used drug known as fluconazole. Furthermore, the extract from garlic has demonstrated the ability to suppress the growth of harmful fungi such as *Biotrytis cinerea*, *Penicillium expansum*, *Neofabraea alba*, *Fusarium* and Rhizopus species.

1.2 Statement of the problem

Studies have been conducted on the possible biocidal effects of garlic and orange extract on a variety of species, including fungus. While these natural extracts have shown some antimicrobial properties, their effectiveness against fungi associated with contaminated stored grains may vary. The problem is to assess the efficacy of orange-peel and garlic extract as potential biocidal agents against fungi commonly found in contaminated stored grains. This involves investigating the antifungal activity of these natural extracts, considering factors such as the specific fungal species involved, the concentrations and application methods of the extracts, and the environmental conditions in which they are applied.

1.3 Aim and objectives of the study

1.3.1 Aim of the study

The aim of this study was to determine the phytochemical and biocidal effects of orange peel and garlic extract on contaminated grains.

1.3.2 Objectives

The objectives of the study include:

i. Phytochemicals analysis of orange-peel and garlic extracts using gas chromatography (GC).
ii. Phenolic profile of orange-peel and garlic extracts using gas chromatography-mass spectrophotometry (GC-MS).

iii. To determine the antioxidant and free radical scavenging potentials of orange-peel and garlic extracts.

iv. To determine the antifungal effects of orange-peel and garlic extract on contaminated grains.

1.4 Justification of the study

Fungal contamination in stored grains poses a significant threat to food safety. *Aspergillus*, *Fusarium*, and *Penicillium* are just a few of the fungi that can generate mycotoxins, which are hazardous compounds that can contaminate grains and pose risks to human and animal health. Understanding the potential of orange-peel and garlic extract as biocidal agents can contribute to developing safer and more sustainable methods for controlling fungal growth and reducing mycotoxin contamination in stored grains. Overall, the study of the biocidal effect of orange-peel and garlic extract on fungi associated with contaminated stored grains has significant implications for food safety, sustainability, grain quality and the utilization of agricultural by-products. It has the potential to contribute to improved storage practices, reduced mycotoxin contamination and a more environmentally friendly approach to fungal control in the agricultural industry.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pest infestation on food

Stored grains are badly damaged by insect pests, resulting in both quality and quantity losses. The key element contributing to stored grains pest is the availability of circumstances that encourage their development and survival. At various processing stages of grains, such as seed germination and maturation, processing in threshing yards, transportation of seed and storage, several insect pests get access to stored grains. Some pests harm the seeds at the ripening stage and continue to do so while they are being stored. Among the principal sources of pest infestation are old containers, storage buildings, and bags (Ahmad *et al.*, 2022). The movement of grains from one area to another either passively or by active flight as particular adult insects have powerful wings, is what disperses and spreads stored grain pests.

Approximately one thousand species are stored grain pests occur globally, posing a danger to diverse types of stored goods. The bulk of stored grain pests belong to two groups: Lepidoptera and Coleoptera (Khare, 1994). Stored grain pests represent a major concern to agricultural product derivatives that are dried, stored, perishable and not meant for human consumption globally. In addition to causing significant post-harvest losses (from about 9% in developed countries to nearly 20% or more in developing countries), stored grains have the potential to contaminate food products by the introduction live insects, insect products such as chemical excretions or silk, dead insects or some other storage structures (Pimentel, 1991).

Globally, 8–10% of stored food products are considered to be lost owing to inadequate storage, accounting for 100 million tonnes of incorrectly stored grains and 13 million tonnes of grains lost to insects. Pests such as various insects, pathogens, mites possess serious

threats and cause severe damage to grains by producing certain enterotoxins and mycotoxins (Morgan & Aldred, 2007). Approximately one-third of the world's production, which values almost $100 billion has been destroyed by almost 20,000 species of field and stored grain pests. Coleoptera and Lepidoptera groups represent roughly 60% and 10%, respectively, of all the stored grain pests according to Atwal and Dhaliwal (2008). The bulk of stored grain pests belong to these two classes. Stored grain pests generally feed on grain, bore into the kernel and then destroy the germ portion, cause heat and then cause deterioration in-stored grain products thus resulting in huge losses mainly due to nutritional depletion and reduction in market value besides causing contamination by their excretory products, that can be extremely hazardous to human health who process and infest the grains so the loss caused by insect pests is not in terms of quantity but mostly in terms of quality. Chemical changes affecting proteins, carbohydrates, amino acids cause stored grains to lose quality and severely affect the nutritional content of grains (Ahmad *et al.*,2022).

2.2 Food shortage

Food shortage refers to a situation where the availability of food is insufficient to meet the demand of a population or a specific region. It occurs when there is a significant disparity between the amount of food required and the amount that is actually accessible or affordable. Food shortages can be caused by several factors, such as natural disasters, severe weather conditions (such as droughts or floods), pests and diseases affecting crops or livestock, conflicts and wars, economic instability, inadequate infrastructure for transportation and storage, and poor agricultural practices (Abdul Monam, 2020).

Food shortages typically results in an increase in food prices, making it more difficult for people to afford a sufficient and nutritious diet. This can result in malnutrition, hunger, and even famine in extreme cases. Food shortages can have severe consequences for the affected

population, particularly for vulnerable groups such as children, the elderly, and those living in poverty.

Addressing food shortages requires a comprehensive approach that includes increasing agricultural productivity, improving infrastructure and storage facilities; promoting sustainable farming practices, enhancing access to credit and markets for farmers, investing in research and development for improved crop varieties, and establishing effective social safety nets to protect the most vulnerable populations. International cooperation and assistance are often crucial in responding to food shortages, providing emergency relief, and supporting long-term strategies for food security.

2.3 Food safety

Food safety refers to the measures and practices taken to ensure that food is safe for consumption and free from any potential hazards that could harm human health. It encompasses food handling, preparation, and storage at every level of the food production and supply chain to prevent foodborne illness (Hanning, O'Bryan, Crandall & Ricke, 2012). Food safety includes several key components:

1. Food handling and hygiene: This involves proper washing of hands, maintaining cleanliness of utensils, equipment, and food preparation areas, and preventing cross-contamination between different foods.
2. Safe storage: Food should be stored at appropriate temperatures to prevent the growth of harmful bacteria. Refrigeration and freezing are important methods to inhibit bacterial growth and maintain food quality.
3. Proper cooking: Thorough cooking kills bacteria, viruses, and other microorganisms that may be present in raw or undercooked food. It is essential to follow recommended cooking temperatures and times for different types of food.

4. Avoiding contamination: Preventing contamination during food production, processing, and distribution is crucial. This includes monitoring the use of pesticides, antibiotics, and other chemicals, as well as implementing food safety protocols in factories, farms, and restaurants.
5. Food labelling and traceability: Clear and accurate labelling of food products helps consumers make informed choices and enables quick identification and recall of potentially unsafe products. Traceability systems allow the tracking of food from its source to the end consumer.
6. Regulation and monitoring: Governments and regulatory agencies establish and enforce food safety standards, conduct inspections, and monitor compliance with regulations to ensure the safety of the food supply.
7. Foodborne illnesses, caused by consuming contaminated food, can result in symptoms ranging from mild discomfort to severe illness and even death.

Common sources of food contamination include bacteria (such as *Salmonella* and *E. coli*), viruses (like *norovirus* and *hepatitis* A), parasites, toxins, and chemical contaminants. Promoting food safety requires the collaboration of governments, food producers, retailers, and consumers. Public education and awareness campaigns play a crucial role in promoting safe food handling practices and fostering a culture of food safety.

2.4 Use of chemical pesticides in food preservation and storage

Grains are integral to daily food requirements and are consumed in large quantities across Africa. Every nation's economic and social development depends on its food security. Africa is not left behind, as their livelihood and sustainability sources are agriculture (Hollinger & Staatz, 2015). Storage of cereal grains and legumes (such as cowpea, millet, wheat, rice,

barley, oats, sorghum, and corn) with chemicals is a common practice amongst African farmers and sellers of agro-produce (Obeng-Ofori, Adarkwa & Ulrichs, 2015).

The food problem in Africa is the lack of preservative mechanisms in handling bountiful harvests after short farming seasons. Global annual storage produces losses are estimated to give 10% of all stored grain, while in sub-Saharan Africa, it ranges from 25 to 40% of grain losses. Africa has become more dependent on pesticides to preserve and store its farm products. The demand for these pesticides keeps increasing due to crop production, which yields bountiful agricultural produce (Bjornlund, Bjornlund & Van Rooyen, 2020). Many farmers have embraced the use of pesticides as the best means of insurance in protecting and preserving their crops against wanton destructions by pests, notwithstanding that many farmers are not adequately equipped with personal protective gadgets and their inability to read labels on pesticide products (Mobolade, Bunindro, Sahoo & Rajashekar, 2019).

Growing evidence shows that farmers and their families may be predisposed to severe and immediate health risks linked with pesticides, although the impacts are undetected in many cases. These chemicals inevitably get to the soil due to dispersal through run-off water, excessive application, and failure on the part of farmers to adhere to their usage guidelines, inevitably distorting the soil ecosystems and microflora. Also, persistent pesticides leach into the water bodies and intermittently cause nuisance and mortality to the aquatic ecological diversities and promote climate change (Damalas & Koutroubas, 2016; Nicolopoulou-Stamati, Maipas, Kotampasi, Stamatis & Hens, 2016) In the terrestrial biosphere, toxic chemicals can affect the skin when dermally absorbed due to splashes and spills during handling (Damalas & Koutroubas, 2016; Okeke, Okagu, Okoye & Ezeorba, 2022).

Health implications from consuming food with pesticide residues beyond the permissible limit are not left out. In addition to the acute effects caused by these chemicals, they also cause chronic effects even if exposed in minute amounts. Although these chemicals are used for storage, some residues have been discovered in cooked foods as well as directly in animal feeds (Sarkar, Bernardes, Keeley, Mohring & Jansen, 2021).

Sadly, people have resorted to using pesticides in suicidal attempts due to stress and psychological factors (Bonvoisin, Utyasheva, Knipe, Gunnel& Eddleston, 2020). About 50% of the pesticides involved in these cases have been classified as highly hazardous by WHO (Adiaha, 2017). The risk factors associated with the usage of pesticides include respiratory, cardiovascular, and endocrine disruptions, metabolic disorders, as well as gastrointestinal, reproductive, and neurological dysfunctions (Nicolopoulou-Stamati *et al.,* 2016).

Unfortunately, Africa has become the dumping site for banned synthetic pesticides rejected by the European Union, and this calls for questions, given the growing evidence that these toxic chemicals pose serious threat to humans and the ecological systems and contravene the United Nations' Sustainable Development Goals on food security. Moreover, pesticide manufacturers are interested in making a profit and argue about the environmental health issues concerning their products rather than the health of the end-users and the ecosystem (Sarkar *et al.,* 2021; Okeke *et al.,* 2022). Currently, herbicides (50%), insecticides (30%), fungicides (18%), and other categories used for rodents and nematode prevention account for about 3 million tonnes used yearly globally (Hollinger & Staatz, 2015; Bjornlund *et al.,* 2020). To worsen the situation, many farmers indiscriminately use these hazardous chemicals on the farm and grain storage to avoid postharvest losses without being mindful of their dangers to human health and the ecosystems.

Using insecticides that come in contact with food grains requires a proper understanding and evaluation of their hazards and benefits (Kumar & Kalita, 2017). The major causes of the abuse of synthetic pesticides in Sub-Sahara Africa lie that most of the farm products are predominantly produced by poor-enlighten farmers who may not be able to afford the cost of safe pesticides. In many African countries, using hazardous chemicals in grain storage is part of the game because no farmers want to experience any damage to their grains by pests while ignoring the consumers' and environmental safety. Africa's high prevalence of pests is much higher, possibly due to its tropical climates. There are tendencies of infrastructural deficiencies leading to poor pest control systems (Nwaigwe, 2019). The abuse of pesticides in food grain storage has caused dangerous health implications, insect pesticide resistance, and other severe environmental complications (Sarkar *et al.,* 2021).

2.4.1 Adverse effects of using chemical pesticides in food storage and preservation

Because of their physicochemical properties and composition, most agro-produce are perishable and can be easily altered by biological, physical, and chemical agents (Martinez & Carballo, 2021). Farmers use different storage chemicals to store cereal grains, which are costly and toxic. Some farmers cannot afford certain chemicals, and those who can abuse them, resulting in environmental and health concerns. Low-income farmers may use cheap but noxious synthetic chemicals to store cereal grains (Anaduaka *et al.*,2023).

Storage of cereal grains and legumes (such as cowpea, millet, wheat, rice, barley, oats, sorghum, and corn) with chemicals is a common practice amongst African farmers and sellers of agro-produce. Generally, chemicals usually used to protect agro-produce from pest infestation and destruction during storage fall into a few classes, including organochlorine (e.g.,dichlorodiphenyltrichloroethane, heptachlor, and hexachlorobenzene), Organophosphate (e.g., Dimethoate, quinalphos, 2, 3-dichorovinyl dimethyl phosphate (sniper), methyl parathion, malathion, diazinon, chlorpyrifos, profenofos, monocrotophos,

and), carbamate (e.g., carbendazim, carbofuran, and carbosulfan) and Pyrethroid (e.g., cyphenothrin and cypermethrin) (Akinneye, Adedolapo & Adesina, 2018; Mdeni, Adeniji, Okoh & Okoh, 2022). The impact of these chemicals on the nutritional composition of cereal grains is of great concern.

Studies have shown the prevalence of these chemical residues in stored products, affecting the health and nutritional value derived from their consumption (da Silva & Camoes, 2010; Adeyosoye *et al.,* 2016; Arowora *et al.,* 2020). The quality of these chemically stored grains is mainly influenced by the duration and method of storage**.** A high concentration of these chemicals may interfere with the nutritional constituents of food and non-food crops (Mulla *et al.,* 2020). The maximum residue limits (MRL) of organophosphate is shown to 0.05–1.00 mg/kg and the MRL of organochlorine pesticides are 0.01–1.00 mg/kg (Arowora *et al.,* 2020). Organochlorine pesticides, also known as lipophilic chemicals, vary in their mechanisms of toxicity because of their differences in chemical structures and their build-up at the higher trophic levels, which leads to bio-magnifications within the food chain (Mulla *et al.,* 2020). Pesticides and other storage chemicals potentially foster the rise of ROS in stored produce, which some studies have highlighted to promote degradation of the protein composition of crops, as well as distortion of the activities of postharvest enzymes (Sharma, Kumar, Thukral & Bhardwaj, 2019).

Chemical treatment of crops for storage may have undesirable effects that lead to decreased nutritional value or altered sensory properties. Little is known about the effect of storage chemicals on the physicochemical properties (such as gel consistency, water absorption, solubility, gelatinization, and bulk density), nutritional content involving proximate profile (crude fat, ash content, moisture content, nitrogen-free extract, and crude protein), carbohydrate (amylase, starch, and amylopectin), total dietary fibre and ash content (Hurtada *et al.,* 2020) of agro-produce in Africa.

Based on the chemical groups present in these chemicals, there is a possibility of the adduct (or metabolites) formation resulting from chemical interactions between the nutrients and phytochemicals present in the crops, thereby reducing or limiting the availability of these nutritional components of crops and inhibiting food enzymes (Chandra, Sharpanabharathi, Prusty, Azeez & Kurakalva, 2021).

Studies on the nutritional composition and pesticide residue levels in some chemically stored cereals grains (wheat, sorghum, maize, and millet) sold in Africa showed the ash content and crude protein, organochlorine, and organophosphate levels were highest in wheat and lowest in maize. In contrast, the fat content was highest in millet and lowest in wheat, with all the organochlorine and organophosphate levels within the Maximum residue limits (MRLs) (Arowora *et al.,* 2020).

Studies have indicated that when 2, 3-dichorovinyl dimethyl phosphate (sniper) is applied at higher concentrations on cowpeas, the amount of its fat, crude protein, and fiber contents is reduced, whereas the ash and carbohydrate contents were significantly increased. Moreover, the decrease in protein, fat, and moisture was accompanied by an increase in microbial activities, resulting in the deterioration of protein and fat and an increase in moisture content (Aremu, Babajide, Ogunlade, Oyeniran & Kadiri, 2015; Tizhe, Dagze, Yusuf, Jacob & Mallum, 2021). The moisture content of chemically treated grains is usually maintained within the desired range since the chemicals effectively control pests' respiratory activities, increasing stored grains' moisture content. The mineral nutrients were observed to increase with increased storage duration and sniper concentration. These storage chemicals add to the bulk of the grain, thus leading to increased ash content (Maaleku & Kotey, 2014). The toxicity of storage chemicals in agric-produce depends on the concentration of pesticides, rate and duration of application, climate conditions, spraying

technique, organization of flora, temperature, pH, texture, humidity, and microbial activity (Sharma *et al.*, 2019).

