Antibacterial Activity of *Anacardium occidentale* (Cashew) Leaf Extracts on *Staphylococcus aureus*, *Escherichia Coli* and *Pseudomonas aeruginosa.*

Akin-Osanaiye, Bukola Catherine & Audu Mohammed Anoze
Department of Microbiology,
Faculty of Science,
University of Abuja,
Abuja. Federal Capital Territory,
Nigeria.
tou_femi@yahoo.com

Abstract
Microorganisms that cause losses are proving to be resistant to most known antibiotics, thereby encouraging the search for naturally occurring antibiotics. This study aimed to perform a phytochemical and antibacterial study of ethanolic and aqueous extracts of leaves of Anacardium occidentale L. The crude extracts were used to for phytochemical evaluation based on the identification of chemical constituents and to evaluate the antibacterial activity; the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using broth dilution method. The ethanol and aqueous extracts of Anacardium occidentale (Cashew) leaves are rich En wide range of secondary metabolites. Alkaloids, flavonoids, tannins, glycosides and phenols were found in both extracts. Both extracts exhibited bactericidal activities against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa with the aqueous extracts recording higher zone of inhibition (3.0 – 10.0 mm) than the ethanol extract (3.0 – 9.0 mm). The MIC recorded was 250 mg/ml for each of the extracts against all the test organisms. The antibacterial analysis in this study also showed that there was no significant difference ($p \leq 0.05$) in the antibacterial effect of ethanol and aqueous extract against the test bacteria. The results indicate that the plant demonstrated considerable antibacterial therapeutic potential.

Keyword*: Anacardium occidentale, Escherichia coli, Ethanol extract, Pseudomonas auregenos*

Introduction

African flora in general and Abuja in particular, have an important reserve of aromatic, food and medicinal plants. It was demonstrated that medicinal plants play an important role in the African pharmacopoeia. About 80% of Africans have recourse to traditional medicine that involves the use of plants' active principles, to treat most of diseases (Alagesaboopathi, 2011). Nigeria is covered with a large number of plant species, some of which have been used for centuries in folkloric medicines to diagnose, prevent and treat various ailments (El-Mahmood *et al*., 2010) some of these plants such as Guava, *Calotropis procera*, *Trema orientalis*, and *Cnidoscolus aconitifolius***,** have been investigated for heir antibacterial properties and have recorded positive results (Akin-Osanaiye *et al*., 2018; Akin-Osanaiye and Okholoma, 2018; Akin-Osanaiye *et al*., 2016; Akin-Osanaiye *et al*., 2015).

Anacardium occidentale has a height of 5-10 m, but in clay land can reach up to 20 m. It has a crooked trunk of 25-40 cm in diameter. The leaves are oval, obovais, leathery, glabrous; rosy when young; it has vinaceas flowers, arranged in terminal panicles (Lorenzi, 2008). The

family is rich in important secondary metabolites with varieties of interesting biological activities (Abu-Reidah *et al*., 2015).

Several ethnobotanical studies have focused on identifying medicinal plants species. Among these plant species, *Anacardium occidentale* L. has an important place (Chabi-Sika *et al*., 2014). Its leaves, bark, roots, stem are traditionally used for the treatment of numerous diseases such as, allergy, cough, stomach ache, diarrhoea, skin infections (Chabi-Sika *et al*., 2013; Chabi-Sika *et al*., 2014). Besides these medicinal uses, cashew plays several other important roles. Its wood is used mainly in carpentry, as firewood, or turned into charcoal, whereas the resins are used in the manufacture of plastics and natural insecticides (Akinwale, 2000).

Phytochemicals are plant metabolites (Sofowora, 1993) which act as natural defence systems for host plants, and also provide characteristic colour, aroma and flavour in specific plant parts. They are a group of non-nutrient compounds that are biologically active when consumed by human. Many phytochemicals are health-promoting and are prevent many diseases (Birt, 2006).

Materials

Plant Material

The leaves of *A. occidentale* L., were collected in Giri, Abuja and was taken for botanical identification in the herbarium of University of Abuja Biological Garden. The leaves were dried under room temperature till constant weight was obtained; the dried leave sample was grinded to powder form using a blending machine for effective extraction with solvents.

