International Journal of Research and Review
Vol.8; Issue: 1; January 2021
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Phytochemical Screening and Anti-Bacterial Activity of Leaves and Stem Bark Extract of *Feretia apodanthera* against Selective Pathogenic Bacteria

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ABSTRACT

Feretia apodanthera (F. apodanthera), is a member of the Rubiaceae family of plants is widely known in literature and renown for the treatment of various preoccupying pathologies in Sub-Saharan African traditional pharmacopoeia. It is usually harvested for local use as food, medicine and cosmetic. This study is aimed to determine the phytochemical content and antimicrobial activities of leaves and stem bark of *Feretia apodanthera* on some *Enterobacteriaceae* which include Escherichia coli, and staphylococcus using disc diffusion method. The result revealed that the leaf and stem bark extract of *Feretia apodanthera* inhibited the growth of the microorganisms to varying proportions with zones of inhibition of leaf ranging from 9 to 32 mm and stem bark ranging from 8.1-20. Furthermore, the phytochemical screening of the leaf extract indicated the presence of saponins, tannins, alkaloids and flavonoids but steroids is absent while stem extracts shows the present of tannins, saponins and flavonoid but steroids and alkaloid are absent. The presence of Saponins, flavonoids and tannins in the extracts could be responsible for the observed antimicrobial activity. The study therefore, confirms the use of *Feretia apodanthera* leaves and stem bark in the treatment of various diseases and infections caused by the test organisms used.

Keywords: Feretia apodanthera, Anti-microbial, Treatment, Infections.

INTRODUCTION

Feretia apodanthera (F. apodanthera); a member of the *Rubiaceae* family of plants is widely known for the treatment of various preoccupying pathologies in Sub-Saharan African traditional pharmacopoeia. It is usually harvested for local use as medicine, cosmetic as well as food (Burkil, *et al.*, 2004). In certain areas the fleshy pulp of the ripe fruit is eaten raw as snack to quench hunger and thirst while the dried leaves are eaten as vegetable (Taiwe, *et al.*,2015). The twigs of *Feretia apodanthera* are used for beehive construction in rural areas of Burkina Faso to foster traditional beekeeping (Paul, *et al.*,2013). It is also the most persistently consumed browse species by cattle in Burkina Faso (Husain, *et al.*, 2008). Medicinally, it is popularly used in various West African Countries carrying various vernacular names to treat various infections and health conditions.

Recently, *Feretia apodanthera* extracts have also been shown to have high antioxidant activity similar to quercetin *and* relatively high flavonoid content (Hansen *et al.*, 2008). The presence of these metabolites may be responsible for the therapeutic effect exhibited by this plant. Investigations with extract of *Feretia apodanthera* in rats also showed a decreased the activity of nuclear factor kappa β and nitric oxide which have been implicated in inflammation (Ene and Atawodi, 2012; Menenga *et al*., 2016).

Antimicrobial drug resistance remains a scourge across multiple sectors including and especially human health. The

lack of vaccines against some pathogenic microorganisms, the overuse and misuse of antibiotics and other antimicrobials in humans, plants and animals and together with the spread of residues of the antimicrobials on land and in water makes the problem more challenging and constantly requiring novel approaches. The *Rubiaceae* family very noticeable for its antiplasmodial activities, most of the plants in this family remain unexplored despite the wealth of information from tradi-practitioners (Karou, *et al.*, 2011).

Feretia apodanthera Del. (*Rubiaceae)* is extensively used in ethnomedicine in Nigeria, Niger and Cameroon for various ailments. The stem bark of *Feretia apodanthera* (Rubiaceae) is being used empirically in traditional medicine in Cameroon to treat epilepsy and diseases related to the brain like agitation, anxiety, infertile convulsions, headaches, pains, insomnia, and schizophrenia according to our traditional healers and the literature (Njimoh *et al.*, 2015). In Senegal, the leaves of *F. apodanthera* are used to treat different urinary and renal infections. The plant is also used to treat stomach aches, nausea, and syphilis, as a calming agent for agitated mental conditions, and for enhancing cognitive performance (Armah *et al*., 2015).