Chemicals used for storage majorly possess insecticidal activities – deterring destructive insect pests and microorganisms from stored agricultural produce (Yigit & Velioglu, 2020). Despite the controversy about their health and environmental safety, these chemicals are still predominantly used by local farmers and agro-wholesalers in developing countries to ensure an all-year supply of several agriproducts, both in and off-season (Thapa *et al.*, 2021). The persistence in using these chemicals despite recent innovations of health and eco-friendly alternatives and government bans and regulations could majorly be due to poor or limited consequential knowledge of local farmers or wholesalers (Onwona Kwakye, Mengistie, Ofosu-Anim, Nuer & Van den Brink, 2018).

2.5 Biopesticides

The population of the globe is anticipated to reach 9.7 billion people by 2050, with the bulk of them living in Asia and Africa. The population is rising at an exponential rate. Due to the rising requirement for inputs in crop production, agriculture and its allied businesses are under a lot of strain to supply the world's food demands. In addition to significantly altering people's surroundings, human activities have also had an unfavourable influence on the environment and ecosystems. These effects include shrinking agricultural areas as a result of construction; a surge in nutrient mining; depletion and contamination of water resources, leading to a shortage of water; the accumulation of xenobiotics in soils; and degradation and deterioration of soil quality, fertility, and efficiency, with implications on soil erosion and climate change.

To conquer these barriers and meet the wants for sustenance and food, agricultural practices' productivity and sustainability had to be raised, and new and improved techniques needed to be found. Increased agricultural production may be accomplished in a variety of methods,

such as by adding manure and organic treatments like biopesticides, or by lowering yield loss brought on by severe environmental variables including biotic and abiotic stressors (Pathak, Maurya, Singh, Häder & Sinha, 2018; Goncalves, 2021). The application of biostimulants and bioeffectors can greatly reduce abiotic stress (Van Oosten, Pepe, De Pascale, Silletti & Maggio, 2017). Biopesticides, and insecticides generated from natural ingredients or living microorganisms, show tremendous potential for lowering yield loss without losing product quality.

Because of their negative side effects, chemical pesticides, which are applied in agricultural regions to minimize the damage caused by pests and illnesses, represent various long-term hazards and threats to living things. Due to their limited biodegradability, they linger in the environment for many years (Kalliora *et al.*, 2002), and are known to generate cancers (Nicolopoulou-Stamati *et al.*, 2016) and fetal impairments (Kalafati, Barouni, Karakousi, Abdollahi & Tsatsakis, 2018). Furthermore, these synthetic pesticides are extensively employed and have a major influence on product manufacture due to their high inhibitory action against pests and various uses (Liu *et al.*, 2021). Research suggests that the worldwide market for synthetic and biopesticide insecticides was valued at USD 61.2 billion in 2017 and is predicted to reach about USD 79.3 billion by 2022 (Lehr, 2014; Chen, 2018). Crops planted on soils extensively treated with chemical pesticides typically experience nutrient loss and a rise in disease incidence (Tripathi, Srivastava, Devi, & Bhadouria, 2020), which is undesirable from the standpoint of agricultural soil management for food and nutritional security. China, the United States, and Brazil are the top three nations in the world in terms of pesticide consumption, according to the Food and Agriculture Organisation (FAO), United Nations (2017– 2018). Additionally, the FAO reported that between 2015 and 2018, the United States accounted for 32.4% of the world's pesticide consumption, followed by

Asia with 52.2%, Europe with 11.8%, Africa with 2%, and Oceania with 1.6% (FAOSTAT, 2021).

Agricultural pests and illnesses are handled with the use of biopesticides, which are naturally occurring compounds originating from plants, animals, and microorganisms including bacteria, cyanobacteria, and microalgae. Biopesticides are "derived from natural materials such as animals, plants, bacteria, and certain minerals," according to the US Environmental Protection Agency. Crop damage can be averted by employing by-products from these biocontrol agents, such as genes or metabolites. Because biopesticides are host-specific and ecologically benign, they are much more beneficial to utilize than their traditional chemical equivalents (Essiedu *et al.*, 2020). Biopesticides can considerably boost the utilization and deployment of agro-based chemicals in the agricultural sector to protect crop plants against invasive and contagious pests (Essiedu *et al.*, 2020).

2.5.1 Types of biopesticides

Biopesticides occur in a number of forms, and they are categorized based on the molecules or compounds employed in their manufacture as well as the sources from which they are extracted (Ruiu, 2018).

2.5.1.1 Microbial pesticides

These are derived by microbes such as viruses, fungi, and bacteria. Certain pest species or entomopathogenic nematodes are combated by the active molecules or compounds that have been isolated from these organisms. Bioherbicides are weed-controlling agents that employ microorganisms, including fungus, to reduce weeds; bioinsecticides, on the other hand, target insects that injure crops. Numerous biopesticides have been found and developed over the past 10 years owing to extensive research on microbial biopesticides, which has also cleared the road for their commercialization (Ruiu, 2018). Many novel microbial species and strains, together with their important toxins and virulence factors that might be helpful

for the biopesticide business, were found as a result of the successful usage of *Bacillus thuringiensis* (Bt) and some other microbial species. Some of these strains have also been turned into commercial goods (Ujváry, 2001; Ruiu, 2018). While fungi include species of *Beauveria, Metarhizium, Verticillium, Lecanicillium, Hirsutella, Paecilomyces,* and other species, important families of bacterial entomopathogens include species of *Pseudomonas, Yersinia* and *Chromobacterium* (Sporleder & Lacey, 2021). Species-specific baculoviruses, whose infectivity is connected to crystalline occlusion bodies that are active against eating insects (Lepidopteran caterpillars), are among the other primary manufacturers of microbial pesticides (Chang *et al.,* 2003). The recombinant *baculovirus, ColorBtrus*, is formed when the baculoviral occlusion body and the Bt toxin join to form a virion. This occlusion body includes the Bt insecticidal Cry1Ac toxin protein, which improves the occlusion body's pathogenicity and rapidity of action compared to its wild-type counterpart. Entomopathogenic nematodes (EPNs) are innocuous to mammals, the environment, and nontarget species. They are usually found in the genera *Heterorhabditis* and *Steinernema* and are connected with mutualistic symbiotic bacteria of the genera *Photorhabdus* and *Xenorhabdus*. Their simplicity of mass manufacture, capacity to utilize in vitro or in vivo methodologies, and absence of registration requirements have made their commercial development as biocontrol agents uncomplicated (Chang *et al.,* 2003).

2.5.1.2 Biochemical Pesticides

Chemical pesticides employ produced chemicals that kill bugs directly, while biochemical pesticides use naturally occurring components and manage pests using safe techniques (Kumar, Ramlal, Mallick & Mishra, 2021). According to whether they employ plant extracts or oils, natural insect growth regulators, or pheromones (semi-chemicals) to control insect pest infestations, biochemical pesticides are further categorized into many classes (Kumar *et al.,* 2021).

a). Insect Pheromones

These are chemicals generated by insects that are cloned and utilized in integrated pest management systems to control insects. These compounds are efficient in stopping insects from successfully mating, which limits the amount of insects that may reproduce. The insects exploited in this method operate as pheromone dispensers that get confused due to the presence of pheromone flumes diffused in the surroundings. According to González-Coloma *et al*. (2013), insect pheromones are not real "insecticides" as they affect an insect's olfactory system without killing the insect. Pheromones are picked up by the antennae of the perceiving insect and then diffuse into the sensilla's interior through microscopic cuticle pores. Pheromone-binding proteins (PBPs) then deliver them to the chemosensory membranes via the hydrophilic sensillum. The pheromone or pheromone–PBP complex subsequently attaches to a particular receptor protein, which is responsible for transforming the chemical signal into an amplified electric signal through the activity of a second messenger system in cooperation with brain machinery (Gurr, Thwaite & Nicol, 1999).

b). Plant-Based Extracts and Essential Oils

Insect pest management has witnessed an increase in the use of plant-based extracts and essential oils as efficient replacements for synthetic pesticides in recent years. Since these pesticides are sourced from plants and include a variety of bioactive compounds, they are naturally occurring insecticides (Magierowicz, Górska-Drabik & Golan, 2020). Plant extracts and essential oils (EOs) have a wide variety of activities against insects, depending on the physiological features of the insect species and the kind of plant. They can work as attractants, repellents, or antifeedants; they can also slow respiration, make it harder for insects to identify their hosts, hinder oviposition, and limit adult emergence through larvicidal and ovicidal activities (Tripathi, Upadhyay, Bhuiyan & Bhattacharya, 2009; Ali

et al., 2017). Their makeup varies widely. Neem and lemongrass oil are two well-known examples in this regard, as they are readily accessible in herbal shops throughout the globe. According to a comprehensive study by Halder, Rai and Kodandaram (2013), neem oil and entomopathogenic bacteria—specifically, *Beauveria bassiana*—worked remarkably well together to control pests that feed on plants. To avoid injuring nontarget species, it is vital to determine the quantity of *azadirachtin* concentration in neem oil (Mordue, Morgan & Nisbet, 2005). To successfully manage the target pests without injuring nontarget insects, an equivalent method for entomopathogenic fungi must be established and supported by complementing laboratory bioassays, station, and/or field investigations (Dannon *et al.,* 2020). In terms of marketability, essential oils really constitute a market worth USD 700.00 million with a total global production of 45,000 tonnes. Additionally, US firms are able to introduce essential oil-based pesticides faster than they can with traditional pesticides.

c). Insect Growth Regulators

Insect growth regulators, or IGRs, kill insects by delaying critical processes that are important to their existence. Moreover, Gurr *et al*. (1999) demonstrated that these drugs exhibit high selectivity and negligible toxicity towards nontarget species. IGRs have lately been split into two classes depending on their mechanisms of action: chemicals that impede the activity of insect hormones (such as ecdysteroids and juvenile hormone analogs) and chitin synthesis inhibitors (CSIs) (Gwinn, 2018). IGRs are not especially harmful to adult insects, but they are efficient in suppressing a range of insects, including fleas, cockroaches, and mosquitoes. Despite their minimal toxicity to humans, they hinder immature insects from breeding, hatching their eggs, and moulting from one stage to the next. When coupled with other insecticides, they can even kill adult insects. (Gwinn, 2018).

2.5.2 Importance of biopesticides

Compared to traditional chemical pesticides, biopesticides have a number of advantages. They are strong enough to take the role of synthetic pesticides for pest control since they are non-toxic to nontarget species, ecologically benign, and target-specific. Because biopesticides may be effectively employed in sustainable agricultural practices, their application has been increasing in recent years (Pathak *et al.*, 2018; Goncalves, 2021). As a crucial part of integrated pest management (IPM) programmes, biopesticides can minimise the usage of conventional pesticides since they are very efficient in small doses and break down fast without leaving harmful residues (Damalas & Koutroubas, 2018). Nevertheless, despite the benefits of utilizing biopesticides, their application has not been as common as anticipated for the reasons listed below:

1. The high cost of producing pesticides is a result of the expenses associated with creating, testing, and obtaining regulatory approval for novel biological agents.

2. Limited shelf life since biopesticides are susceptible to changes in humidity and temperature.

3. Limited field efficacy as a result of geographical and climatic changes in soil type, humidity, and temperature.

4. Farmers have little interest in biopesticides because of their high specificity—that is, the fact that they work solely against the illnesses and pests that they are intended to target. In the field, they must employ a variety of biological agents to manage various infections and pests. These agents are not accessible for every pest or disease and are also perplexing, expensive, and laborious.

2.6 Biocidal effect of orange-peel

Orange peel has natural components that have the power to either kill or stop the growth of germs. This property is known as biocidal effects. Essential oils and other bioactive substances present in the peel are primarily responsible for these biocidal qualities.

1. Antimicrobial activity: Research has shown that the essential oil extracted from orange peels is effective against a variety of germs and fungi. Pathogens including Salmonella enterica, Staphylococcus aureus, Escherichia coli, and Candida albicans can all be inhibited by it. Orange peels have antibacterial properties that make them a viable natural substitute for managing microbial development (Baba, Mohammed, Ya'aba & Umaru, 2018; Oikeh, Oviasogie & Omoregie, 2020).

2. Antifungal activity: Orange-peel extracts have demonstrated antifungal properties against various fungal strains. They can inhibit the growth of common fungi like *Aspergillus*, *Penicillium*, and *Fusarium* species. These antifungal properties can be beneficial for food preservation and controlling fungal infections (Hernández *et al.*, 2020).

3. Insecticidal activity: The essential oil and compounds present in orange-peel have shown insecticidal properties, acting as natural insect repellents and insecticides. They can repel or kill pests like mosquitoes, flies, ants, and termites. This makes orange-peel a potentially useful natural alternative to synthetic insecticides (Ezeonu, Chidume & Udedi, 2001; Oboh *et al.*, 2017).

4. Larvicidal activity: Orange-peel extracts have been reported to exhibit larvicidal activity against mosquito larvae. They can effectively kill mosquito larvae and disrupt their life cycle, helping to control mosquito populations and reduce the

transmission of mosquito-borne diseases (Murugan *et al.*, 2012; Abiodun & Opeyemi, 2022).

2.7 Biocidal effect of garlic

Garlic (*Allium sativum* L.) has acquired a reputation in different traditions as a prophylactic as well as therapeutic medicinal plant. Garlic has played important dietary and medicinal roles throughout history. Garlic has been recognized for its various health benefits and has been used for centuries for its medicinal properties. It contains a compound called allicin, which is responsible for its characteristic smell and many of its therapeutic effects. Allicin is known to have antimicrobial properties, meaning it can inhibit the growth and survival of microorganisms such as bacteria, fungi, and viruses. When garlic is crushed or chopped, the enzyme alliinase converts the chemical compound alliin into allicin. Allicin is a potent biocide that can kill or inhibit the growth of a wide range of microorganisms. It works by disrupting the function of enzymes and proteins necessary for the survival and replication of these microorganisms.

Several studies have demonstrated the biocidal effect of garlic against various pathogens. Garlic has been shown to have antibacterial activity against common bacteria, including *Staphylococcus aureus, Escherichia coli*, and *Salmonella* species. It has also been found to inhibit the growth of certain fungi, such as *Candida albicans*, which can cause yeast infections.

2.8 Phytochemicals

Phytochemicals, often referred to as phytonutrients, are natural bioactive components found in foods such as; vegetables, fruits, whole grain products, nuts and seeds, legumes, tea and dark chocolate. Although there are tens of thousands of phytochemicals, only a small number have been isolated and identified from plants and plant products (Cao *et al.*, 2017;

Singh & Chaudhuri, 2018). Phytochemicals are responsible for the colours, flavours and aromas of plants, and they play a role in protecting plants from environmental threats such as pest, diseases and UV radiation. The most common phytochemicals in food include polyphenols, carotenoids, flavonoids, coumarins, indoles, isoflavones, lignans, organosulfures, catechins, phenolic acids, stilbenoids, isothiocyanates, saponins, procyanidins, phenylpropanoids, anthraquinones, ginsenosides etc. (Xiao, 2017; Zhao *et al.*, 2018).

2.8.1 Flavonoids

Fruits, vegetables, herbs, and other plant-based meals are excellent sources of flavonoids, a form of phytochemicals. They have been explored for their potential medicinal uses and antibacterial characteristics in addition to their range of biological activities. Some essential information on the antibacterial characteristics and medicinal uses of flavonoids are as follows:

1. Antimicrobial activity: Studies have established the antimicrobial activity of flavonoids against a range of pathogens, such as viruses, fungi, and bacteria. They have the capacity to block microbial growth and inhibit microbial enzyme action. Flavonoids may interfere with microbial adhesion, disrupt the integrity of microbial cell membranes, and impede microbial nucleic acid synthesis (Dias, Pinto & Silva, 2021). While several flavonoids have proved to have broad-spectrum antimicrobial effects, others have more specific antibacterial activity.
2. Antibacterial effects: Several flavonoids have shown antibacterial activity against common pathogens, including *Staphylococcus aureus, Escherichia coli, Salmonella* spp., and *Helicobacter pylori*. They can interfere with lipid bilayers by inducing bacterial membrane disruption and inhibit several processes such as biofilm

formation, cell envelope synthesis, nucleic acid synthesis, electron transport chain, and ATP synthesis (Górniak, Bartoszewski & Króliczewski, 2018).

