Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci.7(1): 195-202, February 2013

ISSN 1991-8631

International Journal of Biological and Chemical Sciences

Original Paper

http://indexmedicus.afro.who.int

Antimicrobial activity of the leaf extracts of *Moringa oleifera* and *Jatropha curcas* on pathogenic bacteria

S.I. OKORONDU [1*], C. O. AKUJOBI [1], J.N. OKORONDU [2] and S.O. ANYADO-NWADIKE [3]

[1]*Department of Microbiology, Federal University of Technology Owerri, P.M.B. 1526, Owerri, Nigeria.*
[2]*Department of Chemistry, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria.*
[3]*Department of Biotechnology, Federal University of Technology Owerri, P.M.B. 1526, Owerri, Nigeria.*
[*]*Corresponding author, E-mail: sokorondu@yahoo.co.uk*

ABSTRACT

This study evaluates the antimicrobial activity of the leaf extracts of *Moringa oleifera* and *Jatropha curcas* against *Staphylococcus aureus* and *Escherichia coli*. Different concentrations of the extracts were subjected to these organisms in which *Moringa oleifera* showed a higher zone of inhibition on *Staphylococcus aureus* (2.8 cm) while on *E. coli* (2.4 cm) while *Jatropha curcas* showed a higher zone of inhibition on *E. coli* (2.6 cm) while on *S. aureus* (1.80 cm). The minimum inhibitory concentration (MIC) of *Moringa oleifera* extract on *E. coli* and *S. aureus* were 0.250 mg/ml and 0.125 mg/ml respectively while MIC of *Jatropha curcas* extract on test organisms was 0.125 mg/ml. The quantitative phytochemical screening in g/kg revealed the presence of flavonoid 36 and 21, alkaloids 92 and 39, tannins 7.4 and 5.6, saponins 115.0 and 53.5, cyanogenic glycosides 8.4 and 14.5 for *M. oleifera* and *J. curcas* respectively. The observed antimicrobial properties could be due to the presence of these bioactive compounds and further substantiates the use of *Moringa oleifera* and *Jatropha curcas* leaf extracts in medicine. The extracts in correct doses can successfully be used *in vivo* to inhibit and eventually kill the test bacteria used in this study.

Keywords: *Moringa oleifera*, *Jatropha curcas*, phytochemicals, bacteria, antimicrobial.

INTRODUCTION

Plants have been used for centuries before the advent of Orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines. The medicinal values of these plants depend on their phytochemical components, which produces definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds (Afolabi et al., 2007; Okorondu et al, 2010a, b, 2011).

Moringa is the sole genus in the flowering plant family Moringaceae. The genus *Moringa* in turn is made up of 12 species. *Moringa oleifera* is the most widely cultivated species of the genus Moringa and it is found in many tropical and sub-tropical regions (Sofowora, 1984). *M. oleifera* is cultivated and harvested in many areas of the world where other plants are unable to thrive and virtually every part of the moringa tree

DOI : http://dx.doi.org/10.4314/ijbcs.v7i1i.16

can be used for food, medicine or put some other beneficial use (Farooq et al., 2007). The "moringa" tree is grown mainly in semi-arid, tropical and subtropical areas. It grows best in dry sandy soil, tolerates poor soil, including coasted areas. *M. oleifera*, commonly referred to as the miracle tree, is the most widely cultivated species of the genus Moringa, which is the only genus in the family Moringaceae. It is a fast growing and resistant shrub, native to India but now widely distributed in the tropics and subtropical areas (Oliver-Bever, 2000). In Nigeria, *M. oleifera* is encountered doing well in all ecological zones where it is always in season all the year round. The plant is propagated by both seeds and cuttings. The Moringa tree is grown mainly in semi-arid, tropical and subtropical areas. Today, it is widely cultivated in Africa, Central and South America, Sri- Lanka, India, Mexico, Malaysia, Indonesia and the Philippines. It is considered one of the World's most useful trees, as almost every part of the Moringa tree can be used for food or has some other beneficial property (Fahey, 2005).