Taiwe *et.al.,* in 2016 found that ; the screened the stem-bark extracts against one gram positive *(Staphylococcus aureus*) and four gram negative pathogenic bacteria strains (*Escherichia coli, Proteus vulgaris, Providencia stuartii* and *Pseudomonas aeruginosa*). The aqueous extract, alkaloid fraction and the Ethanol extract were active on all the five bacteria strains tested. The hexane and methylene chloride extracts were not active on any of the strains tested. The alkaloid fraction of *F. apodanthera* had the highest diameter of zone of inhibition (17.8mm) against Staphylococcus aureus. Another study examined the effects of a lyophilized aqueous extract of *F. apodanthera* on the course of kindling development, kindling-induced learning deficit, oxidative stress markers, and cholinesterase activity in pentylenetetrazole (PTZ)-kindled mice. The result showed that pretreatment with the aqueous extract of *F. apodanthera* antagonizes seizures, oxidative stress, and cognitive impairment in PTZ-kindled mice. The aqueous extract of *F. apodanthera* also showed anxiolytic activities, but the inhibition of memory impairment was not attributed to the anxiolytic activities of the plant. These results thus suggest the potential of *F. apodanthera* as an adjuvant in epilepsy both to prevent seizures as well as to protect against seizure-induced oxidative stress and memory impairment.

James and Owolabi (2017), evaluated the in-vitro antioxidant capacity and anti-inflammatory effect of different extracts of *Feretia apodanthera* against right hind paw oedema of albino rats. The phytochemical constituents and antioxidant activity was assayed using DPPH. Anti-inflammatory studies were carried out with ethanol and hexane extracts using carrageenan induced paw oedema in albino rats. The phytochemical screening of extracts revealed the presence of unsaturated steroids, triterpenes, cardiac glycosides, tannins, saponin and alkaloids. Vitamin C had a median inhibitory concentration (IC50) of 0.0383 mg/ml which was lower than IC50 of all the extracts. Of all the extracts, ethanol extract had the lowest IC50 (0.0443 mg/ml) which is comparable to vitamin C. Anti-inflammatory studies showed that all the extracts had a significant ($p<0.05$) inflammation inhibition potential at 400 mg/kg body weight at all hours except at the fifth hour, where the n-hexane extract was significantly ($p<0.05$) lower than all the extracts.

Antimicrobial resistance poses a serious threat to human development, health and security. This coupled with the increasing rate of antimicrobial resistance among health-care associated and community-acquired infections over the past decade has recently prompted world leaders

in United Nations submit to commit to devise action plans on antimicrobial drug resistance (Dieudonne *et al.*, 2018) based on the *Global Action Plan on Antimicrobial resistance* developed by WHO in 2015 in coordination with the FAO and the OIE (WHO, 2015).

Also the rise in bacterial and fungal infections which constitute a major health problem today is due to the fact that there are no vaccines for some infections and the emergence and widespread occurrence of multidrug resistant microbial phenotypes to past and present drug regimens. Plant-based systems have been widely exploited in traditional medicine to treat microbial infections for thousands of years. They are equally the principal sources of most conventional antimicrobials. Natural plant extracts and pure compounds isolated from plants, as well as synthetic compounds obtained by a further bioassay guided fractionation and isolation have been a good source of lead compounds for use as antimicrobials and for drug development.

Similarly, lack of vaccines against some pathogenic microorganisms is causing the overuse and/or misuse of antibiotics and other antimicrobials. Thus, Studying the phyto-constituents in order to observed its potential phytochemical activities is of the plant preparation are necessary for standardization, which helps in understanding the significance of phyto-constituents in terms of their observed activities. The study is confined to the phytochemical screening and antimicrobial activities of *feretia apodanthera* (leaves and stem bark) in order to attach a scientific connotation to its usage in African folk medicine as well as for further exploitation to identify lead compounds for drug development. This study will also screen the leaf and stem bark extract of *Feretia apodanthera* for the presence of active metabolites such as flavonoid, alkaloid, steroid, tannins and saponins as well as access the antimicrobial sensitivity pattern of *E. coli*, S. aureus to the extract.