3. Antifungal effects: Flavonoids have been investigated for their antifungal properties against various fungi, including *Candida species, Aspergillus species*, and dermatophytes. There are several antifungal mechanisms exerted by flavonoids, such as disruption of the plasma membrane, induction of several mitochondrial dysfunctions, and inhibition of cell wall formation, cell division, and RNA and protein synthesis (Al Aboody & Mickymaray, 2020).
4. Antiviral effects: Some flavonoids have shown antiviral activity against different types of viruses, including herpes simplex virus (HSV), human immunodeficiency virus (HIV), influenza virus, and hepatitis C virus (HCV). They can interfere with viral entry into host cells, inhibit viral replication, and modulate host immune responses (Lalani & Poh, 2020).
5. Anti-inflammatory and immunomodulatory effects: Flavonoids have been recognized for their anti-inflammatory properties. They can inhibit the release of inflammatory mediators, such as cytokines and prostaglandins, and modulate immune cell activity. By reducing inflammation, flavonoids may contribute to the management of various inflammatory conditions (Zaragozá, Villaescusa, Monserrat, Zaragozá & Álvarez-Mon, 2020; Payam, Maral, Mozhdeh, Mahmoud & Rezvan, 2021).
6. Antioxidant effects: Many flavonoids exhibit antioxidant activity, which helps to neutralize harmful free radicals and protect cells from oxidative damage. The antioxidant action mechanisms of flavonoids can be by the (a) direct scavenging of ROS, (b) inhibition of ROS formation through the chelation of trace elements (e.g., quercetin has iron-chelating and iron-stabilizing properties), or inhibition of the

enzymes that participate in the generation of free radicals (e.g., glutathione S-transferase, microsomal monooxygenase, mitochondrial succinoxidase, NADH oxidase, and xanthine oxidase), and (c) activation of antioxidant defences (e.g., upregulation of antioxidant enzymes with radical scavenging ability) (Agrawal, 2011; Kumar & Pandey, 2013; Kaleem & Ahmad, 2018; Dias, Pinto & Silva, 2021).

7. Other medicinal roles: Flavonoids have been investigated for their potential roles in various health conditions, including cardiovascular health, cancer prevention, neuroprotection, and diabetes management. They may help improve blood vessel function, reduce the risk of certain cancers, protect against neurodegenerative diseases, and regulate blood sugar levels.

It's important to note that while in vitro and animal studies have shown promising results regarding the antimicrobial and medicinal properties of flavonoids, further research, including clinical trials, is needed to fully understand their effectiveness and safety in humans. Moreover, the bioavailability and pharmacokinetics of flavonoids can vary, and their potential health benefits should be considered as part of an overall balanced diet and lifestyle.

2.8.2 Alkaloids

For thousands of years, plants have been a well-known source of traditional medicine, helping to alleviate human maladies and enhance health (Rupani & Chavez, 2018; Sadia *et al.*, 2018). According to Ishtiyak and Hussain (2017), plants are a rich source of a wide spectrum of active compounds with essential medical benefits, including antiviral, anticancer, analgesic, and antitubercular characteristics. The major secondary metabolites known as alkaloids were originally identified and exploited as early as 4,000 years ago, and they are generally known for having a wide range of medical uses (Amirkia & Heinrich, 2014). Alkaloids are categorized into numerous classes, including indole, purine, quinoline,

isoquinoline, tropane, and imidazole, depending on their heterocyclic ring structure and biosynthetic precursor (Kaur & Arora, 2015; Roy, 2017). Alkaloids exhibit antioxidant, antimicrobial, and antiproliferative characteristics that make them ideal for medicinal development (Qiu *et al.,* 2014). Alkaloids' potential as a medication enhances its industrial usage. Many investigations on the pharmacological features of different alkaloids that are produced by plants have been undertaken.

Alkaloids are groupings of chemical composites that develop spontaneously and mainly consist of simple nitrogen atoms. Additionally, certain neutral or weakly acidic compounds might be present (Manske & Holmes, 2008). A small number of synthesized compounds are likewise recognized as alkaloids. Alkaloids can comprise sulphur in addition to carbon, nitrogen, or hydrogen, and occasionally bromine, phosphorus, or chlorine. Numerous diverse species, such as fungi, bacteria, plants, and mammals, create these secondary metabolites. They are isolated from the crude extract by acid-base extraction due to their wide spectrum of pharmacological activities (anticancer, antimalarial, anaesthetic, stimulant) (Ziegler & Facchini, 2008).

Drug discoveries obtained from natural sources are important to provide a more diversified portfolio of pharmaceuticals for human use. Furthermore, compared to manufactured molecules that may be detected as substrates by active transporters, natural goods are more likely to imitate endogenous metabolites and metabolic intermediates. The demand for natural product-based drugs endures despite modifications in discovery strategies, most notably the emergence of molecular biology-derived medicines. This strategy has been demonstrated to be highly successful.

Alkaloids are crucial to human health as well as an organism's defence systems. Roughly 20% of the secondary metabolites found in plants are alkaloids. Alkaloids in plants govern

growth and give predator defence. Alkaloids are notably well-known for their anaesthetic, cardioprotective, and anti-inflammatory activities when used therapeutically. Quinine, nicotine, morphine, strychnine, ephedrine, and quinine are well-known alkaloids that are exploited in medicinal contexts (Kurek, 2019). Bioactive natural materials have witnessed a recent uptick in interest due to their potential for drug discovery as well as a very proactive development in the field of traditional medicines (ethnopharmacology) (Atanasov *et al.*, 2021).

2.8.3 Tannins

Infections caused by bacteria are treated with antibiotics. Antibiotics are used in many different industries, including agriculture, aquaculture, and cattle breeding, in addition to their medical use. β-lactam antibiotics, macrolides, aminoglycosides, sulfonamides, quinolones, lincosamides, and glycopeptides are the most often used antibiotics. Nevertheless, the overuse and abuse of antibiotics led to the emergence of bacteria resistant to several drugs. When antibiotic resistance develops, the majority of medications are insufficient to treat bacterial infections. Antibiotic resistance is a serious public health issue on a global scale.

Antibacterial agents are necessary to combat pathogenic microorganisms, particularly those that are resistant to drugs. Since natural plant chemicals often have little toxicity to humans and animals, they are regarded as a potential source of effective antibacterial agents. Many plants, including many plant-based foods, contain tannins. Plants that are known to be high in tannins include mimosa, chestnut, quebracho, maple trees, acacia, oak, and eucalyptus. Wine, coffee, and tea are among the drinks that contain tannins. Food tannins are mostly found in fruits, such as pomegranates, blackberries, cranberries, and persimmons. Tannin is present in relatively large amounts in unripened fruits, and its presence shields the fruits from animals that feed on them before they mature. Furthermore, tannins play a critical role

in a plant's defence systems and protect against herbivores and insects, among other predators. Additionally, tannins have an impact on how plants develop. Numerous researches have examined the use of extracts high in tannins in traditional medicine to treat a range of illnesses, including bacterial infections (Bhalodia & Shukla, 2011). Tannins have strong antibacterial properties. Farha *et al.* (2020) conducted a search of the Web of Science Core Collection to locate papers that detailed the antibacterial and antivirulence properties of tannins, along with their potential mechanisms of action.

According to Kumar *et al*. (2013), the main mechanisms of antibiotic resistance in bacteria include altering the drug target site, having enzymes inactivate the antibiotics, reducing the intracellular concentration of antibiotics by decreasing membrane permeability, or expelling the antibiotics through efflux pump mechanisms. Natural products have the ability to impede the mechanisms by which bacteria develop resistance to conventional antibiotics. Tannins have been used for thousands of years in traditional medicine, and their potential therapeutic benefits have been studied. The following are a few recognised medicinal uses for tannins:

1. Antioxidant activity: Tannins possess strong antioxidant properties, meaning they can neutralize harmful free radicals and protect cells from oxidative damage. Oxidative stress is associated with various chronic diseases, including cardiovascular diseases, neurodegenerative disorders, and cancer (Hagerman *et al.,* 1998; Park, Cho, Jung, Lee & Hwang, 2014; Ajah, Unegbu, Alaebo & Odo, 2021). The antioxidant effects of tannins may contribute to their potential health benefits in preventing or managing these conditions.
2. Anti-inflammatory effects: Tannins have been found to exhibit anti-inflammatory activity. They can help reduce the production of inflammatory mediators, such as cytokines and prostaglandins, and inhibit the activity of enzymes involved in the

inflammatory response. By reducing inflammation, tannins may have a positive impact on inflammatory conditions such as arthritis, inflammatory bowel disease, and certain skin disorders (Sugiura, Tanaka, Katsuzaki, Imai & Matsushita, 2013; Park, *et al.*, 2014).

3. Antimicrobial activity: Studies on tannins against bacteria, fungi, and viruses have revealed their antibacterial capabilities (Kaczmarek, 2020). Through the rupture of their cell membranes, interference with enzyme function, and suppression of microbial adhesion, they can hinder the development and survival of bacteria. Tannins' antimicrobial and wound-healing characteristics have made them helpful in traditional medicine.
4. Anti-diarrheal effects: Due to their astringent characteristics, tannins have long been used to treat diarrhoea by toning and tightening the tissues that line the intestines. Tannins can constrict tissues by building complexes with proteins, which can restrict fluid flow and increase the consistency of faeces (Bonelli *et al.*, 2018).
5. Digestive health: By decreasing inflammation in the digestive tract, supporting the formation of beneficial gut flora, and guarding against gastrointestinal infections, tannins may enhance digestive health. Their probable value in treating diseases including diarrhoea, inflammatory bowel sickness, and ulcers has been examined (Audi *et al.*, 1999; Fiori *et al.*, 2013; Lai, Lai, Nalamolu, & Ng, 2016).
6. Cardiovascular health: Studies show that specific tannins, notably those in wine and tea, may be helpful for the cardiovascular system. Along with enhancing endothelial function, decreasing blood pressure, and reducing oxidative stress, they may also help prevent the oxidation of LDL (bad) cholesterol, which is a key factor in the development of atherosclerosis.
7. Potential anti-cancer effects: Tannins' potential anti-cancer properties have been researched. According to Booth *et al*. (2013), they may trigger apoptosis, which is the

termination of a cell cycle, limit the development of cancer cells, and disrupt many signalling pathways that are important in the beginning and spread of cancer. To properly appreciate the mechanisms and prospective therapeutic benefits of tannins in the treatment of cancer, additional investigation is essential.

8. It's vital to realize that the effects of tannins could change depending on their chemical makeup, dosage, and the particular medical condition under research. In addition, a variety of factors, such as how food is cooked and how it interacts with other substances, could alter the bioavailability and absorption of tannins.

2.8.4 Saponins

Most plants naturally contain non-volatile glycosidic compounds called saponins. "Saponins," derived from the Latin word "Sapo," which means "soap," are widely renowned for their propensity to make foam. Like other anti-nutrients such as tannins, phytates, lectins, etc., saponins have both good and negative effects (Sharma, Kumar, Kaur, Tanwar & Goyal, 2021). These are triterpenoid secondary metabolites that have been found and extracted from diverse plant parts (seeds, leaves, flowers, and roots) to date. Approximately sixty unique forms of triterpenoid saponins have been found. They were commonly employed in soaps and other cleaning detergents due to their foaming characteristics. Because they have hydrophilic sugar moieties covalently linked to a triterpene backbone or hydrophobic steroid, they are surface-active by nature (Zhu, Wen, Yi, Cao & Liu, 2019). This makes it appear like a good alternative for natural emulsifiers in the food and pharmaceutical sectors, as well as foaming, stabilizing, and drug delivery agents. The application of saponin as an effective additive for producing and sustaining oil-in-water and nano-emulsions has been the topic of various studies. For example, quillaja saponin is utilized to boost the stability of the nano-emulsion in food and its antioxidant activity (Doost, Van-Camp, Dewettinck & Van der Meeren, 2019).

In addition, they have a range of biological features, including anti-inflammatory, hypocholesterolemic, hypoglycemic, antioxidant, and anticancer actions. Because of their antibacterial, antifungal, anti-parasitic, and molluscicidal effects, saponins are also connected to defense mechanisms in plants (Kitagawa *et al.,* 2016). While dioscoresides, gracillin, and pseudo-protodioscin are used to treat rheumatism and are useful against other chronic illnesses, saponins' dioscoresides make them acceptable for the treatment and prevention of cardiac and cerebrovascular problems (Parama *et al.,* 2020). Ashour *et al.* (2019) revealed that saponins were also functioning as a beginning precursor for the semi-synthesis of steroidal drugs in the pharmaceutical sector.

Numerous favourable effects have been found in saponins, which have been explored for prospective medicinal uses. The following are some accepted applications of saponins in medicine:

1. Effects on cholesterol levels: It has been observed that certain saponins can lower cholesterol (Milgate & Roberts, 1995). They have the capacity to inhibit the intestines from absorbing cholesterol from meals, which decreases blood levels of LDL (bad) cholesterol. Saponins may help lessen the risk of heart disease and increase cardiovascular health by reducing LDL cholesterol.

2. Anti-inflammatory effects: Studies have been undertaken on saponins' probable utility in the treatment of inflammatory illnesses due to their anti-inflammatory characteristics. They have the capacity to reduce the manufacture of inflammatory mediators and change the immune response. According to Hassan *et al.* (2011), saponins have proven potential to decrease inflammation in disorders including skin issues, asthma, and arthritis.

3. Antioxidant activity: A vast variety of saponins have proved to contain antioxidant activity, which enables them to scavenge free radicals and safeguard cells from oxidative stress. Saponins aid in decreasing oxidative stress by scavenging free radicals, which is associated with a variety of chronic illnesses, such as cancer, neurological disorders, and cardiovascular diseases (Tapondjou *et al.,* 2011; Bi *et al.,* 2012).

4. Antimicrobial effects: Studies have revealed the antibacterial activity of saponins against viruses, fungi, and bacteria. They can harm microorganisms' cell membranes, decrease microbial enzymes, and hinder their ability to proliferate. Researchers have looked at the probable use of saponins as natural antibacterial agents, which could assist in the battle against infections brought on by a number of pathogens (Shao, Wang, Zhu, Dang & Yu, 2020; Zhao, Xue, Zhang & Wang, 2018).

5. Immunomodulatory effects: By increasing immunological responses or lowering excessive immune activity, saponins can impact the immune system. They can boost macrophage activity and encourage the growth of immune cells like T cells and natural killer cells. Researchers have looked at the probable use of saponins as adjuvants in vaccinations and immunological illnesses (Lacaille-Dubois, 1999; Bhardwaj *et al.,* 2014).

6. Anticancer properties: Certain saponins have been demonstrated to have anti-tumor effects. They can disrupt signalling pathways implicated with the genesis and spread of cancer, inhibit the growth of cancer cells, and cause apoptosis (programmed cell death) (Xu *et al.,* 2016; Elekofehinti, Iwaloye, Olawale, & Ariyo, 2021). Although greater investigation is necessary to completely appreciate the specific mechanisms underpinning saponins' therapeutic potential in the treatment of different kinds of cancer, their optimal usage is yet unclear.

7. Hepatoprotective effects: Some saponins have been proven to have hepatoprotective capabilities, which means they can help shield the liver from injury brought on by diseases or toxins (Qu, Xin, Zheng, Su, & Ling, 2012; He *et al.*, 2021). They can raise the antioxidant capacity of liver cells, reduce hepatic inflammation, and improve liver function.

Hydroxyl scavengers

A substance or chemical that may react with hydroxyl radicals (OH) to inhibit their damaging effects is referred to as a hydroxyl scavenger, also known as a hydroxyl radical scavenger. Because of their high reactivity, hydroxyl radicals may destroy biological components like proteins, DNA, and lipids by oxidatively stressing them (Patterson, Madamanchi, & Runge, 2000). Hydroxyl scavengers operate by providing the hydroxyl radical an electron or hydrogen atom, which neutralizes its reactivity and inhibits it from inflicting additional harm. These scavengers are widely explored and applied in the field of antioxidant research since they can assist in protecting cells and tissues from oxidative stress. Numerous antioxidants, such as ascorbic acid (vitamin C), tocopherols (vitamin E), flavonoids, and polyphenols are examples of hydroxyl scavengers. These chemicals can scavenge hydroxyl radicals and minimize the damage they inflict on biological systems. Since hydroxyl radicals are transitory and very reactive, it is tough to target them directly *in vivo* with scavengers. Nonetheless, by keeping a healthy cellular redox state, antioxidants with extensive reactivity and the potential to create new antioxidants might help indirectly scavenge hydroxyl radicals.

Nitric oxide scavenger

In vertebrate biology, nitric oxide (NO) has become one of the most fascinating molecules (Reeves, Simakajornboon, & Gozal, 2008). According to Kim, Zamora, Petrosko, and Billiar (2001), NO is a lipophilic and highly diffusible solute that forms inside the cell and has concentration-dependent effects. While NO has been connected to a wide range of

remarkably varied biological processes, its participation in neurotransmission has gotten the greatest attention (Reeves *et al.,* 2008). It has multiple physiological actions, such as modulating blood pressure and synaptic plasticity as well as several cellular roles, such as influencing cell proliferation, differentiation and apoptosis (Lloyd-Jones and Bloch, 1996).

