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Antimicrobial Activity of *Mitracarpus scaber* Leaf Extract against Some Human Pathogenic Microorganisms

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ABSTRACT: The study was carried out with the aim to determine the antimicrobial effect of ethanolic leaf extract of *Mitracarpus scaber* against some clinical isolates. The leaves were screened for some phytocompounds using standard analytical methods.The antimicrobial activity of the leaf extract against *Escherichia coli, Staphylococcus aureus* and *Candida albicans* was carried out using agar well diffusion and broth dilution methods. The results of phytochemical studies revealed the presence of alkaloids, flavonoids, cardiac glycosides, triterpenes, phytosterols, tannins, and saponins. The results of the susceptibility test showed significant ($p \leq 0.05$) inhibitory effect of the leaf extract against all the test isolates with mean zones of inhibition range of 10.75 ± 0.35 -16.25 ± 0.35 mg/ml,12.25 ± 0.35 - 17.75 ± 0.35 mg/ml and 13 ± 0.00 -20.75 ± 1.06 mg/ml for *C. albicans*, *E. coli* and *S. aureus* respectively. The effect was significantly higher against *S. aureus* having Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 6.25mg/ml and 12.5mg/ml followed by *E. coli* (12.5mg/ml and 25mg/ml). The least activity was exhibited against *C. albicans* with MIC and Minimum Fungicidal Concentration (MFC) of 25mg/ml and 50mg/ml. The results showed that the ethanolic leaf extract of *M. scaber* can be used to treat diseases caused by *E. coli*, *S. aureus* and *C. albicans.*

Keywords: Antimicrobial activity, *Mitracarpus scaber, Escherichia coli, Staphylococcus aureus, Candida albicans*

Introduction

Plants are generally endowed with bioactive compounds, some of which include terpenoids, phenolic, lignins, tannins, flavonoids, coumarins, alkaloids (Gracelin *et al*., 2013). These diverse array of natural phytochemicals have complementary and overlapping actions, including antioxidant effects, modulation of detoxification enzymes, stimulation of immune system, reduction of inflammation, modulation of steroid metabolism and antibacterial and antiviral effects (Zahin *et al*., 2010). Traditional Medicine Practitioners (TMPs) through ancient indigenous technology and by a series of "trial and error" over time have successively applied various plant parts to treat diverse sicknesses especially in countries where low-income indigenous populations do not have access to modern medical care (Egharevba *et al*., 2008; Ouadja *et al*., 2018).

Methicillin- Resistant *Staphylococcus aureus* (MRSA) and Carbapenem -Resistant Enterobacteriaceae are able to cause serious diseases that are of public health concern. Fungal infections are emerging opportunistic infections that often occur in immune-compromised people and especially those living with HIV / AIDS. In these people, attacks commonly called mycosis are both superficial (skin) and invasive (systemic). *Candida albicans* is among the most frequently isolated species. Candidiasis attacks are recurrent and therapeutic management is complicated by the emergence of multi-drug resistant (MDR) fungi (Zahin *et al*., 2010).

Most medicinal plants have antimicrobial properties and their use is greatest in tropics where the diversity and growth rates of microorganisms are highest (Zahin *et al*., 2010). Nigeria is richly blessed with many varieties of these medicinal plants whose leaves, roots, fruits, seeds and barks are useful in traditional medicine. Among the numerous plants is *Mitracarpus scaber* (Oghenejobo *et al.,* 2013). *M. scaber* belongs to the family Rubiaceae. The family consists of about 500 genera and 6,000 species distributed all over the world. Some of them are tropical trees and shrubs (erect, struggling or twining) while few members are herbs (erect or decumbent). It is found in Africa, Asia and in Latin America and grows on degraded soils and where there is much water during the rainy season (Oghenejobo *et al*., 2013; Moussa *et al*., 2015).In Nigeria, the plant is locally called "Ogwu Ugwo" (Craw-craw medicine) in the Ibo tribe, "Goga masu" ("Smear spear") in Hausa and "Irawo lle" in Yoruba (Gbile, 2004; Dalziel, 2004).