MATERIAL AND METHOD

Study Area

The study was carried out in Gombe metropolis, Gombe, Gombe state. Gombe is located Northeastern part of Nigeria. The state has an area of 20, 265 km^2 and a population of around 2,365,000 people as of 2006. It has two distinct climates, the dry season (November- March) and the raining season (April-October) with an average rainfall of 850nm.The vegetation and land cover of the state revealed that 33.06% and 34.81% of the land area of Gombe state comprise of the River Basin and plains respectively while 26.65% and 5.48% are of upland and highland areas respectively.

Collection of plant materials:

The fresh leaves and stem bark of *feretia apodanthera* was collected from Gombe metropolis. The identification of the plant taxonomic done in the herbarium of the Department of Biological Sciences, Gombe State University using vouchers and identification key through the help of a plant taxonomist.

Plant extraction:

The plant material of *feretia apodanthera* (leaves and stem bark) was shade dried and milled into fine powder using a mechanical grinding. The grounded plant materials (50g) was macerated and shaked using methanol (500ml) for 48hrs.The extract was filtered through filter paper (Whatman no,1) and dried under vacuum and reduced the pressure using a rotary evaporator at $40^{O}C$. The concentration was then placed in aluminium foli before freeze drying. The residual extract was dissolve in sterile water (1ml).

Phytochemical analysis:

The ethanolic extract was used for screening of active constituents such as flavonoid, tannins, saponins, alkaloid and steroid using standard procedure.

Test for Tannins:

About 0.5 g of the plant extract was mixed thoroughly with 10 ml distilled water and then filtered; 5 ml of the filtrate was added to 1 ml of 5% Ferric chloride solution. The appearance of blue black, greenish or blue green precipitate indicates the presence of tannins (Riss *et al.*, 2016).

Test for Flavonoid:

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red colour indicates the presence of flavonoid (Sofowora, 2006).

Test for Saponins:

About 0.1g of powdered plant material boiled with 10 ml of water for 5 minutes then filtered. After cooling, 5 ml of filtrate was then diluted with water and shaken vigorously. The formation of persistent foam indicated presence of saponin (Sofowora, 1993).

Test for Steroids:

About 1 ml solution of the plant extract was added to 1 ml sulphuric acid. the appearance of red colour indicates the presence of steroid (Sofowora, 2006).

Test for Alkaloids:

About 0.5 g of the extract was stirred with 5 ml of 1% hydrochloric acid on a steam bath and filtered. 1 ml of the filtrate was then treated with few drops of Mayer's reagent. A white or creamy white precipitate considered as an indication for the presence of alkaloids (Tumbarello et al., 2004).

Preparation of Sensitivity Disc

Sensitivity discs of about 6 mm in diameter were punched from Whatman's no. 1 filter paper using a file punch, then put onto Bijou bottle. The sensitivity discs were sterilized in an autoclave at 121^0C for 15 minutes, and then allowed to cool. Various concentration of sensitivity discs were prepared by measuring 0.25mL, 0.50mL, and 0.75mL, of ethanolic leaf extract in different test tubes, and diluted with Dimethyl-sulphoxide (DMSO) to form three concentration, 25% (v/v), 50% (v/v), and 75% (v/v) respectively, while 1ml of the undiluted extract served as 100% (v/v) concentration. This followed by placing the improvised paper disc in each concentration. The discs were then allowed to absorb the solution and kept in refrigerator at 4^0C before use.

Test Organisms

Clinical bacteria isolates of *Escherichia coli* and *Staphylococcus* aureus were collected from Gombe State Specialist Hospital and maintained in agar slants in refrigerator (4 °C) prior to use. Appropriate confirmatory biochemical tests were carried out on each of the isolates after sub-culturing.

Inoculums' Standardization

A loop full of each of the test isolates was picked using sterile wire loop and emulsified onto 3.4 ml of sterile physiological saline. The turbidity of the suspension was then matched with that of 0.5 McFarlands standard (Azwanida, 2015).

Sensitivity Testing

Using sterile swab stick, standardized inocular of each isolate was swabbed onto the surface of Mueller Hinton agar in separate Petri dishes. Disc of the extracts was placed onto the surface of the inoculated media, then the plates was inverted and allowed to stand for 30 minutes for extract to diffuse into the agar, after the plates was incubated aerobically at 35^oC for 18hours. Zone of inhibition formed around each of the extract and standard antibiotic discs 'was measured using meter rule. (NCCLS, 1999).