A substance or molecule that can react with and remove nitric oxide from biological systems is referred to as a nitric oxide scavenger or nitric oxide (NO) scavenger. Although nitric oxide (NO) is a signalling molecule that is involved in many physiological processes, excessive or uncontrolled NO levels have the potential to injure cells and aggravate several disease problems. To reduce the concentration and biological activity of nitric oxide, nitric oxide scavengers react with nitric oxide to form stable molecules. Haemoglobin is one sort of nitric oxide scavenger. Because of its tremendous affinity for nitric oxide, haemoglobin may adhere to it and carry it out of circulation. This haemoglobin feature is vital for managing nitric oxide levels and sustaining the body's equilibrium of the molecule. Certain metal complexes, such as ruthenium complexes, and certain antioxidants, such as ascorbic acid (vitamin C), have also been explored as nitric oxide scavengers. These chemicals can react with nitric oxide to form stable compounds and diminish their concentration. Since nitric oxide is vital for various physiological activities, including vasodilation, neurotransmission, and the immune system, the scavenging of nitric oxide needs to be carefully managed. Consequently, it is crucial to target and manage the employment of nitric oxide scavengers to prevent interfering with these vital functions.

Superoxide scavenger

Animal life is maintained by the free energy that is created by oxidation from organic molecules. Reactive oxygen species (ROS) are created in response to humoral and neuronal inputs during times of heightened cellular metabolism. However, in pathological circumstances such as inflammation and ischemia–reperfusion damage, their production is

further elevated. When an oxygen molecule obtains one electron by enzymatic or non-enzymatic mechanisms, a primary radical known as superoxide is formed (Fridovich, 1995). Since superoxide is rapidly converted into other reactive oxygen species, establishing its specific significance in a given aetiology is tricky. Superoxide dismutase (SOD) undoubtedly performs key functions in the avoidance of ROS-mediated oxidative damage from the onset as the radical electron might kick off chain reactions (Fujii, Homma, & Osaki, 2022).

Overly high amounts of reactive oxygen species (ROS) within cells can cause oxidative damage to critical macromolecules, hastening the aging process and worsening various medical diseases. Reversible oxidative modification of target molecules is how moderate quantities of reactive oxygen species (ROS), notably hydrogen peroxide, impact cellular signalling (Rhee, 2006; Finkel, 2011). As a result, whilst antioxidant chemicals or enzymes might defend against oxidative damage, removing ROS too fast could injure cells by interfering with redox signalling (Lennicke & Cocheme, 2021). Because of these characteristics, redox equilibrium may be disrupted by both high ROS levels and an imbalance between ROS and antioxidants, which can result in health difficulties (Janssen-Heininger *et al.*, 2008).

Superoxide scavengers operate by supplying the superoxide radical electrons or hydrogen atoms, which changes it into a less reactive and non-damaging state. This process minimizes the detrimental effects of the superoxide radical and helps block it from launching oxidative chain reactions. Superoxide scavengers can be found in a range of compounds. Among the occurrences are:

1. Superoxide dismutase (SOD): Cells naturally contain this enzyme, which scavenges superoxide. It catalyzes the transition of less damaging hydrogen peroxide and molecular oxygen from superoxide radicals. There are several isoforms of SOD, such as extracellular SOD, manganese SOD, and copper/zinc SOD.

2. Manganese complexes: Research has revealed the capacity of certain manganese-based compounds, such as manganese porphyrins, to successfully scavenge superoxide radicals. These compounds can replicate the superoxide dismutase function.

3. Synthetic antioxidants: A variety of artificial compounds have been produced as superoxide scavengers, including tempol and MitoQ. These compounds can specifically target and scavenge superoxide radicals in addition to possessing antioxidant characteristics.

4. Natural antioxidants: A few naturally occurring compounds have the potential to scavenge superoxide radicals, including flavonoids, polyphenols, and vitamins (like C and E). These antioxidants can neutralize superoxide radicals' reactivity by giving them an electron.

Hydrogen peroxide scavenger

A substance or chemical that may react with and neutralize hydrogen peroxide in biological systems is referred to as a hydrogen peroxide scavenger, commonly called a hydrogen peroxide (H_2O_2) scavenger. Reactive oxygen species like hydrogen peroxide can injure cells and tissues through oxidative stress if their concentrations grow too high or uncontrolled. To prevent damage, hydrogen peroxide is converted into less reactive forms by hydrogen peroxide scavengers. According to Scaglione, Xu, and Ramanujan (2016), they can either directly react with hydrogen peroxide or help in its breakdown into water and oxygen. This minimizes the potential of hydrogen peroxide's detrimental effects and helps prevent it from building up. A variety of compounds are capable of scavenging hydrogen peroxide:

1. Catalase: A highly efficient enzyme that is found in cells, catalase scavenges hydrogen peroxide. Hydrogen peroxide is broken down into water and molecular oxygen by it.

2. Glutathione peroxidase: This enzyme and other peroxidases can employ reducing equivalents supplied by molecules such as glutathione to catalyze the reduction of hydrogen peroxide. A key aspect of the cellular antioxidant defence system is glutathione peroxidase.

3. Ascorbic acid (vitamin C): Ascorbic acid and hydrogen peroxide can react rapidly to form dehydroascorbic acid and water. Moreover, it replenishes other antioxidants, like vitamin E, which has the potential to scavenge hydrogen peroxide indirectly.

4. Transition metal ions: In the Fenton reaction, hydrogen peroxide is converted into exceedingly reactive hydroxyl radicals. Iron and copper are two examples of transition metal ions that can take part in this process. By scavenging hydrogen peroxide, chelating chemicals like EDTA, which are scavengers for these metal ions, can indirectly limit the generation of hydroxyl radicals.

Complete hydrogen peroxide elimination may not always be desirable, as hydrogen peroxide also works as a signalling molecule in various biological processes. Cellular activity and signalling pathways depend on the appropriate management of hydrogen peroxide levels.

Total antioxidant capacity

The ability of a biological sample to neutralize or scavenge distinct reactive oxygen species (ROS) and other oxidizing agents is assessed by its total antioxidant capacity or TAC. TAC assays are widely performed to measure a biological system's total antioxidant capabilities. Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP), and Oxygen Radical Absorbance Capacity (ORAC) are among the methodologies and tests that may be used to measure TAC. In these tests, the sample's antioxidant content

interacts with a particular oxidizing agent or radical, and the change in absorbance or fluorescence that follows is quantified. TAC assays have been utilized in scientific research to examine the antioxidant capability and putative health advantages of varied substances, such as plant extracts, natural products, and dietary antioxidants. This research typically measures TAC to appreciate the complete antioxidant potential and possible preventative advantages against diseases related to oxidative stress. For instance, Yildirim, Mavi, Kara, and Yildirim (2015) performed the FRAP test to assess the TAC of different plant extracts. The researchers tested the antioxidant capacity of extracts obtained from diverse plant sources and noticed considerable differences in their TAC values, which indicated diversity in their capability to scavenge chemicals that cause oxidation. Prior, Wu, and Shaich (2005) employed the ORAC test in a different experiment to compute the TAC of various fruits and vegetables. To acquire insight into the relative antioxidant capacities of various food sources, the researchers tested the antioxidant capacity of varied samples and compared their TAC values.

The ferric reducing antioxidant power (FRAP)

One technique that is widely used to measure the total antioxidant capacity (TAC) of biological samples is the ferric reducing antioxidant power (FRAP) assay. In the presence of a reducing agent, it examines the sample's antioxidant capacity to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) (Rahman, Islam, Biswas, & Khurshid-Alam, 2015).

Benzie and Strain originally described the FRAP test in 1996. Since then, it has been broadly recognized and employed in various research to measure the antioxidant potential of varied samples, such as meals, beverages, and body fluids. For instance, the FRAP test was employed by Serafini, Del Rio, Yao, Bettuzzi, and Peluso (2012) to evaluate the antioxidant potential of different fruit juices. The researchers analyzed the antioxidant capacity based on the change in absorbance and studied the reduction of the ferric-tripyridyltriazine

complex by antioxidants present in the juices. The obtained FRAP values made it feasible to compare the fruit juices under test for antioxidant capability. The FRAP assay was also utilized by Cao, Chai, and Prior (1997) to examine the antioxidant capacity of tea extracts. To establish the antioxidant capability of the tea's ingredients, the researchers measured the FRAP values and examined each component's power to reduce ferric ions. This test gave information on the various tea varieties' antioxidant capabilities. These examples explain how the FRAP test may be used to analyze and compare the antioxidant capacities of different samples, delivering vital insights into their putative health advantages and decrease of oxidative stress.

2,2-diphenyl-1-picrylhydrazyl (DPPH)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a prominent procedure for measuring a sample's or compound's antioxidant potential. It evaluates an antioxidant's ability to scavenge the stable free radical DPPH, which appears yellow when reduced from a purple state. The DPPH test was used by Aruoma, Halliwell, Aeschbach, and Löligers (1989) to investigate the antioxidant activity of different plant extracts. By evaluating how much DPPH the extracts lowered, the researchers were able to quantify the scavenging activity and determine how much absorbance dropped. This made it feasible to compare the antioxidant properties of the extracts that were studied. The DPPH test was used in research to evaluate the antioxidant activity of herbal extracts. The concentration needed to scavenge 50% of the radicals is indicated by the IC^{50} values, which the researchers utilized to measure the extracts' scavenging activity against DPPH radicals. The relative antioxidant capacities of the examined herbal extracts may be determined thanks to the DPPH test (Huang, Ou & Prior, 2005).

2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

The 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay is a frequently used technique to measure a sample's or compound's antioxidant activity. It analyses antioxidants' capacity to scavenge the ABTS radical cation (ABTS•+), which decreases to a colourless state from a blue-green one. The ABTS test was utilized in research by Benslama, Mouhoubi, Boulkroune, and Madani (2018) to assess the antioxidant activity of different medicinal plant extracts. The extracts were evaluated for their reduction of ABTS+, and the absorbance decrease was utilized to estimate the scavenging activity. This made it feasible to compare the antioxidant properties of the extracts that were studied. Leong, Shui, and An (2020) investigated the antioxidant capacity of various phenolic compounds using the ABTS assay. Trolox Equivalent Antioxidant Capacity (TEAC) values were utilized to transmit the findings of their study of the compounds' scavenging capacity against ABTS+. The findings of the ABTS test shed information on the varied antioxidant properties of the phenolic compounds under research.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals and reagents

The following reagents and chemicals were utilized in this study; Phosphate buffer (0.1M, pH 7.4), H_2O_2 (40mM) in phosphate buffer, EDTA (0.1M containing 1.5mg of NaCN), Nitrobluetetrazolium (NBT – 1.5mM) 17, Riboflavin (0.12mM), Phosphate buffer (0.067M, pH 7.6), Sodium nitroprusside (100mM), Phosphate buffered saline (pH 7.4), Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylethylene, diaminedihydrochloride), Deoxyribose (2.8mM), Ferric chloride (0.1mM), EDTA (0.1mM), H_2O_2 (1mM), Ascorbate (0.1mM), KH_2PO_4-KOH buffer (20mM, pH 7.4), Thiobarbituric acid (1%)

3.1.2 Glassware and equipment

The following glassware and equipment were utilized in this study; water bath, test tubes, BUCK M910 gas chromatograph fitted with flame ionisation detector, spectrophotometer, electric blender, conical flask, bijou bottles, media plates, microscope.

3.1.3 Collection of plant samples

Orange and garlic samples were obtained from vendors at Douglas Road Market (Ekeonuwa Market) in Owerri Municipal Local Government Area, Owerri, Imo State. The samples were identified and authenticated by a plant taxonomist, in the Department of Crop Science and Technology of the Federal University of Technology, Owerri (FUTO). Contaminated grains (rice and maize) were also sourced at Douglas Road Market from wholesalers that store these grains in bags.

3.2 METHODS

3.2.1 Extraction of plant materials

The samples were washed with clean water, peeled and allowed to air dry at room temperature. The dried samples were, pulverized to powder with electric blender and stored in airtight container. The powdered samples were subjected to Soxhlet extraction method. A 500 ml clean boiling flasks was dried in an oven at 105 - 110^{0}C for 30 min. It was transferred into a desiccator and allowed to cool. Weighed 100 g of the sample was poured into the Soxhlet thimble plugged lightly with cotton wool to aid filter the extract. Then the boiling flask was filled with 300 ml of ethanol and soxhlet set up was allowed to reflux for about 4 hr at 60^{0}C. Afterwards the thimble was removed carefully and the extract poured into a volumetric flask and allowed to cool. Finally, the content of the volumetric flask was transferred into a rotatory evaporator to separate the solvent (n-hexane) from the oil.

3.2.2 Determination of bioactive compounds of garlic and orange-peel samples

One gram (1g) of pulverized sample was weighed and transferred in a test tube and 15ml of ethanol was added. The test tube was allowed to react in a water bath at 60^{0}C for 60min. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extract was combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000ul of hexane of which 200µl was transferred to a vial for analysis.

Quantification with GC-FID

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15m x

250µm x 0.15µm) was used. The injector temperature was 280^{o}C with splitless injection of 2ul of sample and a linear velocity of 30cms^{-1}, Helium 5. 0pa.s was the carrier gas with a flow rate of 40 mlmin^{-1}. The oven operated initially at 200^{o}C it was heated to 330^{o}C at a rate of 3^{o}C min^{-1} and was kept at this temperature for 5min. the detector operated at a temperature of 320^{o}C.

Phytochemical were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals was expressed in µg/g.

3.2.3 Determination of chemical constituents using gas chromatography-mass spectrophotometry (GC-MS)

Phytochemical analysis was carried out using the method of Gas Chromatography-Mass Spectrometer (GC-MS) as described by Bezerra and Antoniosi Filho (2014). A weighed 1g of the extract was transferred in a test tube and 25 ml of ethanol was added. The contents of test tube were allowed to react in a hotplate at 60 ^{0}C for 90 min. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane, and all were transferred to the funnel. The extracts were combined and washed three times with 10 ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000 µl of pyridine of which 200 µl was transferred to a vial for analysis.

GC-MS Analysis

The phytochemical analysis was performed on a BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the

carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with acetonitrile was injected in a splitless mode. Relative quantity of the chemical compounds present in each of the extracts was expressed as percentage based on peak area produced in the chromatogram.

Identification of chemical constituents

Bioactive compounds of the plant extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

3.2.4 Determination of radical scavenging potentials of extracts garlic and orange-peel Samples

3.2.4.1 Determination of hydrogen peroxide scavenging activity

The ability of the leaf samples to scavenge hydrogen peroxide was assessed by the method of Ruch *et al.* (1989).

Procedure

A solution of H_2O_2 (40mM) was prepared in phosphate buffer. Leaf samples at the concentration of 10mg/10µl were added to H_2O_2 solution (0.6ml) and the total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer (Genesys 10-S, USA). A blank solution containing phosphate buffer, without H_2O_2 was prepared. The extent of H_2O_2 scavenging of the sample samples was calculated using the formula:

% scavenging of hydrogen peroxide = [(A0 – A1) \A0] × 100 A0 - Absorbance of control

A1 - Absorbance in the presence of sample

3.2.4.2 Determination of superoxide scavenging activity

The superoxide scavenging ability of the samples was assessed by the method of Winterbourn *et al.* (1975).

Principle

This assay is based on the inhibition of the production of nitrobluetetrazoliumformazon of the superoxide ion by the sample samples and is measured spectrophotometrically at 560nm.

Procedure

Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the leaf samples (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the sample samples. All the tubes were vortexed, and the initial optical density was measured at 560nm in a spectrophotometer (Genesys, 10-S, USA). The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

3.2.4.3 Determination of nitric oxide scavenging activity

The extent of inhibition of nitric oxide radical generation in vitro was followed as per the method reported by Green *et al.* (1982).

Principle

Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that are estimated spectrophotometrically at 546nm.

Procedure

The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of leaf samples (50mg) and incubated at 25^{o}C for 30 min. Griess reagent (0.5ml) was added and incubated for another 30 minutes. Control tubes were prepared without the samples. The absorbance was read at 546nm against the reagent blank, in a spectrophotometer (Genesys 10-S, USA).

3.2.4.4 Determination of hydroxyl radical scavenging activity

The extent of hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by Elizabeth and Rao (1990).

Principle

The principle of the assay is the quantification of 2'-deoxyribose degradation product, malondialdehyde, by its condensation with thiobarbituric acid.

Procedure

The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of $FeCl_3$, 0.1ml of EDTA, 0.1ml of H_2O_2, 0.1ml of ascorbate, 0.1ml of KH_2PO_4-KOH buffer and 20ml of samples in a final volume of 1.0ml. The mixture was incubated at 37^{o}C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95^{o}C for 20 minutes to develop the color. After cooling, the TBARS formation was measured spectrophotometrically (Genesys 10-S, USA) at 532nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with that of the samples. The per cent TBARS production for positive control (H_2O_2) was fixed at 100% and the relative per cent TBARS was calculated for the sample treated groups.

3.2.5 Determination of antioxidant capacity of extracts garlic and orange-peel samples

3.2.5.1 Determination of 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH)scavenging ability

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the method of Mensor *et al*. (2001).

