African Journal of Biotechnology Vol. 7 (20), pp. 3665-3670, 20 October, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

In vitro antimicrobial and phytochemical properties of crude extract of stem bark of *Afzelia africana* (Smith)

Akinpelu, D. A.[1, 2], Aiyegoro, O. A.[1] and Okoh, A. I.[1]*

[1]Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa.
[2]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Accepted 29 August, 2008

***Afzelia africana* is used in folklore remedies for the treatment of diarrhoea, gastrointestinal disorders and gonorrhoea among other ailments; hence we assessed the *in vitro* antimicrobial activities of this important medicinal plant. Thirty bacterial isolates as well as four fungal isolates were tested in this study. The crude extract of the stem bark of the plant exhibited antimicrobial activities at a concentration of 25 mg/ml against twenty-one of the bacterial isolates, (i.e. 72.41% of the tested isolates) comprising both Gram positive and Gram negative strains. The zones of inhibition exhibited by the extract against the test bacterial species ranged between 13 and 22 mm. The Minimum Inhibitory Concentrations (MIC) of the extract vary between 1.56 and 12.50 mg/ml while the Minimum Bactericidal Concentration (MBC) ranged between 3.13 and 25.00 mg/ml. However, the extract lacked activity against all four tested fungal species. Phytochemical assay revealed the presence of alkaloids, tannins, flavonoids and saponins in the extract. We conclude that the stem bark of *A. africana* is a promising candidate as source of new antibacterial compounds.**

Key words: *Afzelia africana,* antimicrobial, phytochemical properties, medicinal plant, MIC, MBC.

INTRODUCTION

Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases. The studies of medicinal plants used in folklore remedies have attracted the attention of many scientists in finding solutions to the problems of multiple resistances to the existing synthetic antibiotics. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro-organisms against these drugs. Thus, there is need to search for new and more potent antimicrobial compounds of natural origin to combat the activities of these pathogens which is the basis for this study.

Afzelia africana belongs to the family Caesalpiniaceae. The English name is mahogany. The tree is widely distributed in Africa and Asia (Keay, 1989). It is used as food and plank and widely as folklore remedies among many tribes in Africa. Previous studies have reported the plant to exhibit such bioactive activities as anti-inflammatory and analgesic activities (Akah et al*.,* 2007). Atawodi (2005) investigated and reported the trypanocidal activities of the leaves and stem bark extract of *A. Africana* against the protozoan, *Trypanosoma brucei*. The protozoan was inhibited by the plant extract. Powdered root of *A. africana* mixed with millet beer has been found to serve as treatment for hernia among some tribes in Cote d'Ivoire (Dalziel, 1937). Also, Agbelusi et al. (2007) reported that aqueous extract of chewing stick made from the plant inhibited some microbial isolates obtained from mouth washings, and in addition to this, a novel xyloglucan (carbohydrate) was isolated from the seeds of the plant.

However, to the best of our knowledge there is no report on the antimicrobial property of the stem bark of this plant. In this paper, we report the antimicrobial and phytochemical properties of crude extract of the stem bark of *A. africana* as part of our exploration for new and

*Corresponding author. E-mail: aokoh@ufh.ac.za.

novel bioactive compounds in our group.

MATERIALS AND METHODS

Plant materials

Fresh stem bark of *A. africana* was collected from Abeokuta, Ogun State, Nigeria in the month of April, 2008 and was identified by Dr. H. C. Illoh of the Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria. Voucher sample was prepared and deposited in the Herbarium of the Botany Department, Obafemi Awolowo University, Ile-Ife, Nigeria for reference. The stem bark was air-dried to constant weight, powdered and stored in an air-tight container for further use.

Preparation of extract

Exactly 250 g of the powdered bark of the plant was cold extracted using 70% (v/v) methanol in water for 4 days. The mixture was later filtered and the filtrate was first concentrated *in vacuo* using rotary evaporator to remove the methanol. The aqueous residue left was then lyophilized to obtain the crude extract. The extract was brownish in colour and the yield obtained was 23.6% of the powdered bark.