M. scaber is widely used in the treatment of skin diseases, particularly infectious dermatitis, eczema, ringworm, craw-craw, itching, and scabies caused by bacteria and fungi such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *C. albicans*, *Epidermophyton flocossum*, *Candida neoformans* (Chandra and Baravalia, 2010). In Nigeria, the juice from the crushed plant is applied topically for the treatment of skin diseases or applied to dressings for fresh cuts, wounds and ulcers, an aqueous decoction is also taking for the treatment of diarrhea. And thus, it is claimed that the plant has both antibacterial and antifungal activities (Chandra and Baravalia, 2010).

The high increase in the prevalence of microbial infections worldwide and the increasing rate of resistance of most pathogenic microbes to existing antimicrobials, justifies the need for this study. However, the aim of the study was to determine the antimicrobial activity of *M. scaber* leaf extract against some human pathogenic microorganisms.

Figure1: *M. scaber* in its natural environment (Oghenejobo *et al*., 2013).

Materials and Methods

Collection and preparation of plant material: The fresh leaves of *M. scaber* was collected from a farm at Bachama road, Kaduna. A sample of the plant material was transported in a sterile polythene bag to the section of the Department of Biological Sciences, Kaduna State University (KASU) for authentication. Voucher number (1/6) was assigned. The leaves were washed, cleaned with tap water and air dried under shade for ten (10) days. After which it was ground into a coarse powder using a mortar and pestle. The coarse leaf sample was placed in a sterile container, labelled and kept until required (Oghenejobo *et al.,* 2013).

Plant Extraction: The maceration method described by Azwanida(2015) was employed for the plant extraction. Using 70% ethanol, about 250g of the powdered leaf extract was suspended in 500mls of ethanol in a beaker and tightly sealed. The mixture was allowed to stand for 72 hours at room temperature with frequent agitation before filtration. At the end of the stirring, the mixture was decanted and filtered with Whatman filter No. 1 (Ouadja *et al*.,

2018). The filtrate was concentrated to dryness by evaporation at 40 ^{o}C using water bath. The crude extract obtained was placed in a screw capped tube, labeled and stored at 4^{o}C in the refrigerator until required. The percentage yield of ethanol leaf extract of *M. scaber* was calculated according to the formulae:

$$\text{Yield (\%)} = \frac{W_2 - W_1}{W_0} \times 100$$

W_1= Weight of container (g)
W_2 = Weight of extract and container
W_0= Weight of dried sample used (Ouadja *et al*., 2018).