Procedure

The leaf samples (20µl) were added to 0.5ml of 0.1mM methanolic solution of DPPH and0.48ml of methanol. The mixture was allowed to react at room temperature for 30minutes. Methanol served as the blank and DPPH in methanol, without the leaf samples, served as the positive control while butylatedhydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = 100 - A518\,(\text{sample}) - \frac{A518\,(\text{blank}) \times 100}{A518\,(\text{blank})}$$

3.2.5.2 Determination of ferric reducing antioxidant property (FRAP)

Principle

The principle of the assay is the quantification of ferric degradation product, by its condensation with the extract.

Method

The reducing property of the extracts will be determined as described by Pulido *et al*. (2000).

Procedure

A portion, 0.25 ml of the extracts was mixed with 0.25 ml of 200 mM Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% Potassium ferrocyanide. The mixture was then incubated at 50^{o}C for 20 min, thereafter 0.25 ml of 10% trichloroacetic acid will be added and

centrifuge at 2000 rpm for 10 min, 1 ml of the supernatant will be mixed with 1 ml of distilled water and 0.2 ml of ferric chloride and the absorbance was measured at 700 nm.

3.2.5.3 Total antioxidant capacity (TAC) assay:

Principle

This assay is based on the inhibition of the production of nitrobluetetrazolium formation of the superoxide ion by the plant extracts and is measured spectrophotometrically at 560nm.

Method

The Total Antioxidant Capacity (TAC) of extract in different extracting solvents (absolute ethanol, 70% and 50% ethanol) will be determined by the phosphomolybdate method according to Jayaprakasha *et al*. (2002).

Procedure

An aliquot (30 mL) of different concentrations (20, 40, 60, 80 and 100 mg mLG1) of the test extracts will be mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes will be capped with aluminium foil and incubated in a boiling water bath at 95°C for 90 min. The reaction mixture will be allowed to cool to room temperature and the absorbance of the solution was measured at 695 nm against a blank containing 3 mL of reagent solution and the appropriate volume of the dissolving solvents. The blank will be incubated under the same conditions as the test samples.

Ascorbic acid will be used as standard reference compounds to compare the activities of the extracts.

3.2.6 Determination of antimicrobial activities of extracts garlic and orange-peel samples

3.2.6.1 Preparation of media and diluents

Malt Extract Agar (MEA) and Potato Dextrose agar (PDA) were prepared according to manufacturer's specification. Nutrient agar was used in the isolation of heterotrophic fungi (Cheesbrough, 2000).

Distilled water used as diluents was prepared by dispensing 90 ml and 9 ml portion into conical flask and bijou bottles respectively. Both diluents and media were sterilized in an autoclave at 121^0C for 15min.

3.2.6.2 Preparation of samples and inoculation

Ten grammes (10g) of samples (Rice and Maize) was dispersed in 90 ml of sterile distilled water. Sample was serially diluted decimally by transferring 1 ml from each tube until 10^4 was obtained.

One-tenth milliliter (0.1 ml) of appropriate dilution was inoculated into the pre-sterilized and surface dried media (MEA and PDA). Inoculate were spread evenly to ensure discrete colonies. Plates were incubated at ambient temperature for 3-5 days for heterotrophic fungi (Beishir, 1987; Ameh & Kawo, 2017).

3.2.6.3 Determination of microbial population

Colony counts obtained on the media were expressed as colony forming units per gram (CFU/g) to obtain total population (Harrigan & McCance, 2000).

3.2.6.4 Characterization and identification of microbial isolates

Microbial isolates were characterized based on cultural (colonial) and microscopic methods with reference to standard manuals. The identities of the isolates were cross-matched with reference to standard manuals for the identification of fungi (Barnett & Hunter, 1997; Harrigan & McCance, 2000).

Procedure for staining and identification of fungi (yeasts and moulds)

Moulds were identified using the wet-mount method described as follows: A tiny portion of moulds was picked with the aid of sterile mounting needle onto a pool of lactophenol cotton blue at the center of a grease free slide. This preparation was covered with a sterile coverslip at the center of the slide. Then, excess fluids/stains on the slide were blot-dried with a cotton wool and the slide passed through a flame to burst accumulated gas. Furthermore, the slide was viewed at low magnification of x10and x40. Finally, the identification was done following the arrangement: mycelia/hyphae, septation and spore arrangement.

3.2.6.5 Testing for antifungal activity of plant extracts

Garlic and orange-peel extracts were used as an antifungal agent against two moulds isolated from mouldy (contaminated) rice and maize.

Extracts of the plant materials were incorporated into the medium (PDA) before sterilization. The medium incorporated with plant extract was poured onto pre-sterilized petri dishes and allowed to solidify. The isolates (*Penicillium notatum* and Aspergillus *flavus*) were inoculated at the center of the surfaced dried medium and incubated at ambient temperature for 5 days. Characterization was further done on the colonies and wet mount carried out microscopically to further identify and confirmed the moulds following standard methods. The results of the sensitivity test were captured and displayed in a screenshot. This procedure was repeated for the combined effect of the two extracts on the test organisms.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Phytochemical screening of orange-peel

Table 4.1 presents various alkaloids which include; spartein (11.61±1.07 mg/kg), epihedrine (17.43±0.53 mg/kg), aphylidine (17.10±0.70 mg/kg), dihydrocystine (9.08±0.37 mg/kg), ribalindine (5.79±0.22 mg/kg) and ammodendrine (3.02±0.62 mg/kg). Epihedrine showed the highest concentration of alkaloid. Flavonoids include; proanthocyanidin (15.42± 0.54 mg/kg) which has a highest concentration followed by anthocyanin (12.82±0.34 mg/kg), catechin (7.66±0.28 mg/kg), narigenin (5.44±0.54 mg/kg), flavonones (4.47±0.55 mg/kg), and flavone (2.66±0.35 mg/kg) respectively. This shows proanthocyanidin as the flavonoids with the highest concentration followed by the anthocyanin. The polyphenols are tannin (12.98±0.17 mg/kg) and sapogenin (3.55±0.49 mg/kg) including steroids (6.14±0.35 mg/kg).

Table 4.1: Phytochemical composition of orange-peel

Phytochemicals	Concentration (mg/kg)	Type of Phytochemical
Spartein	11.61±1.07	Alkaloid
Ribalinidine	5.79±0.22	Alkaloid
Epihedrine	17.43±0.53	Alkaloid
Dihydrocystine	9.09±0.37	Alkaloid
Aphylidine	17.09±0.70	Alkaloid
Ammodendrine	3.02±0.62	Alkaloid
Kaempferol	8.82±0.33	Flavonoid
Anthocyanin	12.82±0.34	Flavonoid
Flavonones	4.47±0.55	Flavonoid
Catechin	7.66±0.28	Flavonoid
Flavone	2.65±0.35	Flavonoid
Narigenin	5.44±0.54	Flavonoid
Proanthocyanidin	15.42±0.54	Flavonoid
Tannin	12.98±0.17	Polyphenol
Sapogenin	3.55±0.49	Steroidal alkaloid
Steroids	6.14±0.35	Steroids
Phytate	2.73±0.42	AntiNutrient
Cyanogenic glycoside	1.64±0.28	AntiNutrient
Oxalate	3.75±0.14	AntiNutrient

4.1.2 Phytochemical screening of garlic

Table 4.2 presents phytochemicals of garlic. There are alkaloids which include; spartein (9.50±0.01 mg/kg), epihedrine (7.24±0.49 mg/kg), and ribalindine (3.14±0.07 mg/kg). spartein showed the highest concentration of alkaloid. flavonoids include; kaempferol (2.99±0.13 mg/kg), anthocyanin (3.29±0.56 mg/kg), resveratol (7.09±0.14 mg/kg), epicatechin (14.25±0.65 mg/kg), proanthocyanidin (5.52±0.05 mg/kg), and rutin (9.21±0.35 mg/kg), epicatechin showed the highest concentration flavonoids concentration. The polyphenols were ellagic acid (9.14±0.14 mg/kg), coumaric acid (8.58±0.14 mg/kg), ferullic acid (16.64±0.21 mg/kg), tyrosol (5.45±0.64 mg/kg), hydroxytyrosol (6.49±0.56 mg/kg), with ferullic acid presenting the highest concentration. garlic sample contains also vanillic acid (20.56±0.66 mg/kg) and sapogenin (16.38±0.15 mg/kg).

Table 4.2: Phytochemical composition of garlic

Phytochemicals	Concentration (mg/kg)	Type of Phytochemical
Spartein	9.50±0.01	Alkaloid
Ribalinidine	3.14±0.07	Alkaloid
Epihedrine	7.24±0.49	Alkaloid
Kaempferol	2.99±0.13	Flavonoid
Anthocyanin	3.29±0.56	Flavonoid
Resveratol	7.09±0.14	Flavonoid
Epicatechin	14.25±0.65	Flavonoid
Proanthocyanidin	5.52±0.05	Flavonoid
Rutin	9.21±0.35	Flavonoid
Ellagic acid	9.14±0.14	Polyphenol
Coumaric acid	8.58±0.14	Polyphenol
Ferullic acid	16.64±0.21	Polyphenol
Tyrosol	5.45±0.64	Polyphenol
Hydroxytyrosol	6.49±0.56	Polyphenol
Vanillic acid	20.56±0.66	Phenolic
Sapogenin	16.38±0.15	Steroids

4.1.3: GC-MS results of orange-peel extract

Table 4.3 presents the chemical constituents of orange-peel extract. The analysis revealed that orange-peel consisted of 14 compounds and the main chemical compounds identified were; 4-Ethylcyclohexanol (20.59%), citral (18.92%), 2,6-Octadienal (12.86%), cis-3-Hexenyl cis-3-hexenoate (10.56%), 9,17-Octadecadienal (7.75%) and n-Hexadecanoic acid (5.39%). Other compounds identified include; cis-Vaccenic acid (4.93%), methyl 9-methyltetradecanoate (3.75%), 3-Hexene (3.48%), 4-Hexenoic acid (3.08%), oxirane (2.77%), heptadecanoic acid (2.49%), propyleneglycolmonoleate (2.33%) and citronellol (1.09%).

Table 4.3: GC-MS results of chemical constituents of orange-peel extract

S/N	Retention Time	Area %	Name of compound
1	7.067	12.86	2,6-Octadienal
2	7.691	18.92	Citral
3	9.103	10.56	cis-3-Hexenyl cis-3-hexenoate
4	9.767	20.59	4-Ethylcyclohexanol
5	11.693	3.08	4-Hexenoic acid
6	12.544	3.48	3-Hexene
7	16.982	3.75	Methyl 9-methyltetradecanoate
8	17.631	5.39	n-Hexadecanoic acid
9	18.809	4.93	cis-Vaccenic acid
10	18.972	2.77	Oxirane
11	19.041	2.49	Heptadecanoic acid
12	19.431	7.75	9,17-Octadecadienal
13	19.605	2.33	Propyleneglycolmonoleate
14	20.087	1.09	Citronellol

4.1.4: GC-MS results of garlic extract

Table 4.4 presents the chemical constituents of garlic extract. The analysis revealed that garlic consisted of 22 compounds and the main chemical compounds identified were; Oxalic acid (22.87%), cis-Vaccenic acid (12.69%), n-Hexadecanoic acid (12.12%), 6-methylheptyl vinyl (6.31%), bicyclo[3.1.0]hexane (6.18%), octadecanoic acid (5.64%), D-erythro-pentose (5.45%) and cis-13-Octadecenoic acid (5.41%). Other compounds identified include; Hexadecanoic acid (2.94%), 9-Octadecenoic acid (2.60%), 3-Deoxy-d-mannoic lactone (2.49%), oxazole (2.39%), 7-Pentadecyne (2.33%), chloro-methyl-methoxy-amine (1.70%), 4,7,7-Trimethylbicyclo[2.2.1]hepta (1.63%), 4-Thiazolidinone (1.54%), 2-Trifluoroacetoxypentadecane (1.24%), 1,2-Benzenedicarboxylic acid (1.17%), butoxyacetic acid (0.96%), octasiloxane (0.95%), 2-Propenoic acid (0.75%) and 1-Octenylsuccinic anhydride (0.66%).

Table 4.4: GC-MS results of chemical constituents of garlic extract

S/N	Retention Time	Area %	Name of compound
1	11.877	22.87	Oxalic acid
2	12.46	5.45	D-erythro-Pentose
3	12.699	0.96	Butoxyacetic acid
4	14.435	6.31	6-methylheptyl vinyl
5	14.527	1.7	Chloro-methyl-methoxy-amine
6	14.703	2.49	3-Deoxy-d-mannoic lactone
7	15.474	2.39	Oxazole
8	16.982	2.94	Hexadecanoic acid
9	17.486	1.17	1,2-Benzenedicarboxylic acid
10	17.621	12.12	n-Hexadecanoic acid
11	18.208	0.66	1-Octenylsuccinic anhydride
12	18.8	5.41	cis-13-Octadecenoic acid
13	19.102	6.18	Bicyclo[3.1.0]hexane
14	19.425	12.69	cis-Vaccenic acid
15	19.604	5.64	Octadecanoic acid
16	19.915	1.63	4,7,7-Trimethylbicyclo[2.2.1]hepta
17	20.268	2.33	7-Pentadecyne
18	20.564	0.75	2-Propenoic acid
19	23.602	2.6	9-Octadecenoic acid
20	23.931	1.54	4-Thiazolidinone
21	26.11	1.24	2-Trifluoroacetoxypentadecane
22	29.714	0.95	Octasiloxane

4.1.5: GC-MS results of combined extracts of Garlic and orange-peel

Table 4.5 presents the chemical constituents of combined extracts from garlic and orange peel. The analysis revealed that garlic consisted of 33 compounds and the main chemical compounds identified were; Bicyclo[4.2.0]oct-2-ene (30.011%), oleic acid (12.54%), 1,3-Dioxolane (9.31%), trans-13-Octadecenoic acid (8.69%), butyl 9-tetradecenoate (8.63%), oleic acid (7.17%), 9-Octdecenoic acid (4.54%) and methyl stearate (4.27%). Other compounds identified include; Hexadecanoic acid (3.74%), cyclopropane carboxamide (3.49%), pseduosarsasapogenin-5 (2.74%), 3,7,11-Tridecatrienoic acid (2.64%), bis(trifluoromethylthio)selenide (2.35%), glyceric acid (ISP-TFA) (2.24%), 9,17-Octadecadienal (2.13%), 3-Dodecyne (1.92%), 19-epoxyandrostane-8 (1.71%), citral (1.19%), cyclohexanone (1.15%), benzoic acid (1.09%), 6-Octadecenoic acid (1.08%), octadec-9-enoic acid (1.02%), norethindrone Acetate (0.99%), methyl 2-hydroxydodecanoate (0.96%), cyclotetradecane (0.92%), cyclopropane carboxamide (0.86%), 2,2-Difluoroheptacosanoic acid (0.82%), 1,4-Heptadiene (0.82%), 2,5-Furandione (0.61%), pyridine-3-carboxamide (0.57%), R-Limonene (0.34%) and 2-Propenoic acid (0.20%).

Table 4.5: GC-MS results of combined extracts of Garlic and orange-peel

S/N	Retention Time	Area %	Name of compound
1	7.070	0.82	1,4-Heptadiene
2	7.722	1.19	Citral
3	16.990	3.74	Hexadecanoic acid
4	17.638	6.98	n-Hexadecanoic acid
5	17.866	0.34	R-Limonene
6	18.804	8.69	trans-13-Octadecenoic acid
7	19.051	4.27	Methyl stearate
8	19.217	0.86	Cyclopropane carboxamide
9	19.434	12.54	Oleic acid
10	19.601	7.17	Oleic acid
11	19.795	0.92	Cyclotetradecane
12	19.830	1.02	Octadec-9-enoic acid
13	19.981	4.54	9-Octdecenoic acid
14	20.228	8.63	Butyl 9-tetradecenoate
15	20.328	1.92	3-Dodecyne
16	20.601	9.31	1,3-Dioxolane
17	20.712	3.49	Cyclopropane carboxamide
18	20.807	1.15	Cyclohexanone
19	20.934	2.13	9,17-Octadecadienal
20	21.044	2.35	Bis(trifluoromethylthio)selenide
21	21.646	2.74	Pseduosarsasapogenin-5
22	23.479	2.24	Glyceric acid (ISP-TFA)
23	23.479	0.61	2,5-Furandione
24	23.550	1.08	6-Octadecenoic acid
25	23.601	0.57	Pyridine-3-carboxamide
26	24.481	0.82	2,2-Difluoroheptacosanoic acid
27	26.076	0.99	Norethindrone Acetate
28	26.640	0.20	2-Propenoic acid
29	28.039	0.96	Methyl 2-hydroxydodecanoate
30	28.113	1.09	benzoic acid
31	28.926	1.71	19-epoxyandrostane-8
32	29.751	2.64	3,7,11-Tridecatrienoic acid
33	30.011	30.011	Bicyclo[4.2.0]oct-2-ene

4.1.6: Hydroxyl radical scavenging potentials of orange-peel and garlic extracts

Figure 4.1 shows the hydroxyl radical scavenging activities orange-peel and garlic extracts. The extracts hydroxyl radicals scavenging activities were quantified using 2'-deoxyribose oxidative degradation product by its condensation with Thiobarbituric acid in a concentration dependent manner. The results show that an increase in concentration produced a hyperbolic increase in percentage hydroxyl radical scavenging capacity. A lag was exhibited at higher concentration of 100mg/ml with the standard (ascorbic acid) presenting 83.71%, garlic 82.85% and orange-peel 54.49%. This indicates that the extracts on further increase in concentration may not attain any significant hydroxyl radical scavenging capacity. This effect was observed in orange-peel which showed higher hydroxyl activity at 50 mg/ml (60.49 %) compared to 100 mg/ml (54.49%).