Preparation of test micro-organisms

The test micro-organisms used in this study were obtained from the culture collection of the Applied and Environmental Microbiology Research Group (AEMREG) laboratory, University of Fort Hare, Alice, South Africa, and include the following.

Bacteria

Escherichia coli (ATCC 8739), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884), *Pseudomonas aeruginosa* (ATCC 7700), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 10031), *K. pneumoniae* (ATCC 4352), *Proteus vulgaris* (ATCC 6830), *P. vulgaris* (CSIR 0030), *Serratia marcescens* (ATCC 9986), *Acinetobacter calcoaceticus* (UP), *A. calcoaceticus anitratus* (CSIR), *K. pneumoniae* (LIO), *Bacillus subtilis* (LIO), *Shigella dysenteriae* (LIO), *Staphylococcus epidermidis* (LIO), *P. aeruginosa* (LIO), *P. vulgaris* (LIO), *Enterococcus faecalis* (LIO), *S. aureus* (LIO) *Micrococcus kristinae* (LIO) and *Micrococcus luteus* (LIO).

Fungi

Aspergillus flavus (ATCC 9643), *Aspergillus niger* (ATCC1604), *Candida albicans* (ATCC10231) and *Penicillum notatum* (ATCC 2091).

The bacterial isolates were first sub-cultured in nutrient broth and incubated at 37°C for 18 h while the fungal isolates were sub-cultured on potato dextrose agar medium for 72 h at room temperature.

Antimicrobial activity test

The antimicrobial activity of the crude extract was determined in accordance with the agar-well diffusion method described by Russell and Furr (1977) and Irobi et al. (1994). The bacterial isolates were first grown in nutrient broth for 18 h before use, while the fungal isolates were allowed to grow on potato dextrose agar medium (PDA) at 25°C until they sporulate. The fungal spores were harvested after sporulation by pouring mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with sterile glass rod. The harvested fungal spores and the bacterial isolates were standardized to OD_{600nm} 0.1 before use. One hundred microliter of the standardized bacterial suspension was evenly spread on Mueller-Hinton agar using a glass spreader while the same volume of the fungal spore suspension was spread on Potato dextrose agar. Wells were then bored into the agar media using a sterile 6 mm cork borer and the wells filled with the solution of the extract taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow proper diffusion of the extract into the media. The bacterial isolates were thereafter incubated at 37°C for 24 h after which they were observed for zones of inhibition. Plates containing fungal isolates were incubated at 25°C for 96 h and later observed for zones of inhibition. The effects of the extract on the test bacterial isolates were compared with those of tetracycline and ampicilin standard antibiotics at a concentration of 1 mg/ml and 10 µg/ml, respectively. The effect of the extract on fungal isolates was compared with nystatin at a concentration of 1 mg/ml.

Determination of minimum inhibitory concentration (MIC)

The MIC of the crude extract was carried out using the method of Akinpelu and Kolawole (2004). Two-fold dilutions of the crude extract was prepared and 2 ml aliquot of different concentrations of the solution was added to 18 ml of pre-sterilized molten nutrient agar at 40°C to give final concentrations regimes of 12.5 to 0.391 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry under a laminar flow before streaking with 18 h old bacterial cultures. The plates were later incubated at 37°C for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that will prevent the growth of the test bacteria.

Determination of minimum bactericidal concentration (MBC)

The MBC of the crude extract was determined as described elsewhere (Olorundare et al., 1992). Samples were taken from plates with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar plates and later incubated at 37°C for 48 h. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

Phytochemical analysis of the plant extract

A small portion of the dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods of Trease and Evans (1983) and Harborne (1998) with modifications.

Test for tannins

Exactly 1.0 g of plant extract was dissolved in 10 ml of distilled water and filtered (using Whatman No 1 filter paper). A blue colouration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract.

Test for alkaloids

Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. One millilitre of the filtrate was treated with few drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloid.

Test for flavonoids

About 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the flavonoids.

Test for saponins

Freshly prepared 7% blood agar plate was used and wells were made in it. The extract in methanol was applied with distilled water and methanol used as negative control while commercial saponin (BDH) solution was used as a positive control. The plates were incubated at 35°C for 6 h. Complete haemolysis of the blood around the extract was indicative of the presence of saponin.