Phytochemical screening: Preliminary phytochemical screening was carried out on the crude extracts using the standard methods described by Tiwari *et al.* (2011) to determine the following bioactive ingredients: carbohydrates, glycosides, cardiac glycosides, saponins, alkaloids, tannins, triterpens, steroids and flavonoids.
Detection of carbohydrates: The extract was dissolved separately in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.
Molisch's test: Filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation or non-formation of a violet ring at the junction indicates the presence or absence of carbohydrates.
Benedict's test: Filtrate was treated with Benedict's reagent and heated gently. Formation or non-formation of orange red precipitate indicates the presence or absence of reducing sugars.
Detection of alkaloids (Mayers test): The extract was dissolved in dilute hydrochloric acid and filtered. Filtrate was then treated with Mayer's reagent (Potassium Mercuric Iodide). Formation or non-formation of a yellow coloured precipitate indicates the presence or absence of alkaloids.
Detection of glycosides: The extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.
Modified Borntrager's test: The extract was treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was allowed to cooled and extracted with equal volumes of benzene. The benzene layer that separated was treated with ammonia solution. Formation or non-formation of rose-pink colour in the ammonical layer indicates the presence or absence of anthranol glycosides.
Ferric chloride test: About 3 drops of ferric chloride was added to the portion. Formation or non-formation of a brown to black precipitate indicate the presence or absence of phenolic aglygone (Tiwari *et al.*, 2011).
Legal's test: The extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation or non-formation of pink to blood red colour indicates the presence or absence of cardiac glycosides.
Detection of saponins (Foam test): About 0.5 g of extract was shake into 2 ml of water. Foam produced and persists for ten minutes it indicates the presence or absence of saponins.
Detection of tannins (Gelatin test): To the extract, 1% gelatin solution containing sodium chloride was added. Formation or non-formation of white precipitate indicates the presence or absence of tannins.
Detection of flavonoids (Alkaline Reagent test): The extract was treated with few drops of sodium hydroxide solution. Formation or non-formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence or absence of flavonoids.
Detection of phytosterols: Salkowski's Test: The extract was treated with chloroform and filtered. The filtrate was then treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Formation or non-formation of golden yellow colour indicates the presence or absence of triterpenes.
Libermann Burchard's test: The extract was treated with chloroform and filtered. The filtrate was then treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation or non-formation of brown ring at the junction indicates the presence or absence of phytosterols.
Test for free anthraquinones (Borntrager's test): A small quantity of the extract was shake with 10ml of Benzene and filtered. 5ml of 10% Ammonia solution was added to the filtrate and stirred. Formation or non-formation of pink or violet colour indicate the presence or absence of free anthraquinones.
Collection of test isolates: The clinical isolates of *Escheichia coli, S. aureus and C. albicans* were obtained from 461 Nigeria Air Force Hospital Laboratory (NAFH) Kaduna, Kaduna State. Samples were collected in agar slants and transported to the laboratory section of Microbiology Department, Kaduna State University (KASU) in ice box. The agar slants were kept in the refrigerator at 4^{o}C until required.
Reconfirmation of test isolates: The isolates were subjected to colonial, microscopic and biochemical characterization using standard microbiological methods (Cheesbrough, 2006).The test isolates were grown on selective media to study their colonial morphology. *Escheichia coli* isolate was inoculated in Eosin Methylene blue (EMB) to obtain colonies of *E. coli* isolate. Mannitol Salt Agar (MSA) was used to inoculate the colonies *S. aureus*

and Brilliance Candida Agar (BCA) was used to inoculate the colonies to obtain *C. albicans* isolate. Nutrient agar and Sabouraud Dextrose Agar (SDA) were used to obtain the pure cultures of bacteria and fungi respectively. IMVIC (Indole, Methyl red, Vouges prescaure and Citrate utilization) tests were used for biochemical confirmation of *E. coli* isolate. *S. aureus* was confirmed by coagulase test, while *C. albicans* isolate was confirmed by cultivating the fungus in YEDP (1% yeast extract, 2% peptone and 2% dextrose) to form germ tubes (Cheesbrough, 2006). Muller Hinton agar and Sabouraud Dextrose agar were used for antibacterial and antifungal susceptibility testing respectively while Mueller Hinton broth and Sabouraud Dextrose broth were used to determine the Minimum Inhibitory Concentration and Minimum Fungicidal Concentration for bacteria and fungi respectively.

Standardization of inoculum: Sets of 18-24 hours culture of isolates were used to prepare the inocula. The organisms were suspended in sterile normal saline and the turbidity was adjusted to 0.5 Mc Farland's standard. Bacterial suspensionswere prepared in sterile distilled water from pure culture of 18 hours from nutrient agar. This suspension was compared to the standard of the McFarland 0.5 solution which corresponds to1.0×10^8 CFU/ml (Clinical Laboratory Standards Institute, 2002). Twenty four hours solid culture of the fungal isolates (*C. albicans*) from Sabourad Dextrose Agar plate was also standardized by gradually inoculating in normal saline to compare its turbidity to McFarland standard of 1.0 which is approximately 1.0×10^8 CFU/ml (CLSI, 2002).

Preparation of different concentrations of leaf extract: A stock solution was prepared by dissolving 0.4 g of the extracts in 4ml of distilled water. Serial dilution by half was then carried out in four test tubes containing 2ml of distilled (sterile) water to obtain a concentration of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml respectively. Exactly 2 ml of the stock solution was transferred into 2ml of distilled water into the next test tubes until the final concentration is reached (Cheesebrough, 2006). Ciprofloxacin (10 ug) was used as control for bacteria and ketoconazole (2.5ug) was used as control for the fungus (Cheesebrough, 2006).