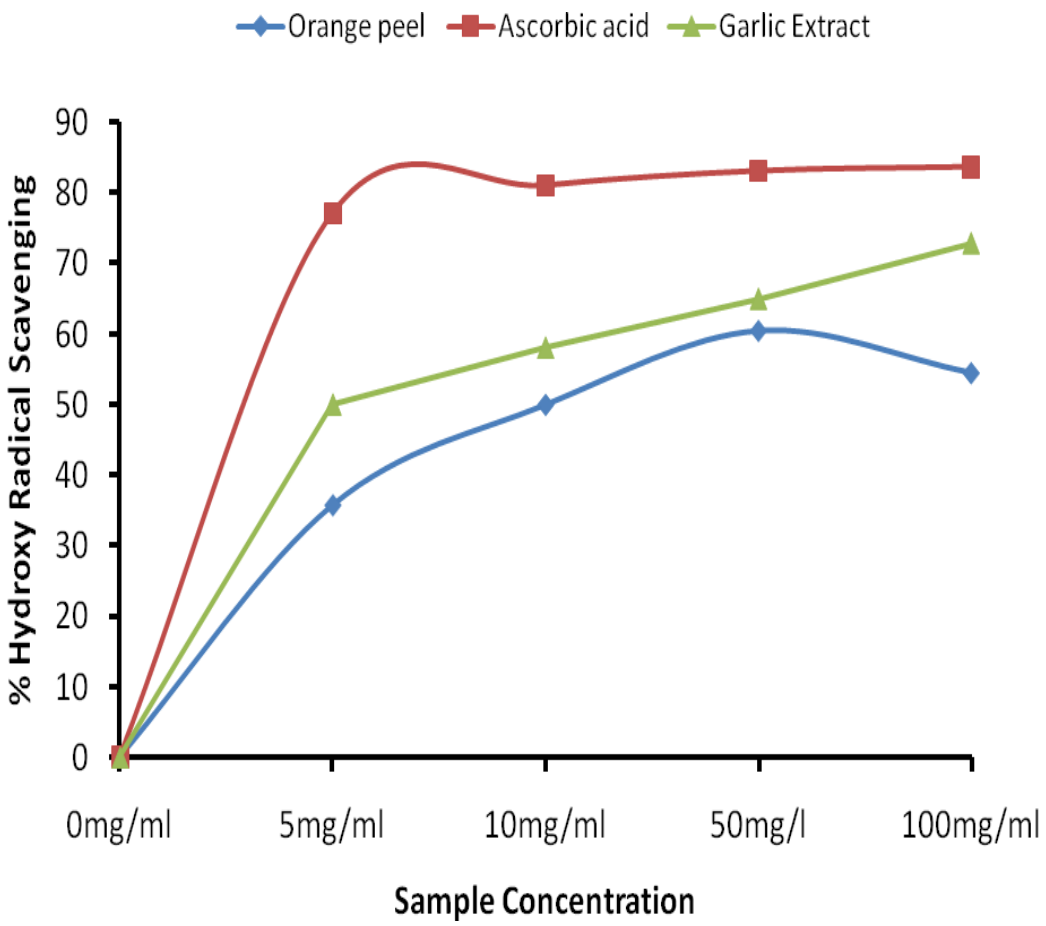


Figure 4.1 Hydroxyl radical scavenging potentials of orange-peel, garlic extracts and ascorbic acid.

4.1.7: Hydrogen peroxide radical scavenging potentials of orange-peel and garlic extracts

Figure 4.2 presents the hydrogen peroxide radical scavenging activities of extracts of orange-peel and garlic. The results show that an increase in concentration produced a hyperbolic increase in percentage hydrogen radical scavenging capacity. Highest inhibitory activities were expressed at 100mg/ml with the standard (ascorbic acid) presenting 86.27%, garlic 82.17% and orange-peel 72.75%.

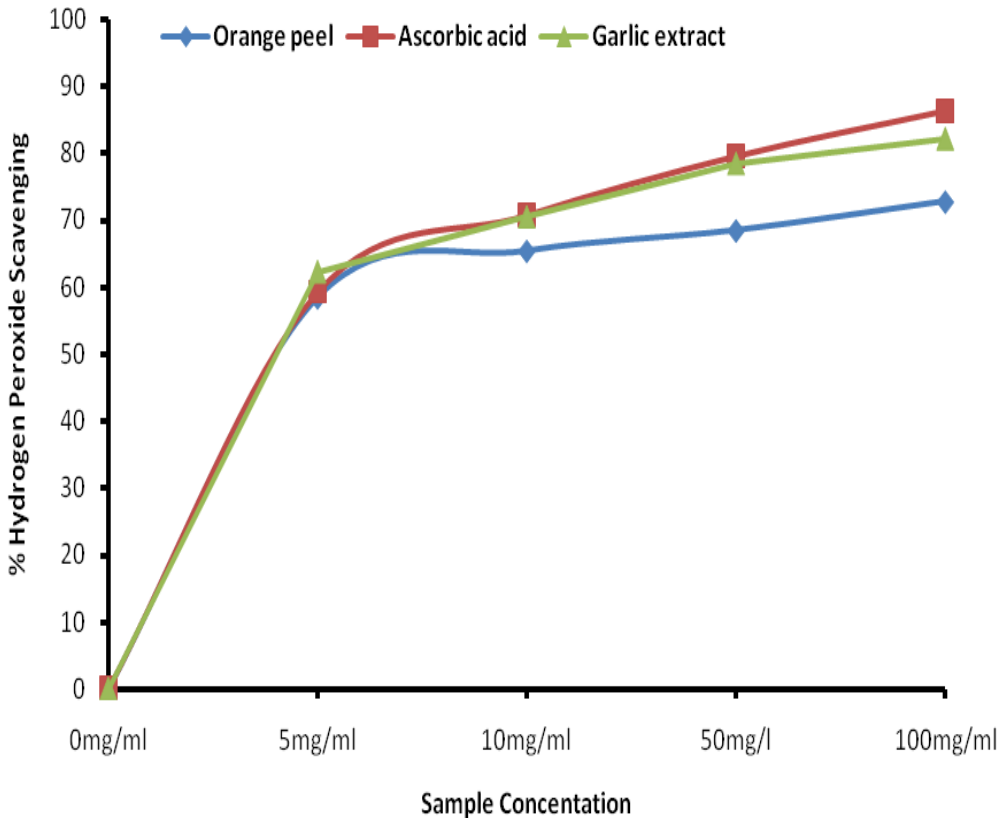


Figure 4.2 Hydrogen peroxide radical scavenging potentials of orange-peel, garlic extracts and ascorbic acid.

4.1.8: Nitric oxide radical scavenging potentials of orange-peel and garlic extracts

Figure 4.3 presents the nitric oxide radical scavenging activities of extracts of orange-peel and garlic. The results show that an increase in concentration produced a hyperbolic increase in percentage nitric oxide radical scavenging capacity. Highest inhibitory activities were expressed at 100mg/ml with garlic and orange-peel expressing 92.34% and 92.34% respectively. However, the highest inhibitory activities were expressed by the standard (ascorbic acid) at 200mg/ml with 96.73%. A plateau was exhibited after 100 mg/ml for garlic and orange-peel extracts. This indicates that the extracts on further increase in concentration may not attain any significant nitric oxide radical scavenging capacity. This effect was observed in orange-peel which showed a reduced activity (91.41%) at 200 mg/ml and garlic which activity (92.34%) was unchanged at 200 mg/ml.

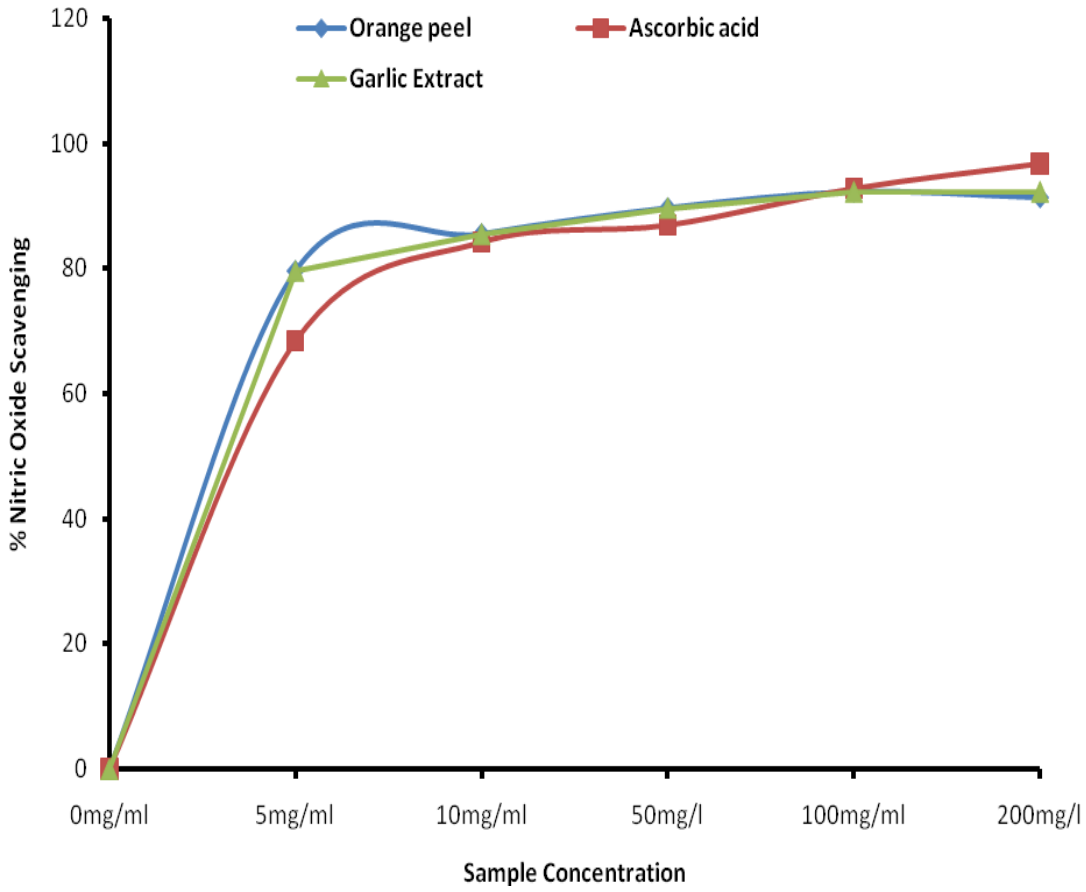


Figure 4.3 Nitric oxide radical scavenging potentials of orange-peel, garlic extracts and ascorbic acid.

4.1.9: Superoxide radical scavenging potentials of orange-peel and garlic extracts

Results presented in Figure 4.4 shows that increased concentrations of ascorbic acid, garlic and orange-peel extracts produced a hyperbolic increase in percentage superoxide radical scavenging capacity. A stationary phase was expressed at higher concentration of 100mg/ml with the standard (ascorbic acid) presenting 82.98%, garlic 85.75% and orange-peel 61.70%. This indicates that the extracts and standard on further increase in dosage may not attain any significant radical scavenging capacity.

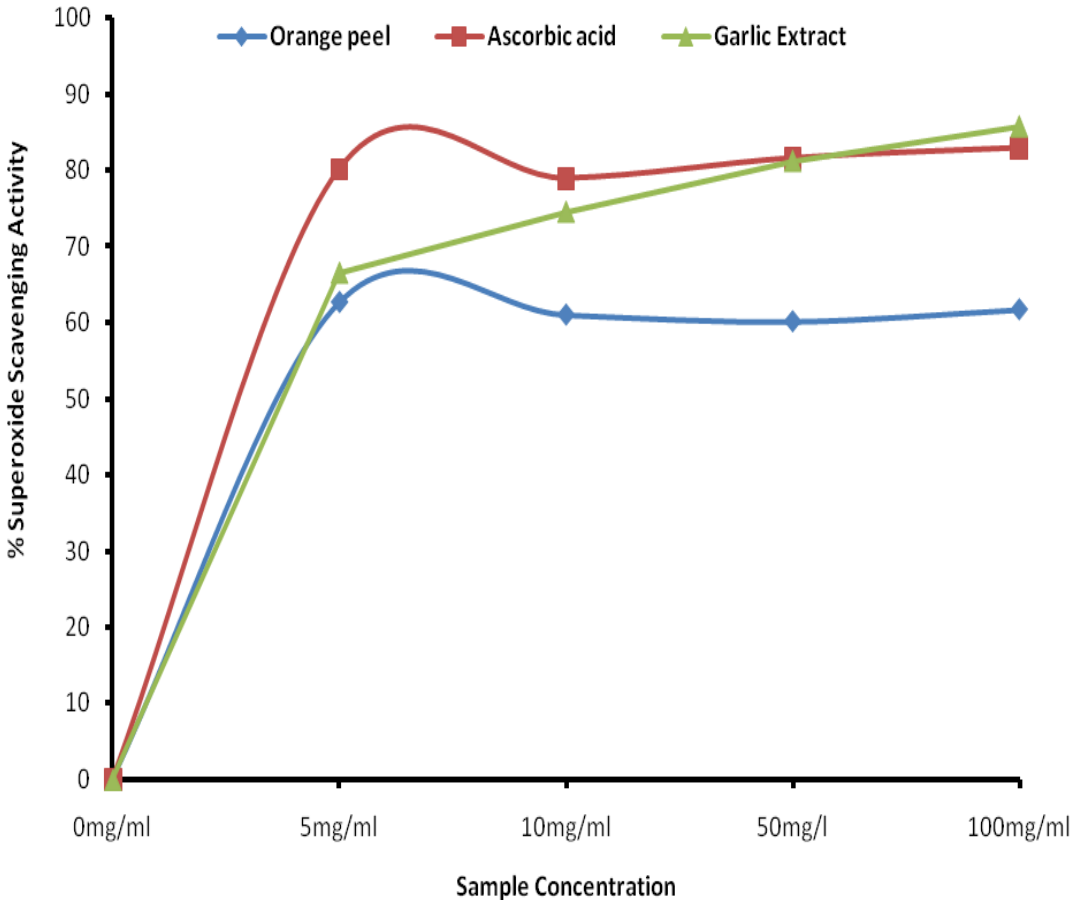


Figure 4.4 Superoxide radical scavenging potentials of orange-peel, garlic extract and ascorbic acid.