Test Organisms

Staphylococcus aureus, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Department of Microbiology, University of Abuja Teaching Hospital.

Methods

Extraction of Plant Material

The powdered *A. occidentale* was extracted using sterile distilled water and ethanol following the procedure described by Bankole *et al*., (2012). One hundred gram of *A. occidentale* leaf powder was soaked in 500 ml of 96% ethanol at room temperature. For the aqueous extraction, 100 g of *A. occidentale* leaf powder was soaked in 500 ml of sterile distilled hot water. The mixtures were allowed to stand for 24 h after which they were filtered using a fine mesh cloth. The solvent was evaporated to dryness using water bath at 40^{o}C.

Phytochemical Constituents

All the extracts were subjected to standard phytochemical qualitative screening for secondary metabolites as described by Trease and Evans (2002), Sofowora (1993) and Harborne (1998).

Confirmation of purity and Viability of test Organisms

The pure isolates of *Staphylococcus aureus* was sub-cultured from nutrient agar slants on Mannitol Salt Agar and observe for growth after 24 h at 37°C, yellow colonies was seen on the mannitol salt agar. Pure isolates of *Escherichia coli* was subcultured from nutrient agar slants onto Eosine Methyl Blue Agar and observe for growth after 24h at 37°C, colonies with metallic green sheen was seen. Pure isolate of *Pseudomonas aeruginosa* was subcultured from nutrient agar slants onto Cetrimide Agar and observe for 24 h at 37 °C, blue-green colonies was seen.

Preparation of Test Inoculum

The test bacteria from the agar slant was subcultured on nutrient agar by stocking and incubated at 37 ^{o}C for 18-24 h. A bacteria suspension was made using the 18-24h culture in 4 ml of physiological saline; its density was adjusted to match the 0.5 McFarland density.

Antimicrobial Activity of plant Extract

The ethanol and aqueous extracts were reconstituted in sterile distilled water into different concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml) by doubling dilution. The antibacterial activity of the different concentrations of the extracts was carried out using agar well diffusion assay technique of Bankole *et al*. (2012).

Aliquot (20 µl) of the bacterium in physiological saline was spread on already solidified Mueller Hinton agar surface using a sterile cotton swab. The surface of the medium was allowed to dry for 3 min and sterile 6mm cork borer was used to bore holes on agar plate (two each), the base of each hole was sealed with a drop of molten agar to avoid diffusion of extract under agar. Some (20 µl) of each extract concentrations was dispensed into each hole. The plates were allowed to undisturbed for about 15 min before they were incubated at 37°C for 24 h. The diameter of zones of inhibition was measured using a transparent ruler and the zone of inhibition was measured in mm.

Minimum Inhibitory Concentration (MIC)

The MIC of *A. occidentale* extract was determined using broth tube dilution method described by Kacaniova *et al*., (2011). Eight (8) sterile test tubes were labelled 1 through 8 and arranged in a rack. *A. occidentale* extract control tube and growth control tube was used as a quality control. Nine (9) ml of sterile nutrient broth was added to each tube, and then 1 ml of *A. occidentale* extract was added to test tube number 1 and growth control tube with sterile pasture pipettes. Then serial dilutions were performed by transferring 1 ml of *A. occidentale* extract into the second tube with separate sterile pasture pipette and vortexed for homogenization. After thorough mixing, 1 ml was transferred with another sterile pasture pipette from tube 2 and tube 3. The procedure was repeated until the 7th tube. The growth control tube received no *A. occidentale* extract. One (1) ml of the culture of 0.5 McFarland standard of test bacteria was added to each tube. Tubes were then incubated at 37 °C for 24 h and observed by visual inspections for the presence and absence of growth.

Minimum Bactericidal Concentration (MBC)

To determine the MBC, incubated tubes showing no visible sign of growth/turbidity in MIC, were sub-cultured onto sterile Mueller Hinton agar plates by streak plate method and incubated at 37 °C for 24 h. The least concentration of extract that did not show growth of test organisms was considered as the minimum bactericidal concentration (Kacaniova *et al*., 2011). The inoculated plates were scored as bactericidal if no growth occurred after 24 h; bacteriostatic if there is light to moderate growth.

Data Analysis

Data obtained were expressed as the mean ± standard error of mean. One-way analysis of variance (ANOVA) was used to determine significant differences of antibacterial effect of aqueous and ethanol extracts at $p < 0.05$.