Test for steroids

About 0.5 g of the extract was dissolved in 3 ml of chloroform and filtered. Concentrated H_2SO_4 was carefully added to the filtrate to form lower layer. A reddish brown colour at the interface was taken as positive for steroid ring.

RESULTS AND DISCUSSION

The antimicrobial activities of *A. africana* stem bark extract was investigated against some microbial isolates and found to possess bioactivity against some of the test organisms. The crude extract at a concentration of 25 mg/ml was found to inhibit the growth of 21 out of 30 test bacteria comprising both Gram-negative and Gram-positive organisms (Table 1). The zones of inhibition exhibited by the crude extract ranged between 13 mm for *E. coli* (ATCC 8739) and 22 mm for *A. calcoaceticus anitratus* (CSIR). The fungal isolates were not susceptible to the activity of the plant extract. The bacterial isolates used in this study include such pathogens as *E. coli* known to cause urinary tract infections; *Shigella* which are associated with diarrhoea and food infections; and *K. pneumoniae* the causative agent of pneumonia (Pelczar et al*.,* 2006). All these pathogens were susceptible to the plant extract used in this study, thus supporting the use of *A. africana* in folklore remedies in the treatment of diseases caused by these micro-organisms. The extract was observed to inhibit the growth of both Gram positive and Gram negative organisms and thus show it to possess a broad spectrum activity. When the activity of the plant extract was compared with that of the standard antibiotics, tetracycline and ampicilin used in this work, it was observed that the plant extract compared favourably with those of these standard antibiotics.

The MIC of the crude extract was also determined and this ranged between 1.56 and 12.50 mg/ml while that of the tetracycline control ranged between 0.02 mg/ml and 1.0 mg/ml (Table 2). Also, the minimum bactericidal activity (MBC) of the extract ranged between 3.13 and 25.00 mg/ml (Table 2). Considering that the extract is in the crude form, this observation has promise as a veritable source of active pure antimicrobial compounds as we suggested in our previous report (Sibanda and Okoh, 2008). Thus improvement on such extract by pharmaceutical industry to produce antimicrobial drug of natural source will go a long way in healthcare delivery. Investigation on the phytochemical compounds of *A. africana* stem bark extract revealed the presence of tannins, flavonoids, alkaloids, steroids and saponins (Table 3). These phytochemical compounds are known to be biologically active and thus aid the antimicrobial activities of *A. africana*. Phytochemicals exert anti-microbial activity through different mechanisms; tannins for example, act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes (Scalbert, 1991) in microbial cells. Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003) thus exhibiting antimicrobial activity. The presence of tannins in *A. africana* supports the traditional medicinal use of this plant in the treatment of different ailments. Motar et al. (1985) revealed the importance of tannins for the treatment of inflamed or ulcerated tissues. Li et al. (2003) reviewed the biological activities of tannins and observed that tannins have remarkable activity in cancer prevention and anticancer, thus suggesting that *A. africana* has potentials as a source of important bioactive molecules for the treatment and prevention of cancer. In addition to its antimicrobial, anticancer activities, tannins have roles such as stable and potent antioxidants (Trease and Evans, 1983). The observations above support the use of *A. africana* in herbal cure remedies.

Alkaloid is another phytochemical compound observed in the stem bark extract of *A. africana*. Alkaloids have been associated with medicinal uses for centuries and other possible roles have not been examined. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities

Table 1. Antimicrobial activity profile of the crude extract of stem bark of *A. africana*.