Jatropha curcas is a plant which belongs to the family Euphorbiaceae originated from Mexico and South Africa (Tint and Mya, 2009). The plant was introduced to Africa by the Portuguese in 1500 and was found growing sparsely in the wild in Nigeria and was use as hedge (Lozan, 2007). *J. curcas* is a small shrub plant which grows to a height of between 3 m and 5 m. The bark exudates white coloured latex. The leaves of the plant were arranged alternatively and of large size with green to pale-green colour (Tint and Mya, 2009). *J. curcas* grows almost anywhere-even on gravelly, sandy and saline soils. It can thrive on the poorest stony soil. It can grow even in the crevices of rocks (Tint and Mya, 2009). The fruits are produced in winter, or there may be several crops during the year if soil moisture is good and temperatures are sufficiently high. The seeds are mature when the capsule changes from green to yellow (Lozan, 2007). *J. curcas* is a drought – resistant perennial plant, growing well in Marginal / poor soil. It is easy to establish and grows relatively quickly producing seeds for 50 years. *J. curcas* has limited natural vegetative propagation and is usually propagated by seed. The oil from *J. curcas* seeds is used to treat rashes and parasitic skin diseases. Mixture of oil from the seeds with benzyl benzoate is effective against scabies and dermatitis (Belewu, 2008). The oil can be as a biodiesel as it burns with clear smoke–free flame. The oily seeds are processed into oil, which may be used directly ("Straight Vegetable Oil") to fuel combustion engines or may be subjected to trans-sterification to produce biodiesel (Belewu, 2008). *Jatrophas curcas* is commonly called physic nut, purging nut or pig nut. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism (Oliver-Bever, 2000). Aiyelaagbe (2001) reported the anti-parasitic activity of the sap and crushed leaves of *J. curcas*.

The main objective of this project is to determine the antimicrobial activity of the ethanol leaf extracts of *Moringa oleifera* and *Jatropha curcas* on selected pathogenic gram positive and gram negative bacteria (*Staphylococcus aureus* and *Escherichia coli*).

MATERIALS AND METHODS

Sample collection and identification

Healthy leaves of *Moringa oleifera* and *Jatropha curcas* were collected from school of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Nigeria in September; 2011. The taxonomical identification of the plant was confirmed by Dr. S.O. Ojiako, a plant taxonomist of the Department of Crop Science, Federal University of Technology Owerri, Nigeria.

Preparation of plant material

The fresh leaves of *Moringa oleifera* and *Jatropha curcas* were harvested, rinsed with tap water and air-dried under shade for 7

days. The dried leaves were reduced to fine powder using a mechanical grinder and stored in a sterile container until when needed.

Preparation of the extract

These were carried out using soxhlet extraction method. 30 g of the grounded powdered plant material of *Moringa oleifera* and *Jatropha curcas* leaves respectively were subjected to sequential extraction using 150 ml ethanol each for 3 hours. The ethanol extract was concentrated into dryness by evaporation of the solvent in a water bath and the weight was noted. The extract was stored in a sterile container at 4 $^{\circ}$C in a refrigerator until required for use.

Phytochemical analysis

Phytochemical screening was carried out on the powdered sample for the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins and cyanogenic glycosides using standard procedures as described by Harborne (1973) and AOAC (1984).

Test for alkaloid

A measured weight (2 g) of each processed sample was dispersed in 100 ml of 10% acetic acid in ethanol solution to stand for 4 hours at room temperature with shaking every 30 mins. At the end of this period, the mixture was filtered through Whatman filter paper. The filtered extract was treated with addition of concentrated ammonia solution in drops to precipitate the alkaloids. The precipitated alkaloid was filtered using Whatman filter paper. After washing with 1% NH_4OH solution, the precipitated alkaloid was dried at 6 $^{\circ}$C and weighed after cooling in a desiccator.

Test for flavonoid

A measured weight (5 g) of the processed samples was boiled in 100 ml of 2 M HCl solution for 40 minutes. It was allowed to cool to room temperature before being filtered through Whatman filter paper to obtain the extract. Flavonoid in the extract was then precipitated by addition of concentrated ethyl acetate in drops until in excess. The flavonoid precipitate was recovered in weighed filter paper following filtration. After drying in the oven and cooling in a desiccator, the weight of flavonoid was obtained.