Results and Discussion

The phytochemical and antibacterial screening of *A. Occidentale* was carried out. The yields of the extracts were recorded. The percentage yield of ethanol extract was more than that of

the aqueous extract (Figure 1). The ethanol and aqueous extracts showed the presence of alkaloids, flavonoids, tannins, glycosides, carbohydrates, and phenols as the phytochemicals present in the plant extracts. However, quinones, terpenoids, flavones and steroids were observed to be absent in both extracts (Table 1). The antibacterial effect of the aqueous and ethanol extracts against the investigated bacteria is shown in Table 2. Also, Table 3 shows the minimum inhibitory concentration of the extracts against the test organisms. The minimum inhibitory concentration (MIC) of the ethanol and aqueous extracts against *Staphylococcus aureus* was at 250mg/ml. The MIC of the ethanol extract against *E. coli* was at 250 mg/ml and 125 mg/ml for the aqueous extract. The MIC for the ethanol extract against *P. aeruginosa* was at 250 mg/ml and 500mg/ml for the aqueous extract.

Table 5 shows the bactericidal activity of the ethanol and aqueous extracts against test bacteria. The minimum bactericidal concentration (MBC) of the aqueous extract against *S. aureus* was at 250 mg/ml and 500 mg/ml for the ethanol extract against the same bacteria. Against *E. coli*, the MBC of both ethanol and aqueous extracts was at 250 mg/ml. against *P. aeruginosa*, the MBC of the ethanol extract was at 250 mg/ml and at 500 mg/ml for the aqueous extract against the same bacteria.

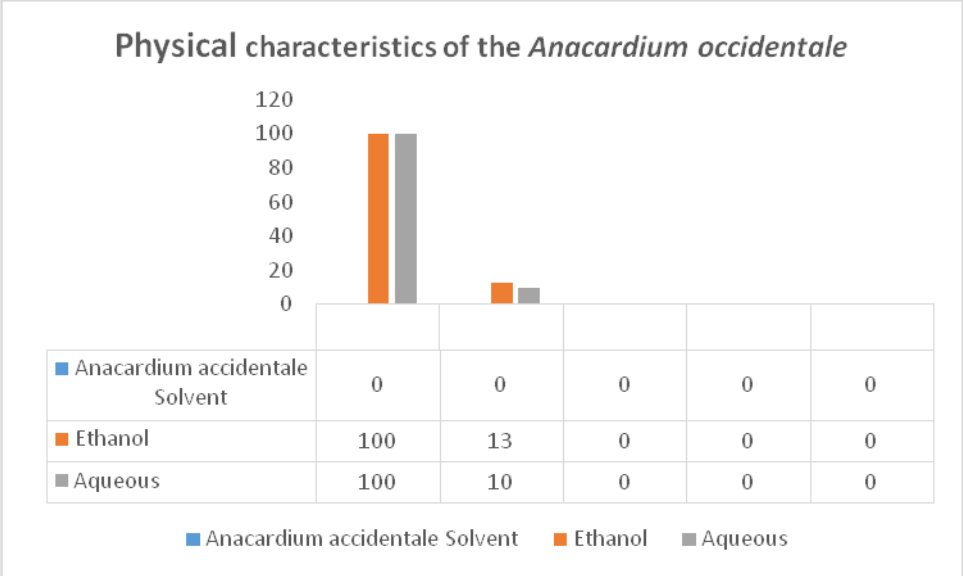

Figure 1: **Showing the Physical characteristics of the *Anacardium occidentale* (Cashew Leaf) Extract**

Table 1: Phytochemical Screening of *A. occidentale* extracts

Phytochemical	Ethanol Extract	Aqueous Extract
Akaloids	+	+
Flavonoids	+	+
Tannins	+	+
Quinone	-	-
Terpenoid	-	-
Glycosides	+	+
Carbohydrates	+	+
Steroids	-	-
Phenols	+	+
Flavones	-	-

Key: + presence of compound
-Absence of compound

Table 2: Antibacterial susceptibility of Ethanol extracts showing diameter zone of inhibition (mm) against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*