Microorganism	Zones of inhibition (mm)*		
Bacterial isolates	***A. africana* extract (25 mg/ml)**	**Tetracycline (1 mg/ml)**	**Ampicilin (10 µg/ml)**
Escherichia coli (ATCC 8739)	13.00	17.00	18.00
Escherichia coli (ATCC 25922)	20.00	28.00	25.00
Pseudomonas aeruginosa (ATCC 19582)	0.00	0.00	0.00
Staphylococcus aureus (ATCC 6538)	20.00	0.00	24.00
Streptococcus faecalis (ATCC 29212)	13.00	19.00	17.00
Bacillus cereus (ATCC 10702)	20.00	24.00	24.00
Bacillus pumilus (ATCC 14884	20.00	27.00	23.00
Pseudomonas aeruginosa (ATCC 7700)	0.00	0.00	0.00
Enterobacter cloacae (ATCC 13047)	0.00	0.00	0.00
Klebsiella pneumoniae (ATCC 10031)	17.00	23.00	14.00
Klebsiella pneumoniae (ATCC 4352)	0.00	25.00	13.00
Proteus vulgaris (ATCC 6830)	15.00	17.00	18.00
Proteus vulgaris (CSIR 0030)	0.00	14.00	15.00
Serratia marcescens (ATCC 9986)	14.00	13.00	17.00
Acinetobacter calcoaceticus (UP)	0.00	0.00	0.00
Acinetobacter calcoaceticus anitratus (CSIR)	22.00	17.00	13.00
Klebsiella pneumoniae (LIO)	15.00	15.00	13.00
Bacillus subtilis (LIO)	18.00	22.00	27.00
Shigella flexineri (LIO)	13.00	0.00	0.00
Salmonella spp. (LIO)	0.00	10.00	15.00
Staphylococcus epidermidis (LIO)	20.00	10.00	17.00
Pseudomonas aeruginosa (LIO)	0.00	0.00	0.00
Proteus vulgaris (LIO)	15.00	22.00	19.00
Enterococcus faecalis (LIO)	20.00	22.00	21.00
Escherichia coli (LIO)	0.00	17.00	17.00
Staphylococcus aureus (LIO)	20.00	0.00	22.00
Staphylococcus aureus (OK2)	16.00	0.00	13.00
Staphylococcus aureus (OK3)	20.00	0.00	17.00
Micrococcus kristinae (LIO)	20.00	18.00	18.00
Micrococcus luteus (LIO)	15.00	17.00	13.00
Fungal isolates		**Nystatin (1mg/ml)**	
Aspergillus flavus (ATCC 9643)	0.00	15.00	
Aspergillus niger (ATCC 16404)	0.00	15.00	
Candida albicans (ATCC 10231)	0.00	20.00	
Penicillum notatum (ATCC 2091)	0.00	13.00	

mm* = Mean of three replicates in mm; ATCC = American Typed Culture Collection; OK2 and OK3 are clinical Isolates.

have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994). In addition, alkaloids possess anti-inflammatory, anti-asthmatic, and anti- anaphylactic properties with consequences of altered immunological status *in vivo* (Ganguly and Sainis, 2001; Gopalakrishnan et al*.,* 1979; Staerk et al*.,* 2002). Furthermore, alkaloid which is one of the largest groups of phytochemical in plants has amazing effect on humans and this has led to the development of powerful pain killer medications (Raffauf, 1996). Flavonoids which is also one of the constituents of *A. africana* stem bark extract exhibit a wide range of biological activities which are antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic effects, cytostatic and antioxidant properties (Hodek et al., 2002). Flavonoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy-radicals highlights many of the flavonoid health-promoting functions in organism, which are important for prevention of diseases associated with oxidative damage of mem-

Table 2. The MIC and MBC regimes of crude extract of stem bark of *Afzelia Africana*.

Microorganism	*A. africana* extract (mg/ml)		Tetracycline (mg/ml)
	MIC	MBC	MIC
Escherichia coli (ATCC 8739)	12.50	25.00	0.13
Escherichia coli (ATCC 25922)	3.13	12.50	0.13
Staphylococcus aureus (ATCC 6538)	3.13	6.25	0.13
Streptococcus faecalis (ATCC 29212)	6.25	12.50	0.02
Bacillus cereus (ATCC 10702)	1.56	6.25	0.25
Bacillus pumilus (ATCC 14884)	6.25	12.50	1.00
Klebsiella pneumoniae (ATCC 10031)	6.25	12.50	0.50
Proteus vulgaris (ATCC 6830)	6.25	12.50	0.50
Serratia marcescens (ATCC 9986)	6.25	12.50	0.25
Acinetobacter calcaoceticus anitratus (CSIR)	1.56	3.13	0.25
Klebsiella pneumoniae (LIO)	12.50	25.00	0.50
Bacillus subtilis (LIO)	1.56	6.25	0.25
Shigella flexineri (LIO)	12.50	25.00	0.25
Staphylococcus epidermidis (LIO)	1.56	3.13	0.25
Proteus vulgaris (LIO)	6.25	12.50	0.50
Enterococcus faecalis (LIO)	3.13	6.25	0.50
Staphylococcus aureus (LIO)	3.13	6.25	0.13
Staphylococcus aureus (OK 2)	1.56	3.13	0.13
Staphylococcus aureus (OK 3)	1.56	6.25	0.13
Micrococcus kristinae (LIO)	3.13	12.50	0.02
Micrococcus luteus (LIO)	6.25	12.50	0.02