Susceptibility test of M. Scaber leaf extract: The agar well diffusion method described by Balouiri *et al*. (2016) was used to determine the susceptibilities of isolates to extract of *M. Scaber*. It was evaluated using Mueller-Hinton Agar for the bacteria and Sabouraud Dextrose Agar for the fungi (Oghenejobo *et al.,* 2013).

Muller-Hinton agar and Sabouraud Dextrose Agar containing chlorampenicol were poured into sterile Petri-dishes, and allowed to solidify for 30 minutes. The standardized inoculum of each test organism was inoculated onto the sterile agar plates and spread using a sterile cotton swab so as to achieve a confluent growth.

Adopting the agar well diffusion method, a sterile cork borer (8mm) was used to bore holes on each agar plate. The bottom of each well was then sealed with the appropriate molten agar. On each agar plate, about 0.3mls of *M. scaber* leaf extract of varying concentration was added to the wells bored on the surface of Sabouraud Dextrose Agar seeded with prepared fungi or Mueller-Hinton Agar seeded with prepared bacterial suspension. Ciprofloxacin was used as control for the bacteria while ketoconaole was used as control for the fungi. The plates were incubated for 2 days at 25 ^{o}C for fungal isolate and 24 hours at 37 ^{o}C for bacterial isolates. The presence of zone of inhibition around the wells indicated microbial inhibition by the extracts and was measured to the nearest millimeter using a well calibrated meter ruler (Cheesebrough, 2006).

Determination of minimum inhibitory concentration (MIC) of *M. Scaber*

Leaf extract against the test isolates: The tube dilution (macrodilution) method described by Balouiri *et al*. (2016) was used. The Minimum Inhibitory Concentrations (MICs) was determined using a standardized culture. The sterile Mueller-Hinton broth was used for the bacteria and Sabouraud dextrose broth was used for the fungi isolates. Fifteen (15) tubes labelled 1-5 were used for each organism and 2 ml each of Muller-Hinton broth and Sabouraud dextrose broth was dispensed into each set of test tubes for both bacterial and fungal isolates. About 2 ml of the crude extract from concentration 50 mg/ml was introduced into tube one for each of the organism and mixed thoroughly and 2 ml of the content of tube one was transferred into tube two and mixed thoroughly. The procedure was repeated for the remaining test tubes to give a concentration of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively while tube 5 contained no plant extracts served as control. About 0.1 ml of each microbial standardized suspension of the test organisms was separately introduced into each set of test tubes. All the test tubes were incubated for 2days at 25 ^{o}C for fungi and 24 hours at 37 ^{o}C for bacteria. The MIC was recorded as the lowest concentration of extract that did not permit any visible growth when compared with the drug free broths containing either fungal or bacterial suspension (Cheesebrough, 2006).

Determination of Minimum Bactericidal Concentrations (MBCs) and Minimum Fungicidal Concentration (MFC) of M. scaber Leaf Extract against the Test Isolates: The tubes with mixture of the organism and extract in MIC test which shows no visible growth after 2days or 24 hours of incubation, was sub cultured onto SDA and nutrient agar and incubated for 2days at 25 ^{o}C for fungal and 24 hours at 37 ^{o}C for antibacterial activity. The MBC was taken as the lowest concentration of the extract that did not yield a single bacterial colony during subculture on nutrient agar

plate after 24 hours of incubation period. The MFC was taken as the lowest concentration of the extract that did not show any fungal colony growth after 2days incubation with SDA (Cheesebrough, 2006).

Results

Phytochemical constituents of ethanol leaf extract of M. scaber: The phytochemical compounds detected in the ethanol extract of *M. scaber* is presented in Table 1. Alkaloids, flavonoid, cardiac glycosides, phytosteroids, triterpenes, steroids, tannins, saponins were found to be present in the leaf extract of *M. scaber*while carbohydrates, anthraquinones and glycosides were absent.