Test for saponins

5.0 g of dry ground samples was weighed into a thimble and transferred into a soxhlet extractor chamber fitted with a condenser and a round bottom flask. 10% acetic acid is poured into the flask. The sample was exhaustively extracted of its lipid and interfering pigments for 3hours by heating the flask on a hot plate and the solvent distilled off. A pre-weighed round bottom flask was fitted into the soxhlet apparatus (bearing the sample containing thimble) and ethanol poured into the flask. The saponin is then exhaustively extracted for 3 hours by heating the flask on a hot plate after which the solvent is distilled off. The flask is re-weighed. The difference between the final and initial weights of the flask represents the weight of saponin extracted.

Test for cyanogenic glycoside

1.0g dry ground sample was weighed into a 250 ml round bottom flask. 200 ml of distilled water was added and allowed to stand for 2 hours. An antifoaming agent (silicon oil) was added before distillation. Full distillation was then carried out and 150-170 ml of distillate was collected in a 250 ml conical flask containing 20 ml of 2.5% NaOH. To 100 ml of the distillate containing cyanogenic glycoside, 8 ml of 6N NH_4OH and 2 ml of 5% KI was added, mixed and titrated with 0.02N silver nitrate ($AgNO_3$) using a micro burette.

Test for tannin

Measured weight of the samples (5.0 g) was dispersed in 100 ml of distilled water. The mixture was shaken for 30 minutess at room temperature and filtered using Whatman

filter paper. The residue was washed further with the distilled water until 100 ml filtrate was obtained. An aliquot of the extract (2 ml) was mixed with equal volume of folin-Dennis reagent in a 50 ml volumetric flask. 2 ml of saturated sodium carbonate solution was added. The mixture was diluted to the 50 ml mark and allowed to incubate for 90 minutes at room temperature. A standard tannin solution was prepared with tannic acid. After incubation, the absorbance of the standard and samples were measured at 760 nm in a spectrophotometer.

Isolation and identification of test organisms

Two pathogenic bacterial isolates of *Escherichia coli* and *Staphylococcus aureus* obtained from the Microbiology department of Federal Medical Centre, Owerri, Nigeria were used for the study. They were isolated and purified on nutrient agar plates, characterized and identified (Cheesbrough, 2002).

Antimicrobial screening

Two approaches were used for the evaluation of the antimicrobial activities of the plant extracts as described by Okigbo et al. (2003).

Antimicrobial susceptibility test/ Zone of inhibition

Antimicrobial activity of the extracts was determined by Agar- well diffusion method using the test organisms; *E. coli* and *S. aureus*. An agar media was prepared and a sterile cork borer of about 6 mm in diameter was used to bore holes on the agar media. The already characterized test organisms were swabbed on the solid agar media. Aliquots of 0.2 ml of the extract was introduced in the holes made on the agar media containing each isolate of organisms, at different concentrations of (1, 0.5, 0.25, 0.125, 0.0625 mg/ml) of the two (2) extracts (*M. oleifera and J. curcas*). A control hole where the solvent used for the extraction was added and the plates were incubated at 37 $^{\circ}$C for 24 hrs in the incubator. The ability of the various extracts to inhibit growth of the clinically significant bacteria was measured and recorded as diameter of the zone of inhibition in cm.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined by incorporating constant volumes (0.2 ml) of each dilution of the extract into the punch-holes made on the agar medium. Aliquot of the extract 0.2 g was dissolved in 100 ml of sterile distilled water to obtain 2.0 mg/ml. This 2.0 mg/ml concentration was then doubly diluted in sterile distilled water to obtain concentrations of 1, 0.5, 0.25, 0.125, 0.0625 mg/ml. The minimum inhibition concentration was determined by recording the least concentration of the extracts (mg/ml) or the highest dilution that inhibited the growth of the organisms.