Test Organisms	Conc (mg/ml)	Ethanol Extract	Aqueous Extract	Chloram.(30μg)
S. aureus	500	7.0 ± 0.1^{a}	8.0 ± 0.0^{a}	19.0± 0.0
	250	6.0 ± 0.0^{a}	7.0 ± 0.5^{a}	
	125	5.0 ± 0.1^{a}	5.0 ± 0.1^{a}	
	62.5	3.0 ± 0.0^{b}	4.0 ± 0.7^{b}	
E. coli	500	8.0 ± 0.0^{a}	10.0 ± 0.4^{a}	22.0± 0.0
	250	6.0 ± 0.1^{a}	6.0 ± 0.1^{b}	
	125	4.0 ± 0.1^{b}	5.0 ± 0.0^{b}	
	62.5	3.0 ± 0.0^{b}	3.0 ± 0.1^{c}	
P. aeruginosa	500	9.0 ± 0.0^{a}	10.0 ± 0.0^{a}	17.0± 0.0
	250	8.0 ± 0.1^{a}	8.0 ± 0.1^{a}	
	125	6.0 ± 0.0^{b}	7.0 ± 0.1^{b}	
	62.5	NA	NA	

Key: Values are Zone Diameter of Inhibition ± Standard error of mean
NA: No Activity
Zones with different alphabets are significantly different ($p<0.05$)

Table 3: Minimum inhibitory concentration

Test Organisms	Concentration (mg/ml)	Ethanol Extract	Aqueous Extract
S. aureus	500	-	-
	250	-	-
	125	+	+
	62.5	+	+
E. coli	500	-	-
	250	-	-
	125	+	-
	62.5	+	+
P. aeruginosa	500	-	-
	250	-	+
	125	+	+
	62.5	+	+

Key: + growth
- No growth

Table 4: Minimum bactericidal concentration

Test Organisms	Concentration (mg/ml)	Ethanol Extract	Aqueous Extract
S. aureus	500	-	-
	250	+	-
	125	+	+
	62.5	+	+
E. coli	500	-	-
	250	-	-
	125	+	+
	62.5	+	+
P. aeruginosa	500	-	-
	250	-	+
	125	+	+
	62.5	+	+

Key: + growth - No growth

Discussion

This study was aimed at evaluating the phytochemical constituents and antibacterial efficacy of *A. occidentale* (Cashew) leaves extract against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, glycosides, carbohydrates, and phenols I both ethanol and aqueous extracts of *A. occidentale* leaves. Ayepola and Ishola (2009) reported the presence of alkaloids; tannins and saponins in *A. occidentale* stem extract. Several studies have reported rich variety of secondary metabolites in *A. occidentale* extracts (Rajesh *et al*., 2009). The pharmacological properties of medicinal plants have been attributed to their rich secondary metabolites (Mahesh and Satish, 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Shahidi *et al*., 2008; Varaprasad *et al*., 2009).

The antibacterial analysis in this study showed that there was no significant difference ($p \leq 0.05$) in the antibacterial effect of ethanol and aqueous extract against the test bacteria. This result is in disagreement with report of Arekemase *et al*., (2011) who reported that ethanolic extract was more effective than aqueous extract. Aderiye and David (2014) reported potent antibacterial effect of cold and hot water extract of *A. occidentale* against *E. coli* O167: H7 and methicillin resistant *S. aureus* (MRSA). Their study showed that the zone of inhibition of *E. coli* O157: H7 by the cold water extracts ranged between 201.06 and 615.75 mm while those of MRSA were from 0 to 314.16 mm.

The minimum inhibitory concentration (MIC) of extracts against test *S. aureus, E. coli* and *P. aeruginosa* in this study (Table 3) are higher than MIC reported by Chabi *et al*., (2014). The authors reported MIC of 0.313 and 0.625 mg/ml for reference strain of *S. aureus* and *E. coli* and 1.25 mg/ml against *S. aureus* isolated from food as against the 250 mg/ml recorded in this study. Onuh *et al*., (2017) reported appreciable antimicrobial effect of the ethanol extract of *A. occidentale* against E. *coli*, *S. mutans*, *B. cereus*, *S. typhi*, and *C. albicans*. The authors also reported varying levels of phytochemicals in the leaves and stem bark of *A. occidentale*.
The minimum bactericidal concentration (MBC) of the aqueous extract against *S. aureus* was at 250 mg/ml and 500 mg/ml for the ethanol extract against the same bacteria. Against *E. coli*, the MBC of both ethanol and aqueous extracts was at 250 mg/ml. against *P. aeruginosa*, the MBC of the ethanol extract was at 250 mg/ml and at 500 mg/ml for the aqueous extract against the same bacteria. This result is in similar to report of Arekemase *et al*., (2011) who reported that the ethanolic extract was found to be bactericidal to all the test bacteria, while the aqueous extract was found to be bacteriostatic to the test bacteria.