4.1.10: DPPH radical scavenging activity (%) of orange-peel and garlic extracts

Figure 4.5 presents the DPPH scavenging activity of garlic and orange-peel at different concentrations of extract (mg/ml) compared to a standard BHT (butylatedhydroxytoluene). The results show that the scavenging activity of the extracts produced a hyperbolic increase in percentage DPPH radical scavenging capacity. A stationary phase was expressed at concentration of 40mg/ml with the standard (ascorbic acid) presenting 100 %, garlic 88.44% and orange-peel 89.25%. This indicates that the extracts and standard on further increase in concentration may not attain any significant radical scavenging capacity

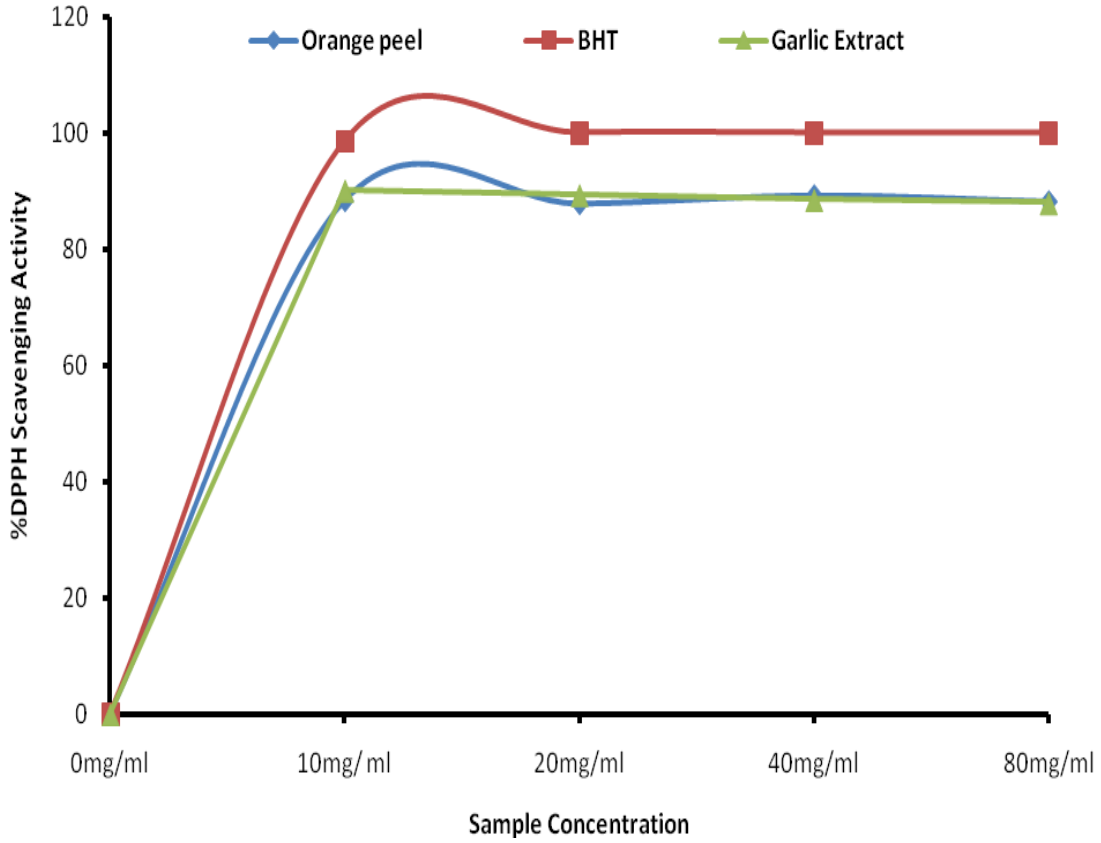


Figure 4.5: DPPH radical scavenging activity (%) of garlic, orange-peel extract and BHT (butylatedhydroxytoluene)

4.1.11: Ferric reducing antioxidant power (FRAP) of orange-peel and garlic extracts

In Figure 4.6 the FRAP analysis presents the result of a study of antioxidant activity of orange-peel and garlic in comparison to a standard (ascorbic acid). At a concentration of 5mg/ml, the orange-peel extract has the highest antioxidant activity with 70.09% inhibition, while garlic extract and standard showed antioxidant activity of 58.20% and 58.52% respectively. The activities of garlic extract and standard showed steady increase with increasing concentration up to 100 mg/ml to 75.24% and 74.60 % respectively. However, orange-peel extract deep at 10 mg/ml and 50 mg/ml but increased to 79.74% at 100 mg/ml.

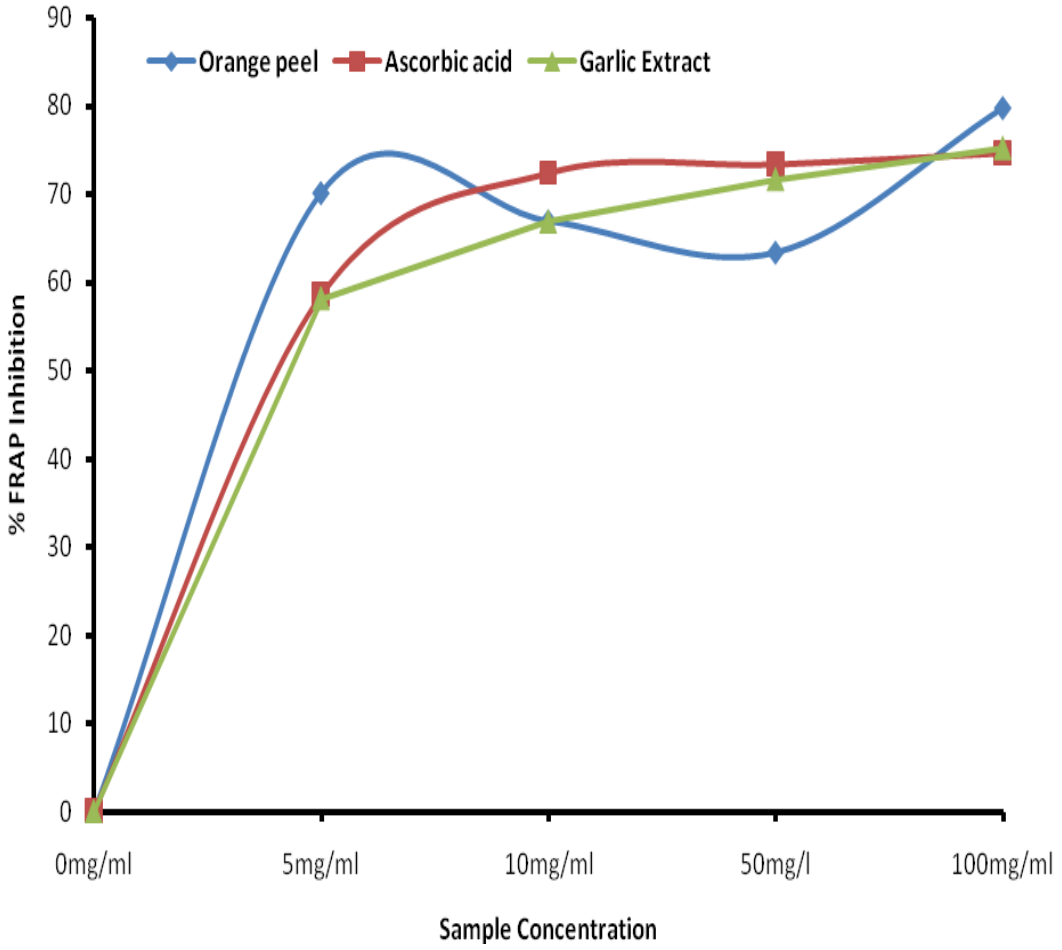


Figure 4.6: Ferric reducing antioxidant power (FRAP) of garlic, orange-peel extracts and ascorbic acid

4.1.12: ABTS scavenging activities of orange-peel and garlic extracts

Results presented in Figure 4.7 shows that increased concentrations of ascorbic acid, garlic and orange-peel extracts produced a hyperbolic increase in percentage ABTS radical scavenging activities. A stationary phase was expressed at higher concentration of 100mg/ml with the standard (ascorbic acid) presenting 79.28 %, garlic 82.66% and orange-peel 61.31%. This indicates that the extracts and standard on further increase in dosage may not attain any significant radical scavenging capacity.

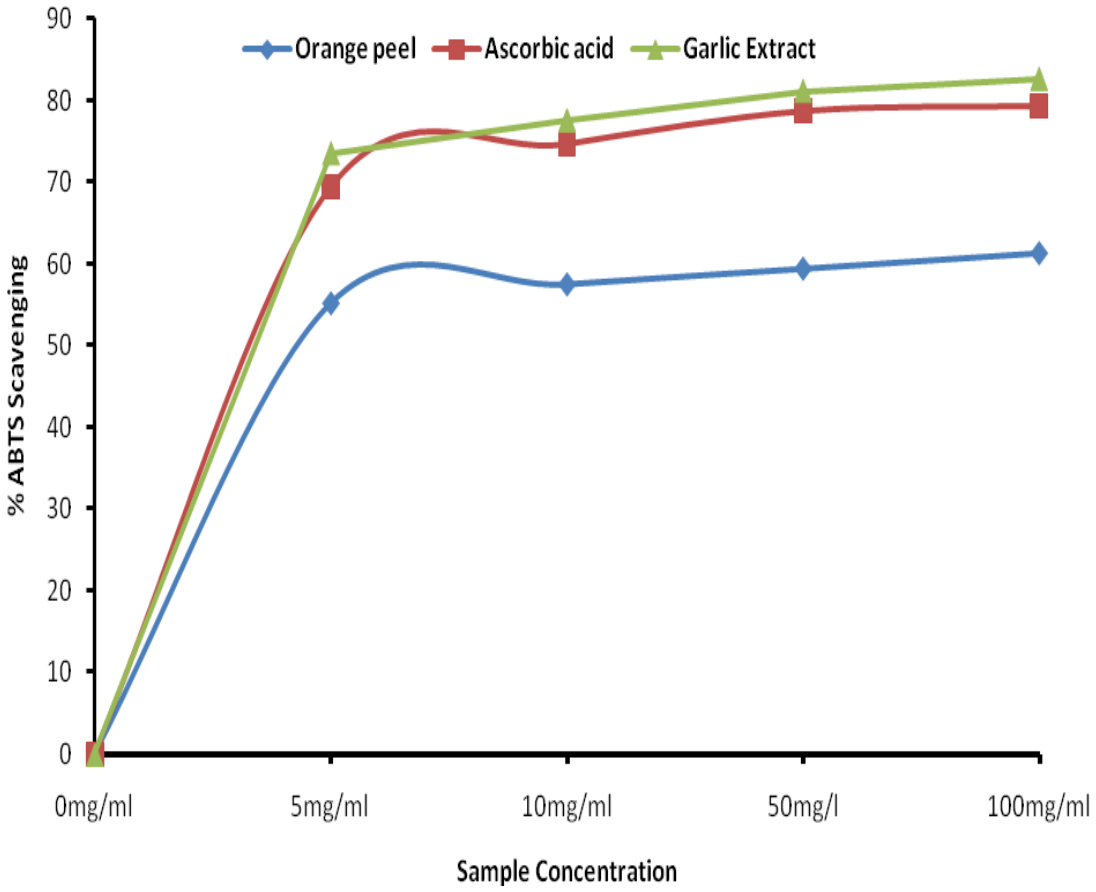


Figure 4.7: ABTS scavenging activities of garlic, orange-peel extracts and ascorbic acid

4.1.13: Total antioxidant capacity (TAC) of orange-peel and garlic extracts

Figure 4.8 presents the results of TAC of garlic orange-peel extract and standard ascorbic acid. At sample concentration of 5mg/ml, orange-peel and garlic extract recorded TAC of 49.88% and 52.44, respectively, compared to the ascorbic acid at 70.70%. The result showed that increased concentrations of ascorbic acid, garlic and orange-peel extracts produced a hyperbolic increase in TAC. At a concentration of 10mg/ml, the orange-peel has a total antioxidant capacity of 68.53%, which is significantly lower than the ascorbic acid (81.40%). Furthermore, at concentration of 20mg/ml, the orange-peel and garlic extracts recorded a total antioxidant capacity of 62.35% and 65.58%, respectively, compared to the standard ascorbic acid which recorded TAC of 88.99%.

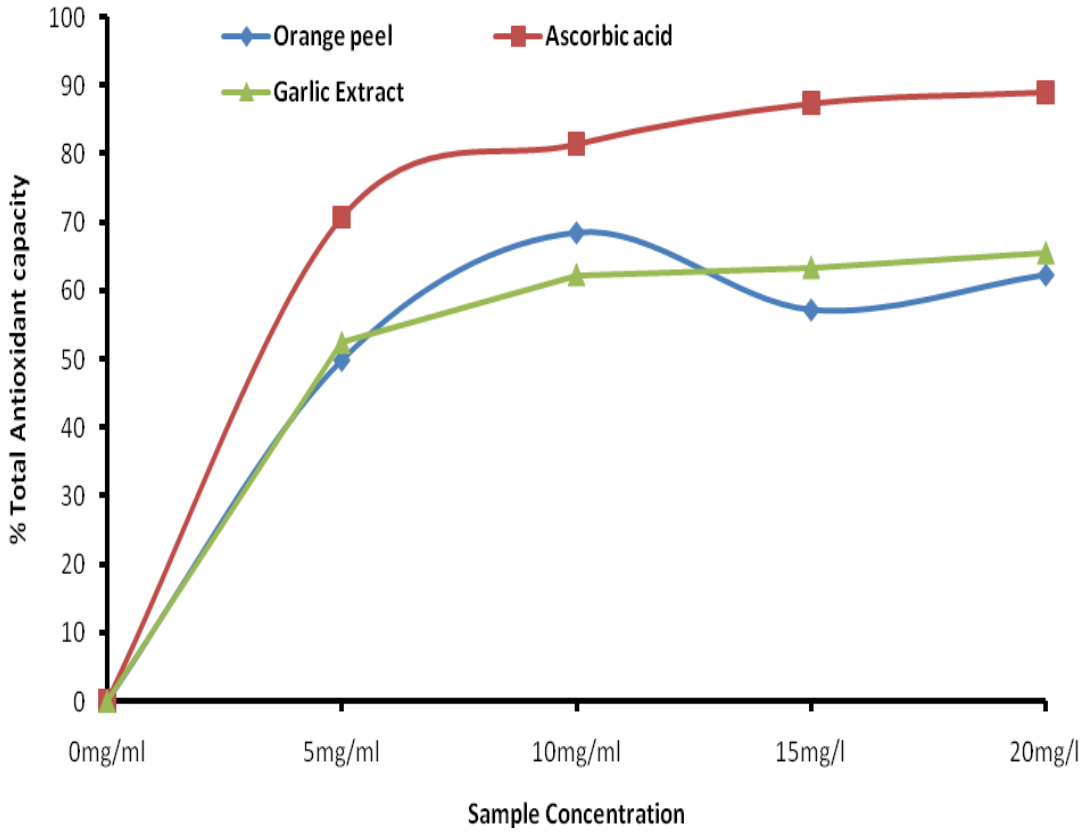


Figure 4.8: Total antioxidant capacity (TAC) of garlic orange-peel extract and standard ascorbic acid

4.1.14: Colonial characteristics of fungi isolated on potato dextrose agar

Table 4.6 presents an overview of the colonial characteristics of fungi from sample of rice and maize isolated on potato dextrose agar. The isolated fungi were identified as *Penicillium notatum* and *Aspergillus flavus.* The results showed the total fungal counts, colony types, colonial characteristics and microscopic characteristics of the isolated fungi.

Table 4.6: Colonial characteristics of fungi isolated on potato dextrose agar on rice and maize

Sample code/Locations	Total Fungal Counts (CFU/g)	Colony Types	Colonial Characteristics	Microscopic Characteristics	Identity of Isolates
Rice	7.0×10^5	Rx	Dirty green spores enclosed in a white patch	Septate conidia arranged like a mob head	*Penicillium notatum*
Rice	5.0×10^5	Ry	Lemon green spore attached on a white hypha	Conidia is septate with visicle attached to phialides	*Aspergillus flavus*
Maize	1.2×10^6	Mx	Lemon green spore attached on a white hypha	Conidia is septate with visicle attached to phialides	*Aspergillus flavus*
Maize	1.1×10^6	My	Dirty green spores enclosed in a white patch	Septate conidia arranged like a mob head	*Penicillium notatum*

4.1.15: Colonial characteristics of fungi isolated on malt extra agar

Table 4.7 presents an overview of the colonial characteristics of fungi from sample of rice and maize isolated on malt extra agar. The isolated fungi were identified as *Penicillium notatum* and *Aspergillus flavus.* The total fungal counts, colony types, colonial characteristics and microscopic characteristics of the isolated fungi are summarized in the table.

Table 4.7: Colonial characteristics of fungi isolated on malt extra agar

Sample code/Locations	Total Fungal Counts (CFU/g)	Colony Types	Colonial Characteristics	Microscopic Characteristics	Identity of Isolates
Rice	1.0×10^6	Ra	Lemon green spore attached on a white hypha	Conidia is septate with visicle attached to phialides	*Aspergillus flavus*
Rice	1.9×10^6	Rb	Dirty green spores enclosed in a white patch	Septate conidia arranged like a mob head	*Penicillium notatum*
Maize	3.3×10^6	Ma	Lemon green spore attached on a white hypha	Conidia is septate with visicle attached to phialides	*Aspergillus flavus*
Maize	2.1×10^6	Mb	Dirty green spores enclosed in a white patch	Septate conidia arranged like a mob head	*Penicillium notatum*

4.1.16 Antifungal activity of orange-peel and garlic extract

Presented in plate 4.1 is the antifungal activity of orange-peel and garlic extract against *Aspergillus flavus* and *Penicillium nonatum*. Laboratory tests of biotic activity allowed to determine the direct effect of plant extracts on the growth dynamics of the fungi. Each fungal species reacted differently to the addition of plant extracts in the substrate, their concentration and time of exposure.