RESULTS

The percentage yield of the ethanol leaves extract of *M. oleifera* and *J. curcas* were shown in Table 1. Crude extract (3.19 g) of *Moringa oleifera* and 3.04 g of *Jatropha curcas* were extracted from 30 g powder of samples. These were converted in percentage to give 10.63% and 10.13% respectively. Table 2 shows the quantitative analysis of bioactive compounds of ethanol leaf extract of *M. oleifera* and *J. curcas*. The phytochemicals present in g/kg are flavonoid 36 and 21, alkaloids 92 and 39, tannins 7.4 and 5.6, saponins 115 and 53.5, cyanogenic glycosides 8.4 and 14.5. These values are for *M. oleifera* and *J. curcas* respectively. Table 3 shows the antimicrobial activities of leaf extracts of *M. oleifera* and *J. curcas* on the test organisms (*Escherichia coli* and *Staphylococcus aureus*). At concentrations of 1.00 mg/ml, 0.50 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml, *M. oleifera* extract inhibited *E. coli* by 2.40 cm, 2.00 cm, 1.60 cm, 0.00 cm and 0.00 cm respectively while *J. curcas* extract inhibited

E. coli by 2.60 cm, 2.20 cm, 1.40 cm, 1.00 cm and 0.00 cm respectively. In the same manner *M. oleifera* extract inhibited *S. aureus* at the same concentrations by 2.80 cm, 2.20 cm, 1.80 cm, 1.00 cm and 0.00 cm respectively while *J. curcas* extract inhibited *S. aureus* by 1.80 cm, 1.40 cm, 1.20 cm, 0.80 cm and 0.00 cm respectively. Table 4 shows the minimum inhibitory concentrations (MIC) of leaf extracts of *M. oleifera* and *J. curcas* on *E. coli* to be 0.1250 mg/ml and 0.125 mg/ml respectively while on *S. aureus* the MIC results are 0.125 mg/m and 0.125 mg/ml respectively.

Table 1: Ethanol extraction yield of *Moringa oleifera* and *Jatropha curcas* leaves.

Leave extract	Weight of crude extract (g)	Percentage yield (%)
Moringa oleifera	3.19	10.63
Jatropha curcas	3.04	10.13

Table 2: Quantitative estimation (%) of bioactive compounds of ethanolic leaves extract of *M. oleifera* and *J. curcas.*

Secondary metabolites	Concentration of phytochemicals present (g/kg)	
	M. oleifera	***J. curcas***
Flavonoids	36.0	21.0
Alkaloids	92.0	39.0
Tannins	7.4	5.6
Saponins	115.0	53.5
Cyanogenic glycosides	8.4	14.5

Table 3: Antimicrobial activity of the leaf extracts of *Moringa oleifera* and *Jatropha curcas* on the test organisms at different concentrations.

Plant extract	Conc. of crude extract (mg/ml)	Diameter zones of inhibition (cm)	
		E.coli	***S. aureus***
M. Oleifera	1	2.4	2.8
	0.5	2.0	2.2
	0.25	1.6	1.8
	0.125	-	1.0
	0.0625	-	-
J. curcas	1	2.6	1.8
	0.5	2.2	1.4
	0.25	1.4	1.2
	0.125	1.0	0.8
	0.0625	-	-

Key: - = No zone of Inhibition.

Table 4: The minimum inhibition concentration of leaf extracts of *Moringa oleifera* and *Jatropha curcas* (mg/ml).

Test Organism	*M. oleifera*	*J. curcas*
Escherichia coli	0.250	0.125
Staphylococcus aureus	0.125	0.125