RESULTS

Yield of extracts

The yield obtained were 3.4% for the stem extract and 7.5% for the leaf extract.

Phytochemical screening result leaf and stem extracts of *Feretia apodanthera*

The phytochemical screening of leaves extract of *Feretia apodanthera* show the present of saponins, tannins, alkaloids and flavonoids but steroids is absent while stem extracts shows the present of tannins, saponins and flavonoid but steroids and alkaloid are absent.

Table 1: Phytochemical screening of leaves and stem bark of *Feretia apodanthera*

S/N	Phytochemical constituents	leaves	stem bark
1	Saponins	+	+
2	Tannins	+	+
3	Alkaloids	+	-
4	Steroids	-	-
5	Flavonoids	+	+

Key: +=Present, - =Absent

Table 2: Antibacterial activity of leaves (*Feretia apodanthera) against S.aureus and E.coli*

TEST ORGANISM	100	75	50	25	C
ZONES OF INHIBITION (MM)					
Ecoli	13	12	10	9	32
S.aureus	16.5	13	10	9	18

Key C= Control. The above shows the result of antibacterial testing indicating zone inhibition measured in millimeter the positive control used was Ciprofloxacin and Augmentin an Antibiotics.

Table 3: Antibacterial activity of stem bark (*Faretia apodanthera) against S.aureus and E.coli*

TEST ORGANISM	100	75	50	25	C
ZONES OF INHIBITION (MM)					
Ecoli	14.3	12.2	10.2	8.1	18
S.aureus	13	12	9	8.2	20

Key C= Control. The above shows the result of antibacterial testing indicating zone inhibition measured in millimeter the positive control used was Ciprofloxacin and Augmentin an Antibiotics.

DISCUSSION

Phytochemical screening of the leaf extract of *Feretia apodanthera* reveal that saponins, tannins, Alkaloid and flavonoid are present but the steroid is absent. While the ethanolic extract of stem bark reveal that saponins, tannins and flavonoid are present while Alkaloid and steroid are absent. This finding is in accordance with that of Ancolio *et al.*, (2012) who states the presence of tannins, Alkaloid, flavonoid and saponins. In terms of sensitivity it opposes the result of Bukar *et al.*, (2009) who found that the leaf and stem bark of *Feretia apodanthera* was sensitive to *staphylococcus aureus and Escherichia.* The antimicrobial activity observed may be attributed to the presence of active metabolite present in *Feretia apodanthera* extracts (leaf and stem bark) (Santana et al., 2015).

CONCLUSION

The leaf and stem extracts of *Feretia apodanthera* showed the present of various phytoconstituents such as saponins, tannins, alkaloid, and flavonoids. The stem-barks of *Feretia apodanthera* possess antimicrobial activities especially against human pathogenic bacteria and are worthy of further exploration to identify novel compounds for antimicrobial drug development.

REFERENCE

1. Ancolio C., Azas N., Mahiou V., Ollivier E, Di Giorgio C., Keita A., Timon-David P,Balansard G.(.2012) Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali and Sao Tome. Phytother Res16:646–9.
2. Armah F.A., Francis A., Kofi Annanb, Abraham Y. (2015) Erythroivorensin: A novel anti-inflammatory diterpene from the root-bark of Erythrophleum ivorense (A Chev.), Fitoterapia. 105 37–42.
3. Azwanida N.N., (2015) A Review on the extraction methods use in medicinal plants, principle, strength and limitation, Med. Aromat. Plants. 4 196.
4. Burkil H.M. (2004) The useful plants of West Tropical Africa, Royal Botanic Gardens, Kew. 1985-
5. Ene A.C., Atawodi S.E. (2012) Ethnomedicinal survey of plants used by the Kanuris of North-eastern Nigeria. Indian J Tradit Knowl.;11(4):640–5.
6. Hansen H.H., L. Sanou, B.M.I. Nacoulma, (2008). Tree leaves in the diet of free-ranging ruminants in three areas of Burkina Faso, Livest. Res. Rural Dev. 20(3)
7. Hussain A. saraiva, L.R., Ferrero, D.M., Ahuja, G., Krishna V.S., Liberles, S.D.,Korsching S.I. (2013) Biochemical characterization and identification of bacterial strains isolated from drinking water sources of Kohat, Pakistan, Afr. J. Microbiol. Res. 7(16) 1579-1590.