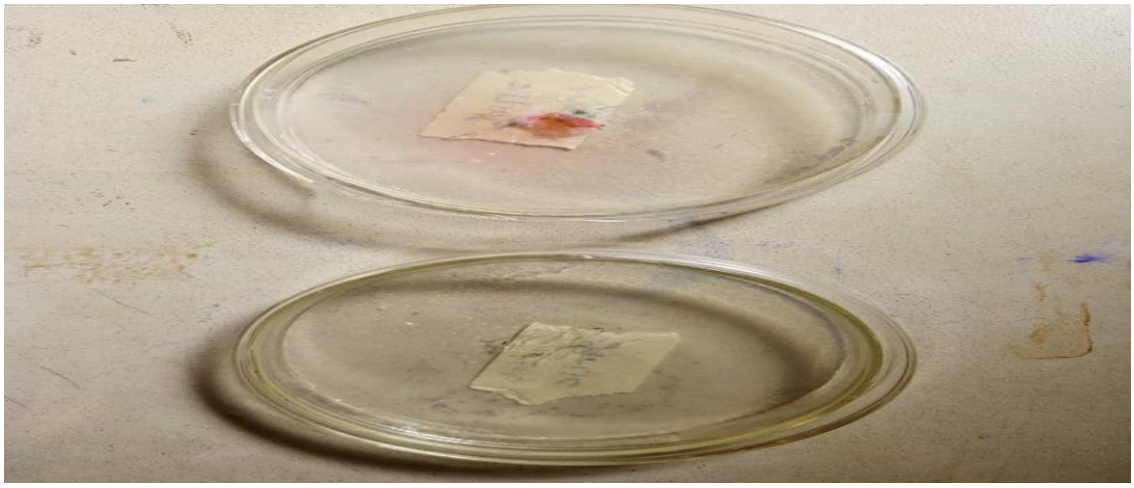
a. Copious growth *Aspergillus flavus* and *Penicillium notatum* on orange-peel extract

b. No growth of *Aspergillus flavus* and *Penicillium notatum* on garlic extract

c. Scanty growth of *Aspergillus flavus* and *Penicillium notatum* on mixture of garlic and orange-peel extracts

a.

b.



c.

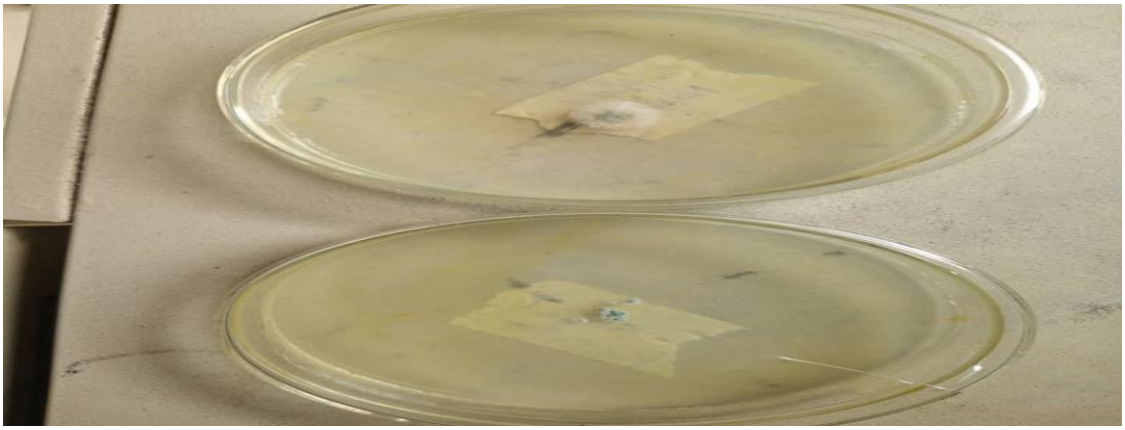


Plate 4.1: Growth of *Aspergillus flavus* and *Penicillium notatum* on extracts

4.2 DISCUSSION

The quantitative GC-FID determination of phytochemicals of garlic and orange-peel presented common phytochemicals (Table 4.1 and 4.2) such as spartein, ribalinidine, epihedrine, kaempferol, anthocyanin, proanthocyanin and sapogenin and all recorded higher concentration in orange-peel compared to garlic except sapogenin which was significantly higher in garlic extract. Furthermore, orange-peel presented these unique phytochemicals; dihydrocystine, aphylidine, ammodendrine, flavanonone, catechin, flavone, naringenin, and tannin. The unique phytochemicals found in garlic include; resveratol, epicatechin, rutin, ellagic acid, coumaric acid, eerullic acid, tyrosol, hydroxytyrosol, and vanillic acid.

Garlic and orange-peel extract recorded appreciable amount of alkaloids which exhibited antiproliferation, antibacterial, antiviral, insecticidal, and anti-metastatic activities (Dey *et al.,* 2020). Therapeutically alkaloids are known anesthetics, cardioprotective and anti-inflammatory agents (Henrich, Mah, & Amirkia, 2021). Alkaloids play vital pharmacological activities, acting as human therapeutic arsenal, such as antioxidant compounds, antitumoral drugs, analgesics, anti-inflammatories and stimulants (Bhambhani, Kondhare, & Giri, 2021). Garlic and orange-peel extract presented significant amount of flavonoids which are secondary metabolites, consisting a benzopyrone ring bearing a phenolic or polyphenolic group at different positions. Flavonoids are applied extensively as anticancer, antimicrobial, antiviral, antioxidant and anti-proliferating agent (Assad *et al.*, 2020).

Steroids and polyphenols (tannin) are active antioxidant, antitumor, and antimicrobial agents (Abeysinghe, Kumara, Kaushalya, Chandrika & Alwis, 2021). Tannins may accelerate blood clotting in certain conditions, reduce blood pressure, decrease serum lipid level, anti-microbial defence and modulate immunoresponse (Marcińczyk, Gromotowicz-Poplawska, Tomczyk & Chabielska, 2022). Polyphenols protects against development of

certain cancers, cardiovascular diseases, diabetes, and neurodegenerative diseases (Cory, Passarelli, Szeto, Tamez & Mattei, 2018; García-Aguilar, Palomino, Benito, & Gullen, 2021). These results indicate that the extracts are potential sources of useful drugs in medicines.

Furthermore, orange-peel extract recorded some antinutrient compounds such as phytate, Cyanogenic glycosides and Oxalate. These antinutrient are compounds that interfere with intake, absorption and utilization of nutrients. Antinutrients may further elicit very harmful biological responses while some are used as pharmacologically active agents. Appreciable amount of phytate was recorded in orange-peel and it is known that phytate has a strong affinity for calcium, magnesium, iron, copper, and zinc preventing absorption. Oxalic acid are presents in many plants, they bind calcium and prevents its absorption. Glucosinolates interfere with the uptake of iodine, chelate metals and flavonoids thus reducing absorption (Lee, Kim, & Woyengo, 2020).

Individually these phytochemicals presented varying biochemical and pharmacological activities. Proanthocyanidins are indicated to protect animal (human) skin from possible damage by sun's radiations, and also improves vision, flexibility in joints, arteries, body tissues such as heart and cardiovascular system. Proanthocyanidins also elicit anti-microbial, anti-carcinogenic, anti-inflammatory properties (Rauf *et al.*, 2019). For anthocyanins, they have shown antidiabetic, anti-cancer, anti-inflammatory, anti-microbial and anti-obesity activities and are found useful in managing cardiovascular diseases (Khoo, Azlan, Tang & Lim, 2017). Catechins are used in the management of inflammatory bowel disease, and have found to be useful in the regulation of the infiltration and proliferation of immune related cells (Fan, Sang, & Jiang, 2017; Musial, Kuban-Jankowska, & Gorska-Ponikowska, 2020). The naringenins reduce glucose levels, lipids in serum and the activity of alpha glucosidase. It also increases antioxidant enzymes, reduces brain vascular diseases

and secondary effects of cancer (Calderón-Oliver & Ponce-Alquicira, 2018). The flavanones and flavones present anti-cancer, antioxidant, anti-inflammatory, antimicrobial properties and neuroprotective and cardio protective effects (Zuiter, 2014; Ullah *et al.*, 2020).

Hydroxyl radical is known as the most reactive of all the reduced forms of dioxygen, that easily initiates cell damage *in vivo* (Duan, Wu, & Jiang, 2007; Sowndhararajan, & Kang, 2013). In this study 50% and 35.77% hydroxyl radical scavenging of garlic and orange-peel extract respectively, were observed at low concentration (5 mg/ml) indicating a good start compared to the standard ascorbic acid. Scavenging of hydroxyl radical is a very critical antioxidant activity due to high reactivity of OH radical. If allowed it has the ability to react with a wide range of molecules such as sugars, amino acids, lipids, and nucleotides (Wang, Gao, Zhou, Cai & Yao, 2008). In the present study, the hydroxyl radical scavenging activity of garlic was higher than orange-peel extract.

Hydroxyl and superoxide radicals are two major reactive oxygen species that are continuously formed in the process of reduction of oxygen to water. Hydroxyl radicals are highly reactive species that can cause oxidative damage to biomolecules such as DNA, proteins, and lipids. These properties implicate hydroxyl radicals in various disease conditions including cancer, neurodegenerative diseases, and cardiovascular disease. Hydroxyl radicals are shown to reduce disulphide bonds of proteins, resulting in its unfolding and scrambled refolding into abnormal spatial configurations (Lipinski 2011). Antioxidants scavenge hydroxyl radicals by donating an electron or hydrogen atom to the hydroxyl radical, neutralizing its reactivity and preventing any further damage of exposed molecules. Flavonoids and polyphenols which are abundantly present in garlic and orange-peel have shown the ability to scavenge hydroxyl radicals and reduce its harmful effects on biological molecules and systems.

The extracts of garlic and orange-peel demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. The results of this study indicated that extracts of garlic and orange-peel effectively scavenged hydrogen peroxide at 82% and 72%, respectively, and this could be credited to the bioactive components especially the phenolic groups which readily donate electrons to hydrogen peroxide, subsequently neutralizing it into water. Hydrogen peroxide are reactive oxygen species that can cause oxidative damage to cells and tissues if its levels become excessive or uncontrolled. Hydrogen peroxide scavengers work by converting hydrogen peroxide into less reactive and non-damaging forms. They can either directly react with hydrogen peroxide or facilitate its breakdown into water and oxygen (Agrawal, Agrawal, Arora, Lahange, & Kshirsagar, 2021). This helps prevent the accumulation of hydrogen peroxide and reduces its potential harmful effects. If allowed hydrogen peroxide rapidly cross cell membranes, react with Fe^{2+} and Cu^{2+} ions forming hydroxyl radicals and this may be the origin of many of its toxic effects (Sharma & Singh, 2012). The appreciable amount of phytate in orange-peel may contribute in chelating metal ions, such as iron and copper, that participate in the Fenton reaction, where hydrogen peroxide is converted into highly reactive hydroxyl radicals thereby prevent the generation of hydroxyl radicals.

Orange-peel showed lower level of antioxidant activity, indicating that garlic expressed better radical scavenging capacity than the standard ascorbic acid and orange-peel extract. A superoxide radical scavenger acts via donation of electrons or hydrogen atoms to superoxide radical, thereby converting it superoxide radicals to less reactive and non-damaging form (Yen & Duh, 1994; Sharma & Gupta, 2008). This process is shown to prevent the radical from initiating oxidative chain reactions and thereby reducing its harmful effects. Phytochemicals such as flavonoids, polyphenols, and others abundantly present in garlic and orange-peel extracts have shown the ability to scavenge superoxide radicals.

Excessive or uncontrolled levels of NO can lead to cellular damage and contribute to certain pathological conditions. Nitric oxide scavengers work by reacting with nitric oxide to form stable products, thus reducing its concentration and biological activity. In the present study, the extract of garlic and orange-peel significantly inhibited nitric oxide radical production at 92% for 100 mg/ml extract concentration. It is important to note that the development and search for compounds to prevent the overproduction of nitric oxide is now a new research target (Venkatachalam & Muthukrishnan, 2012).

The results of the present study showed that extracts of garlic and orange-peel recorded comparable TAC of 62.35% and 65.58 %, respectively, as seen at concentration of 20 mg/ml. TAC serves as an important indicator of the antioxidant defence system's effectiveness in combating oxidative stress. Total antioxidant capacity (TAC) is the measure of the amount of free radicals scavenged by a test solution, being used to evaluate the antioxidant capacity of biological samples (blood, serum or tissue) (Rubio, Hernández-Ruiz, Martinez-Subiela, Tvarijonavicuite, & Ceron, 2016).

Ferric Reducing Ability of Plasma (FRAP) assay based on the reduction of Fe (III) to Fe (II) by antioxidants in the presence of tripyridyltriazine tridentate ligand and measured spectrophotometrically (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016). The result of FRAP assay in this study indicates that bioactive compounds in garlic and orange-peel demonstrates excellent antioxidant capacity when compared to the reference standard. The results of antioxidant capacities provide valuable information on the potential health benefits and oxidative stress mitigation of the plant extracts.

The DPPH is a stable radical readily scavenged by antioxidant (Lu & Yeap-Foo, 2001). The radical scavenging capacity of garlic and orange-peel extract showed significant DPPH radical scavenging even at low concentration of 10 mg/ml, though lower than the standard BHT which presented 100 % DPPH radical scavenging at 10 mg/ml. The ability of the

bioactive compounds (antioxidants) of garlic and orange-peel extract to scavenge DPPH radical shows good capacity to scavenge radicals by donating hydrogen to free radicals, even the lipid peroxides or hydroperoxide radicals implicated as the major propagators of the chain autoxidation of lipids, and to form non-radical species, resulting in the inhibition of propagating phase of lipid peroxidation (Bamforth, Muller, & Walker, 1993).

A significant percentage ABTS radical scavenging activities was recorded in 5 mg/ml of garlic when compared to orange-peel extract and ascorbic standard. This corroborates the assertion by Hagerman *et al*. (1998) which reported that high molecular weight phenolics have greater ability to scavenge free radicals ($ABTS^{\bullet+}$). The result of phytochemical showed that garlic presented more phenolics than orange-peel, therefore the possible reason for the increased ABTS radical scavenging activities.

In this study the results of the investigation of antifungal activities shows varying antimicrobial capacity of garlic and orange-peel extract to inhibit the growth of *Aspergillus flavus* and *Penicillium notatum* isolated from rice and corn. The recorded antifungal activities of plant extracts on stored grains microorganisms can be attributed to various mechanisms expressed by the bioactive compounds present in these extracts.

Plant extracts such as saponins, essential oils, and fatty acids recorded in garlic and orange-peel extracts have the capacity to disrupt the integrity of fungal cell membranes. When membrane disruption occurs, it could lead to the leakage of essential cellular components, causing cell death. For example, the essential oils in plants like cinnamon, thyme, and cloves have been shown to disrupt fungal cell membranes.

The phytochemical screening of garlic and orange-peel recorded appreciable amount of secondary metabolites. These secondary metabolites, such as alkaloids and phenolics, have shown capacity to inhibit fungal growth by interfering with numerous microbial metabolic processes. These secondary plant compounds may have disrupted the fungal enzyme

systems, interfere with DNA replication, or cause the inhibition of synthesis of essential molecules necessary to carryout metabolic activities. The numerous antioxidant compounds in garlic and orange-peel extracts, such as flavonoids and polyphenols, have shown capacity to protect grains from oxidative damage caused by fungal infections.

Some plant extracts have shown the ability to create an unfavorable environmental condition for fungal growth thereby altering the pH or other environmental conditions in storage spaces. This can make it more challenging for fungi to thrive and reproduce. Garlic and orange-peel extracts may have also interfered with fungal spore germination, thereby preventing the establishment of new fungal colonies. The inhibition of spore germination may be triggered by the secondary metabolites in the extracts by disrupting essential processes. The results of the inhibition of fungal growth by garlic and orange-peel extracts indicated that the effectiveness of plant extracts as antifungal agents on stored grains can vary depending on factors such as the plant extract, type fungal species present, the concentration and composition of the plant extract. It is important to state here that proper grain storage practices, such as maintaining appropriate temperature and humidity levels, are essential for preventing fungal contamination in stored grains.

CHAPTER FIVE

CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 CONCLUSION

The goal is to determine if orange-peel and garlic extract can effectively inhibit fungal growth, reduce spore germination and control fungal contamination in stored grains, with the ultimate objective of improving grain quality and safety. Phytochemical screening of extracts of orange-peel and garlic showed the presence of flavonoids, alkaloids, polyphenols, phenols and steroids. GC-MS analysis revealed the presence of over 33 chemical constituents that contribute to the biological activities of the extracts. The results of our analysis also showed that extracts of orange-peel and garlic manifested antioxidant and free radical scavenging potentials and can be a potent source of natural antioxidants.

Our results demonstrated that each of these extracts, when used individually, effectively suppressed the growth of molds, highlighting their potential as natural agents for fungal control in stored grains. This research study also revealed that the combination of orange-peel and garlic extract can effectively inhibit the production of harmful aflatoxins and produce a biocide. The combined effect of the extracts showed scanty growth of the molds, which indicates that there was a reduction in activity and hence was not active in its combined state.

5.2 RECOMMENDATIONS FOR FURTHER STUDIES

1. Further studies can explore optimal application methods and concentrations to maximize their effectiveness.
2. Further researches are required to validate the activity of the combined extract through the ratio proportion of each individual extract.

3. Trials on the biocidal effect of garlic extract and another extract, since the combination of orange and garlic was not active.

5.3 CONTRIBUTION TO KNOWLEDGE

This study has shown that orange-peel and garlic extract have promising biocidal potential and can be effective in controlling postharvest pathogens of food crops. It was observed that garlic extract was more effective than the orange-peel extract, garlic extract had more antifungal activity and therefore can be used as a natural fungicide. The study also showed the extracts exhibited antagonistic characteristics in combined form.

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