Table 1: Phytochemical Constituents of Ethanol Leaf Extract of *M. scaber*

Bioactive Compounds	Ethanolic extract
Alkaloid	+
Flavonoid	+
Glycosides	-
Carbohydrates	-
Cardiac glycosides	+
Phytosterols	+
Triterpenes	+
Tannins	+
Saponins	+
Anthraquinones	-

Keys +: Present
-: Absent

Reconfirmation of test isolates: *E. coli* appeared as green metallic sheen on Eosin Methylene Blue Agar with round, smooth, non- mucoid colonies. *S. aureus* appeared golden yellow on Mannitol Salt Agar with circular, smooth, mucoid colonies. The gram reaction and biochemical test kit confirmed *E. coli* as a Gram negative rod, positive for indole, methyl red and negative for citrate and voges proskauer test. *S. aureus* appeared as Gram positive cocci, catalase and coagulase positive while *C. albicans* appeared dark green on Brilliance Candida Agar and positive for germ tube.

A*ntimicrobial activity of ethanol leaf extract of M. scaber against test isolates*: The result of antimicrobial activity of ethanol leaf extract of *Mitracarpus scaber* against test isolates is presented in Table 2. The mean and standard deviation of inhibition zoneof ethanol leaf extract of *M. scaber* ranged from 12.25 ± 0.35 - 17.75 ± 0.35 mg/mland 13 ± 0.00 - 20.75 ± 1.06 mg/ml for *E. coli* and *S. aureus* respectively while the mean and standard deviation of inhibition zone produced by *C. alblicans* ranged from 10.75 ± 0.35 - 16.25 ± 0.35 mg/ml which were comparable with that of ciprofloxacin and ketoconazole used as controls. The activity of extract at varied concentration was significant at $p \leq 0.05$.

Table 2: Antimicrobial activity of ethanol leaf extract of *M. scaber* against test isolates

Organisms	Zone of inhibition (mm)				Control	
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Ciprofloxacin	Ketoconazole
E.coli	12.25 ± 0.35	14.5 ± 0.00	16.75 ± 0.35	17.75 ± 0.35	25 ± 0.00	
S. aureus	13 ± 0.00	14.5 ± 0.71	17.25 ± 0.35	20.75 ± 1.06	22 ± 0.00	
C. albicans	10.75 ± 0.35	13.5 ± 0.00	15 ± 0.71	16.25 ± 0.35		18.5 ± 0.00

Values are presented as Mean ± Standard deviation, $P \leq 0.05$

Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal (MBC) and Minimum Fungicidal Concentration (MFC) of M. scaber against Test Isolates: The Minimum Inhibitory Concentrations (MICs), Minimum Bactericidal/Minimum Fungicidal Concentrations (MBC/MFC) of ethanol leaf extract of *M. scaber* against the test organisms are presented in Table 3. The MIC and MBC of leaf extract against bacterial isolates were 12.5 mg/ml and 25 mg/ml for *E. coli* and 6.25 mg/ml and 12.5 mg/ml for *S. aureus* respectively while the MIC and MFC of the leaf extract against *C. albicans* were 25 mg/ml and 50 mg/ml.

Table 3: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) of Ethanol Leaf Extract of *M. scaber* against Test Isolates

Organisms	MIC (mg/ml)	MBC (mg/ml)	MFC (mg/ml)
E. coli	12.5	25	
S. aureus	6.25	12.5	
C. albicans	25	-	50

Key: MIC= Minimum Inhibitory Concentration
MBC= Minimum Bactericidal Concentration
MFC= Minimum Fungicidal Concentration

Discussion

The result of phytocompounds detected in this study is consistent with the findings in other studies conducted in Nigeria which identified the presence of tannins, flavonoids, saponins and phenols from extracts of *M. scaber* (Onawunmi *et al*., 2012; Ubani *et al*., 2012;). In a similar study conducted by Ameh *et al*. (2011), anthraquinone and glycosides were detected in the aerial parts of *M. scaber.* The variation in the composition of phytochemical compounds among plants could be due to the geographical conditions, harvest processing, postharvest processing time, moisture content, drying method, storage condition, method of extraction and the solvent used in the extraction of specific plant parts (Nwodu *et al*., 2011). Phytochemical studies have shown that plants with antimicrobial activities contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins which are responsible for the biological properties of plants (Thamaraiselvi *et al*., 2012). All these facts could be some of the reasons why *M. scaber* is widely used for the treatment of many ailments among many tribes in Nigeria.