DISCUSSION

The result of this investigation revealed that the leaf extracts of *Moringa oleifera* and *Jaropha curcas* possesses appreciable antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The extracts inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* at various concentrations. The minimum inhibition concentration of the both leaf extracts on the test organisms ranged between 0.250 mg/ml and 0.125 mg/ml. Quantitative phytochemical screening in percentage of the dried leaf extracts of *M. oleifera* and *J. curcas* revealed the presence of some phytochemical constituents (flavonoids, alkaloids, tannin and saponins). Rehilla et al. (1994) observed that there is need for local herbs to be evaluated for phytochemistry to determine the potential of indigenous source of medicine. Studies elsewhere (Bukill, 1995; Sofowora, 1996 and Timwesizy, 1996) associated medicinal property of plants extract to the presence of alkaloid and essential oil. Harborne (1973) found flavonoid to have antimicrobial effect against wide range of microorganisms *in vitro*, also Lipophilic flavonoids were found to disrupt microbial membranes (Tsuchiya et al., 1994). Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agent for their analgesic and antibacterial effects (Stray, 1998). Phytochemicals with bitter taste such as alkaloids and flavonoids has been found in this study to possess antimicrobial properties. Anthropologists theorize that animals evolved a tendency to seek out bitter plant parts in response to illness (Huffmann, 2003). Hutching et al. (2003) reported that sick animal tend to forage plant rich in secondary metabolites such as tannin and alkaloids. Flavonoids have been reported to have *in vitro* antimicrobial activity against a wide range of microorganism because lipophilic flavonoids disrupt microbial membranes (Tsuchiya et al., 1994). Plants are useful as drugs because of the chemically active ingredients that they contain (Singh, 2004). Janovska et al. (2003) reported that antimicrobials of plant origin are not usually associated with any side effects and have enormous therapeutic potentials to heal many infectious diseases. The results obtained showed that these secondary metabolites in the plant extracts are responsible for the inhibition of the test bacteria. These plant secondary metabolites exhibit various biochemical and pharmacological actions in animals and microorganisms when ingested (Calixto, 2000), and have potentially significant application against human pathogens (El-Mahmood et al., 2008). Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (Afolabi et al., 2007). *Moringa oleifera* and *Jatropha curcas* contain 8.4ppm and 14.5ppm respectively of hydrogen cyanide. FAO/WHO, (1991) recommended 10ppm as safe level for cyanide toxicity. Cardoso et al. (2005) reported 40ppm to 46ppm after heap fermentation of cassava and Okorondu et al. (2008) reported 18.85ppm hydrogen cyanide in *Mucuna pruriens* (velvet bean) after 4 days fermentation and 1hr boiling. The cyanide level in the extracts of *Moringa oleifera* and *Jatropha curcas* are within safe level.

The effects of the different concentrations of both plant extracts on the bacterial isolates with respect to the zone of

inhibition gave a good linearization response for both organisms. In general, the extracts showed a concentration dependent inhibition effect on the bacterial isolates. This finding correlates with the reports of Ekpendu et al. (1994), Ogbeche et al. (1997) and Aiyelagbe et al. (2001), who independently found that various plants inhibited the growth of some clinical bacterial isolates.

Conclusion

In conclusion, the leaf extract of *Moringa oleifera* and *Jatropha curcas* have antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. This inhibitory action of the extracts could be attributed to the presence of the phytochemical constituents in the plant extracts such as alkaloid, flavonoid, and saponin. The extracts in correct doses can successfully be used *in vivo* to inhibit and eventually kill the test bacteria used in this study. Some antibiotics have become almost obsolete because of the problem of drug resistance caused by microbial mutation over the years, the consequence of drug resistance implies that new drugs both synthetic and natural must be sought to treat disease for which known drugs are no longer effective. Further research probably involving *in vivo* assay would be needed to establish the relationship between the MIC's obtained in this study and the effective doses at which the herbs can be applied in traditional practice. Investigation can combine the different plant extract for possible synergistic effect. These plant extracts should be tested on other human pathogens to ascertain their spectrum of effectiveness. Following rampant grazing by animals especially cow flocks and indiscrimination bush burning followed by urbanization and deforestation, there is need to have medicinal plant garden where medicinal plants can be properly kept and maintained. The production can be expanded and extensive research carried out on them.

REFERENCES

Afolabi C, Akinmoladun EO, Ibukun I, Emmanuel A, Obuotor EM, Farombi EO. 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of *Moringa oleifera*. *Scientific Research and Essay*, **2**(5): 163-166.

AOAC (Association of Official Analytical Chemists). 2007. Official methods of analysis (14th edn). AOAC: Washinton D. C.

Belewu O. 2008. Natural Plant chemicals sources of industry and mechanical materials. *Science*, **27**: 1154-1162.

Bukill HM. 1995. *The Useful Plants of West Tropical Africa* (2nd edn, vol. 3). Families E.I. Royal Betanical Gardens: Kew; 636p.

Cardoso AP, Mirione E, Ernesto M, Massaza F, Cliff J, Haque MR, Bradbury JH. 2005. Processing of cassava roots to remove cyanogens. *J. Food Composition Analysis*, **18**: 451-460.

Calixto JB. 2000. Efficacy; safety and quality control, marketing regulatory guidelines for herbals (Phytotherapeutic agents). *Brazilian Journal of Medical Biological Research*, **33**: 179-189.

Cheesbrough M. 2002. *District Laboratory Practices in Tropical Countries* (2nd edn). Cambridge, London; 100-181.