The antibacterial effect of the ethanol and aqueous extract against the test bacteria in this study could be attributed to the presence of the phytochemicals. Flavonoids have been reported to significantly affect the cell wall of the microorganisms which may invariably lead to the collapse of the cell wall and overall, affect the entire mechanism of the microbial cell (Nwinyi *et al.,* 2009). Alkaloids have also been reported to be involved in antimicrobial activities (Punitha *et al.,* 2005).

Conclusion

The ethanol and aqueous extracts of *Anacardium occidentale* (Cashew) leaves are rich in wide range of secondary metabolites. Alkaloids, flavonoids, tannins, glycosides, carbohydrates, and phenols were found in both extracts. Both extracts exhibited bactericidal activities against *S. aureus*, *E. coli* and *P. aeruginosa*. The antibacterial efficacy of the leaves extracts of *A. occidentale* lends credence to ethnomedicinal use of the plant to treat various ailments.

References

Abu-Reidah, I.M., Ali-Shtayeh, M.S., Jamous, R.M., Arraez-Roman, D. and Segura-Carretero, A. (2015). HPLC-DAD-ESI-MS/MS screening of bioactive components from Rhuscoriaria L. (Sumac) fruits.*Food Chemistry*, 166:179-191.

Aderiye, B.I. and David, O.M. (2014).*In vitro* Antibacterial Activity of Aqueous Extracts of Cashew (*Anacardium occidentale* L.) Fruit Peels Using Bioautography Method. *European Journal of Medicinal Plants*, 4(3): 284-291.

Akin-Osanaiye, B.C. and Okhomina, L. (2018). Phytochemical and Antibacterial Activity of Leaf Extracts of *Calotropis procera* on some Selected Bacteria. *D. Res. J. Biotechnol.*, 4(2):16-21.

Akin-Osanaiye, B.C., Ghali, A.A. and Ugoh, C. (2018). Phytochemical Activity and

Antibacterial Screening of the Root of Guava. *Int. J. Trend Res. Devt.*, 5(3):182-188.

Akin-Osanaiye, B.C**.,** Gabriel, A.F., Omoniyi, A.O. and Ezeani, S.C. (2016). Scientific Approach on the Antimicrobial Potentials of Bioactive Phytochemical of *Trema orientalis* Leaves and Stalk. *Euro.Aca. Res.*, 3(12):12972-12981.

Akin-Osanaiye, BC, Adeniran, O.I., and Aderemi, A.S (2015). Auspicious Antimicrobial Potentials of Ethanol Extracts of Stem and Root of *Cnidoscolus aconitifolius*. *Int. J. Medicinal Plant Natural Products*, 1(1):15-19.

Akinwale, T.O. (2000). Cashew apple Juice: its use in fortifying the nutritional quality of some tropical fruits. *European Food Research Technology*, 211: 205-207.

Alagesaboopathi, C. (2011). Antimicrobial screening of selected medicinal plants in Tamilnadu, India. *African Journal of Microbiological Research*, 5(6): 617-6 21.

Arekemase, M.O., Oyeyiola, G.P. and Aliyu, M.B. (2011).Antibacterial activity of *Anacardium occidentale* on some enterotoxin producing bacteria.*International Journal of Biology*, 3(4): 92-99.

Ayepola, O.O. and Ishola, R.O. (2009).Evaluation of Antimicrobial Activity of *Anacardium occidentale* (Linn.).*Advances in Medical Dental Sciences*, 3(1): 1-3.

Bankole, H.A., Anjorin, A.A., Kazeem, M.I., Ogbeche, M.E. and Agbafor, U. (2012).Antibacterial activity of *Ocimum gratissimum* and *Gongronema latifolium* on *Staphylococcus aureus* and *Salmonella typhi*. *East African Journal of Science and Technology*, 2(1): 114-128.