Table 3. Phytochemical compounds present in the crude extract of stem bark of *Afzelia Africana*.

Compound	Reaction
Tannins	Positive
Alkaloids	Positive
Flavonoids	Positive
Saponins	Positive
Steroids	Positive

brane, proteins and DNA (Ferguson, 2001). Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms (Hodek et al., 2002). All these facts support the usefulness of *A. africana* in folklore remedies and one of the reasons why this plant is widely used for the treatment of many diseases among many tribes in Africa. In addition to the antimicrobial activities exhibited by flavonoids, it also exhibit antitrypanosomal and antileishmanial activities (Tasdemir et al., 2006). Epidermiological studies suggest that the consumption of flavonoids is effective in lowering the risk of coronary heart diseases (Rice-Evans and Miller, 1996). All these facts suggest that A. *africana* can as well be used to treat coronary heart disease. Furthermore, several flavonoids exhibit antiviral activities (Xu et al., 2000). Lastly, saponins which are responsible for numerous pharmacological properties (Estrada et al., 2000) were also tested positive in *A. africana* stem bark extract. Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects (Liu and Henkel, 2002). Saponins are known to produce inhibitory effect on inflammation (Just et al., 1998). These observations cited on phytochemical compounds support our findings on the usefulness of *A. africana* in traditional medicament.

Conclusion

A. africana is used among many tribes in Africa especially in West Africa to prepare decoction for the treatment of different ailments ranging from diarrhoea, gonorrhoea, gastro-intestinal tract infections among others. Although the crude extract of the stem bark of the plant does not appear to be antifungal, this study has confirmed the antibacterial potentials of the plant, thus supporting its folklore application as a medical remedy for some ailments.

ACKNOWLEDGEMENTS

We wish to thank Govan Mbeki Research and Developmental Centre (GMRDC) of the University of Fort Hare,

Alice, South Africa for the postdoctoral fellowship granted the first author. We are also grateful to the National Research Foundation (NRF) for the grant that supported this study. Dr. HC Illoh of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, is appreciated for identifying the plant used in this study.

REFERENCES

Agbelusi GA, Odukoya OA, Otegbeye AF (2007). *In vitro* screening of chewing stick Extracts and sap on oral pathogens: Immune compromised infection. Biotechnology 6(1):97-100.

Akah PA, Okpi O, Okoli CO (2007). Evaluation of the anti-inflammatory, analgesic and antimicrobial activities of *Afzelia africana*. Niger. J. Nat. Prod. Med. 11: 48-52.

Akinpelu DA, Kolawole DO (2004). Phytochemical and antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum.). Sci. Focus J. 7: 64-70.

Atawodi SA (2005). Comparative *in vitro* trypanocidal activities of petroleum ether, Chloroform, methanol and aqueous extracts of some Nigeria savannah plants. Afr. J. Biotechnol. 4(2): 177-182.

Dalziel JM (1937). The useful plants of West Tropical Africa. Crown Agents for the colonies, London, p. 612.

Dharmananda S (2003). Gallnuts and the uses of Tannins in Chinese Medicine. In: Proceedings of institute for Traditional Medicine, Portland, Oregon.

Estrada A, Katselis GS, Laarveid B, Barl B (2000). Isolation and evaluation of Immunological adjuvant activities of saponins from *Polygaja senega* L. Comparative Immunology. Microbiol. Infect. Dis. 23: 27-43.