8. Karou S.D. Saito, Takayuki, Satoru (2011) Sub-Saharan Rubiaceae: A review of their traditional uses, phytochemistry and biological activities, Pak. J. Biol. Sci. 14(3) 149-169.
9. NCCLS (1990). Antimicrobial Susceptibility Testing/SC3, National Committee for Clinical Laboratory Standards, Villanova, Pa, USA, 3rd edition,
10. Njimoh D.L., Jules Clement N. Assob, Seraphine Ebanye Mokake. (2015) Antimicrobial activities of a plethora of medicinal plant extracts and de coopération internationale en recherche agronomique pour le développement/Muséeum
11. Paul S., Issa, N., Kwame, A., Joseph, B. (2013) Plants used in traditional beekeeping in Burkina Faso, Open Journal of Ecology.
12. Riss T.L., Moravec R.A.,Niles A.L., (2016) Cell Viability Assays, Assay Guidance Manual.three areas of Burkina Faso, Livest. Res. Rural Dev. 20(3) thropleum ivorense stem bark in experimental animals, European J. Biomed. Pharm. Sci.traditional medicine in Mali, Phytotherapy Research. 16(7) 646-649.
13. Santana D.B., Raphaell C.C., Laila S.E., (2015) Activity of Fabaceae species extracts against fungi and Leishmania:
14. Sofowora A (2006). Medicinal plants and traditional medicine in Africa.Spectrum Books Limited, Ibadan.
15. Sofowora, E. A. (1993): Medicinal Plants and Traditional Medicine in Africa. Textbook Chapter 1 and 2.
16. Taiwe G.S. Dabole B., Tchoya T.B., Menanga J.R., Dzeufiet P.D.D., De (2015) Effects of a lyophilized aqueous extract of Feretia apodanthera Del. (Rubiaceae) on pentylenetetrazole-induced kindling, oxidative stress, and cognitive impairment in mice, Epilepsy & Behavior. 43 100-108.
17. Taiwe G.S., Dabole B., Tchoya T.B., Menanga J.R., Dzeufiet P.D.D., De (2016) Waard M.Anticonvulsant effects of iridoid glycosides fraction purified from Feretia apodanthera Del.(Rubiaceae) in experimental mice models of generalized tonic-clonic seizures. BMC Complement Altern Me.;16(1):285.
18. Taiwe G.S., Dabole B., Tchoya T.B., Menanga J.R., Dzeufiet P.D.D., De (2016) Extracts of Feretia apodanthera Del. demonstrated anticonvulsant activities against seizures induced by chemicals and maximal electroshock, Epilepsy Research.,127 30-39.
19. Menanga J.R., Dzeufiet P.D.D., De (2016) Anticonvulsant effects of iridoid glycosides fraction purified from Feretia apodanthera Del. (Rubiaceae) in experimental mice models of generalized tonic-clonicseizures, BMC Complement Altern. Med. 16 285.
20. Tumbarello M., Sanguinetti M, Montuori E, Trecarichi E.M., Posteraro B, (2004), ESBL-producing multidrug-resistant Providencia stuartii infections in amacrostachyum Benth (Marantaceae), medicinal plants from Gabon, Int. J. Biosci. 8(6) Management Unit, Nairobi, Microbiol.
21. WHO, Global Action plan on Antimicrobial Resistance, 2015. (Online). Available: http://www.who.int/antimicrobial-resistance/global-action-plan/en/.
22. WHO, Global Strategy for Containment of Antimicrobial Resistance (Online). Available: http://www.who.int/csr/resources/.

How to cite this article: Musa MM, Alhassan A. Phytochemical screening and anti-bacterial activity of leaves and stem bark extract of *feretia apodanthera* against selective pathogenic bacteria. *International Journal of Research and Review*. 2021; 8(1): 448-453.

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