Ekpendu TO, Akahomeju AA, Okogun JI. 1994. Anti-inflamatory and antimicrobial activities of Mitrocarpusscaber extracts. *International Journal of Pharmacology*, **32**(2): 191-196.

El-Manhood AM, Doughari JH, Ladan N. 2008. Antimicrobial Screening of Stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microogganism. *African Journal of Pharmacy and Pharmacology*, **2**(5): 89-94.

Fahey ON. 2005. Antibacterial activities of crude extract of Sennaalata. *Bioscience Research Communications*, **10**(3): 181-184.

FAO/WHO. 1991. Joint FAO/WHO Food Standard Programme. Codex

Alimentarius Commission XII, Supplement 4, FAO, Rome, Italy.

Harborne JB. 1973. *Phytochemical Methods*: *A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall Ltd: London; 279p.

Huffmann MA. 2003. Animal self-medication and ethomedicine: exploration of the medicinal properties of plants. *Proc. Nutr. Soc*., **62**(2): 371-381.

Hutchings MR, Athanasiadous S, Kynazakis I. and Gordon IJ. 2003. Can animals use foraging behaviour to combat parasites? *Proc. Nutr. Soc*., **62**(2): 301.

Janovska D, Kubikova K, Kokoska L. 2003. Screening for Antimicrobial activity of some medicinal plant species of traditional Chinese medicine. *Czech J. Food Sci.*, **21**: 107-110.

Lozan DE. 2007. Evaluation of diacetyl antimicrobial activity against *E. coli*: L. monocytogens and S. aureus. *Food Microbiology*, **20**: 557-543.

OgbecheAK, Ajayi GO. andOnyeneta P. 1997. Antimicrobial activities of the leaf extract of Ageratum conizodes. *Nigerian Journal of Medicine*, **7**: 397-399.

Okigbo RN, Emeogene AO. 2003. Effect of leaf extracts of three plant species on Mycospherellafijiensis, the causal organism of black sigatoka disease of banana (Musa acuminate). *Nigerian Journal of Plant Protection*, **19**: 10-15.

Okorondu SI, Aririatu LE, Okorondu MMO. 2008. The effects of Fermentation and Boiling on the level of hydrogen cyanide in *Mucuna pruriens* (velvet bean).*International J. Tropical Agriculture*, **2**: 268-271.

Okorondu SI, Aririatu LE, Chinakwe EC, Braide W. (2010a). Antibacterial Properties of *Picralima nitida* Seed Extract. *Current Trend in Microbiology*, **6**: 13-19.

Okorondu SI, Mepba HD, Okorondu MMO, Aririatu LE. 2010b. Antibacterial Properties of *Musa paradisiaca* Peel Extract. *Current Trend in Microbiology*, **6**: 21-26.

Okorondu SI. 2011. Evaluation of the Antifungal Properties of *Picralima nitida* Seed Extracts. *International Journal of Natural and Applied Sciences*, **7**(1): 41-46.

Oliver-Bever DC. 2000. *Medicinal Plants in Tropical West Africa*. Cambridge University Press: London.

Rehilla T, Rukhasandra N, Zaichi AA, Shamshila R. 1994. Phytochemical Screening of medicinal plants belonging to family Eupheribiaceae. *Pak Vert J.*, **14**: 100 – 112.

Singh BD. 2004. *Biotechnology* (5^{th} edn). Kalyani publishers: New Delhi, Calcutta, India.

Sofowora PK. 1984. Antimicrobial and Chemo-preventive properties of herbs and spices. *Current Medical Chemistry*, 1951-1960.

Sofowora AE. 1996. Research on medicinal plants and traditional medicine in Africa. *J. Alt. Compl. Med.*, **2**(3): 365-372.

Stray F. 1998. *The Natural Guide to Medicinal Herbs, and Plants*. Tiger Books International: London; 12-16.

Tsuchiya H, Sato M, Linuma M, Yokoyama J, Ohyama M, Tanaka T, Takase I, Namikawa I. 1994. Inhibition of the growth of carcinogenic bacteria *in vitro* by the plant flavonones. *Experimentia*, **50**: 846-849.

Timwesizy O. 1996. Bumetha Rukarere: Integrating modern and traditional health care in south west Uganda. *J. Alt. Compl. Med*., **3**(3): 373-376.