Ferguson LR (2001). Role of plant polyphenols in genomic stability. Mutat. Res. 475: 89-111.

Ganguly T, Sainis KB (2001). Inhibition of cellular immune response by *Tylophora indica* In experimental models. Phytomed. 8(5): 348-355.

Gopalakrishnan C, Shankaranarayan D, Kameswaran L, Natarajan S, (1979). Pharmacological investigations of tylophorine, the major alkaloid of *Tylophora indica*. Indian J. Med. Res. 69: 513-520.

Harborne JB (1998). Phytochemical Methods - A Guide to Modern Techniques of plant analysis. Chapman and Hall, London.

Hodek P, Trefil P, Stiborova M (2002). Flavonoids-Potent and versatile biologically active compounds interacting with cytochrome P450. Chemico-Biol. Int. 139(1): 1-21.

Irobi ON, Moo-Young M, Anderson WA, Daramola SO (1994). Antimicrobial activity of the bark of *Bridelia ferruginea* (Euphorbiaceae). Int. J. Pharmacog. 34: 87-90.

Just MJ, Recio MC, Giner RM, Cueller MJ, Manez S, Bilia AR, Rios JL (1998). Anti-Inflammatory activity of unusual lupine saponins from *Bupleurum fruticescens*. Plant Med. 64: 404-407.

Keay RWJ (1989). Trees of Nigerian. Oxford University Press.

Li H, Wang Z, Liu Y (2003). Review in the studies on tannins activity of cancer prevention and anticancer. Zhong-Yao-Cai. 26(6): 444-448.

Liu J, Henkel T (2002). Traditional Chineese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities? Curr. Med. Chem. 9: 1483-1485.

Motar MLR, Thomas G, BarbosaFillo JM (1985). Effects of *Anacardium occidentale* stem bark extract on *in vivo* inflammatory models. J. Ethnopharmacol. 95(2-3): 139-142.

Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA (1994). Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature 368(6473): 753-756.

Olorundare EE, Emudianughe TS, Khaar GS, Kuteyi SA, Irobi DN (1992). Antibacterial properties of leaf extract of *Cassia alata*. Biol. Res. Com. 4: 113-117.

Pelczar MJ, Chan EC, Kruz NR (2006). Microbiology. 5th edition. Teta McGraw-Hill Publishing Company Ltd., New Delhi.

Raffauf RF (1996). Plant Alkaloids: A Guide to Their Discovery and Distribution. Hawkworth Press, inc., New York.

Rice-Evans CA, Miller NJ (1996). Antioxidant activities of flavonoids as bioactive Components of food. Biochem. Soc. Trans. 24: 790-795.

Russel AD, Furr JR (1977). The antibacterial activity of a new chloroxylenol preparation containing ethylenediamine tetraacetic acid. J. Appl. Bacteriol. 43: 253.

Scalbert A (1991). Antimicrobial properties of tannins. Phytochem. 30: 3875-3883.

Sibanda T, Okoh AI (2008). *In vitro* antibacterial regimes of crude aqueous and acetone extracts of *Garcinia kola seeds*. J. Biol. Sci. 8(1): 149-154.

Staerk D, Lykkeberg AK, Christensen J, Budnik BA, Abe F, Jaroszewki JW (2002). *In vitro* Cytotoxic activity of phenanthroindolizidine alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanake* against drug-sensitive and multidrug-resistant cancer cells. J. Nat. Prod. 65(9): 1299-1302.

Tasdemir D, Kaiser M, Brun R, Yardley V, Schmidt TJ, Tosun F, Reudi P (2006). Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *In vitro*, *in vivo*, structure-activity relationship, and quantitative structure-activity relationaship studies. Antimicrob. Agents Chemother. 50(4): 1352-1364.

Trease GE, Evans WC (1983). Textbook of Pharmacognosy, 12th edition (Balliere, Tindall, London), pp. 57-59; pp. 343-383.

Xu HX, Wan M, Dong H, But PPH, Foo LY (2000). Inhibitory activity of Flavonoids and tannins against HIV-1 protease. Biol. Pharm. Bull. 23: 1072-1076.