Birt, D. A. (2006). Phytochemicals and cancer prevention: from epidemiology to mechanism of action. *Journal of the American Dietetic Association*, 106: 20 – 24.

Chabi-Sika, S.K., Adoukonou, S.H., Ahoton, L.E., Roko, G.O., Saidou, A., Adeoti, K., Ahanchede, A. and Baba-Mousa, L. (2014).Antibacteria activity of A. occidentale L. leaves and barks extracts on pathogenic bacteria. *African Journal of Microbiology Research*, 8(25): 2458-2467.

Chabi-Sika, S.K., Adoukonou, S.H., Ahoton, L.E., Roko, G.O., Saidou, A., Adeoti, K., Ahanchede, A. and Baba-Mousa, L. (2013).Indegenious knowledge and traditional management of cashew (*Anacardium occidentale*).Genetic resources in Benin.*Journal of Experimental Biology and Agricultural Science*, 1(5): 375-382.

Das, K., Tiwari, R. K. S. and Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4(2): 104-111.

El-Mahmood, A. M., Doughari, J. H. and Kiman, H. S. (2010).*In vitro* antimicrobial activity of crude leaf and stem bark extracts of *Gmelinaarborea* against some pathogenic species of Enterobacteriaceae. *African Journal of Pharmacy and Pharmacology* 4(6):355-361.

Harbone, J.B. (1998). Phytochemicals Methods. A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall Publishing, London, United Kingdom. Pp 286.

Kacaniova, M., Vukovic, N., Bobkova, A., Fikselova, M., Rovna, K. and Hascik, P. (2011) Antimicrobial and antiradical activity of Slovakian honeydew honey samples. *Journal of Microbiology, Biotechnology and Food Science*, 1(3):354– 360.

Lorenzi, H. (2008). Árvoresbrasileiras: manual de identificação e cultivo de plant asarbóreasnativas do Brasil. 5 ed. São Paulo: Instituto Plantarum de Estudos da Flora.

Mahesh, B. and S. Satish (2008). Antimicrobial Activity of some Important Medicinal Plant against Plant and Human Pathogens. *World Journal of Agricultural Sciences*, 4(S):839-843.

Nwinyi, O. C., Chinedu, N. S., Ajani, O. O., IkpoChinwe. O and Ogunniran, K. O. (2009). Antibacterial effect of extracts of *Ocimumgratissimum* and *Piper guineense* on

Escherichia coli and *Staphylococcus aureus.African Journal of Food Science*, 3 (3): 77 – 81.

Onuh, J.O., Idoko, G., Yusufu, P. and Onuh, F. (2017). Comparative Studies of the Phytochemical, Antioxidant and Antimicrobial Properties of Cashew Leaf, Bark and Fruits Extracts. *American Journal of Food and Nutrition*, 5(4): 115-120.

Punitha, I.S.R., Shirwaika, A., Shirwaikar, A. (2005). Anti-diabetic activity of benzyl tetraisoquinoline alkaloid, berberine, in streptozocin-nicotinamide induced type 2 diabetic rats. *Diabetologian Croatia*, 34(4): 117-121.

Rajesh, K.V., Sumathi, C.S., Balasubramanian, V. and Ramesh, N. (2009).Elementary chemical Profiling and antifungal properties of cashew (*Anacardium occidentale* L.) nuts. *Botany Research International*, 2: 253-257.

Shahidi, F., McDonald, J., Chandrasekara, A. and Zhong, Y. (2008). Phytochemicals of foods, beverages and fruit vinegars: chemistry and health effects. *Asia Pacific Journal of Clinical Nutrition*, 17: 380-382.

Sofowora, L. A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Book Ltd, Ibadan, pp. 55 – 71.

Trease, G.E. and W.C. Evans (2002). A Textbook of Pharmacognosy. 15th Edn. Sauders, Pp 214-393.

Varaprasad, B., Katikala, P.K., Naidu, K.C. and S. Penumajji (2009). Antifungal activity of Selected Plant extracts against Pytopathogenic Fungi *Aspergillus niger*. *Indian Journal of Science and Technology*, 2(4):87-90.