The antibacterial and antifungal activities of ethanol leaf extract of *M. scaber* could be due to the presence of active chemical constituents detected in the extracts. This may be associated with the high polarity of the solvent which allows it to penetrate well into the plant tissues and extract more active compounds that are responsible for the inhibition observed (Musa *et al*., 2016).Previous studies showed that the extract of *M. scaber* possess phytochemicals responsible for antibacterial activity against *S. aureus and also* exhibited antifungal activities against *C. albicans* and *Cryptococcus neoformans* (Okunade *et al*., 2005).

The test organisms showed a degree of susceptibility, the order of sensitivity being *S. aureus* which is more susceptible to extract than *E. coli* and *C. albicans.* The differences in anti-bacterial and anti-fungal activities could be due to the mode of actions and structural properties of the organisms. The cell wall of Gram positive bacteria are more sensitive to antimicrobial chemical compounds. Lipopolysaccharides layer and periplasmic space of Gram negative bacteria are the reason of relative resistance of gram negative bacteria (Koohsari *et al*., 2015). In a similar study of Razik *et al.* (2012) the antibacterial effects of *Plantago major* has more effects against gram positive bacteria such *S. aureus* while *Escherichia coli* and *Enterococcus* were less sensitive. *C. albicans* have the ability to form biofilm and the extracellular polysaccharide of the biofilm could serve as an inhibitor to diffusion of an antimicrobial agent (Koohsari *et al*., 2015). This result corresponds with the findings of Fineboy *et al*. (2019) who conducted similar studies.

The lower MIC exhibited by *M. scaber* against both the bacteria and the fungal isolates was attributed to the effectiveness of the plant extract, indicating both the bacteriostatic and fungistatic nature of the extracts. The values of the MBC and FBC test were greater than those of MIC test. This is in line with the study of Ekpendu *et al*. (2006).

The fact that extract of *M. scaber* inhibited commonly encountered microorganisms explains the popularity of the plant among the local folks in the treatment of some infections. In addition, the ability of the extracts to inhibit the growth of *C. albicans* explains the rationale for the use of the plant-drug in fungal skin infections, especially eczema (Fineboy *et al*., 2019).

It is a common clinical experience that many microorganisms are acquiring resistance to the routine antimicrobial agents with broader therapeutic spectrum potency. Thus, there is a need to search for new antibiotics. Plants continue to prove rich sources of therapeutic agents and the results of this study reveal the potential value of *M. scaber* and has added yet new potential antimicrobial agents (Fineboy *et al*., 2019).

Conclusion

The phytochemicals present in the crude extract of *Mitracarpus scaber* are alkaloids, flavonoids, cardiac glycosides, triterpenes, tannins, saponins and phytosterols. The extract showed significant inhibitory effect against isolates of *E. coli*, *S. aureus* and *C. albicans*. The extract was found to be more effective against *S. aureus*. However, *Mitracarpus scaber* leaf extract can be used in the treatment of diseases caused bybacteria and fungi and could be more effective against Gram positive bacteria. The phytochemicals detected in the leaf extract of *M. scaber* could be responsible for the observed antimicrobial activities. Further research should be carried out on the leaf extract of *M. scaber* to isolate the active components present in the leaf extract.

References

Ameh SJ, Obodozie OO, Abubakar MS, Garba M, Gamaniel KS: Herbal drug regulation illustrated with Niprifan® antifungal phytomedicine. In: Modern approaches to Quality Control pp367-382. 2011. http://www.intechopen.com. Accessed on 9th Sept. 2019.

Azwanida N: A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicina Aromat Plants, 4: 196. 2015.

Balouiri M, Sadiki M, Ibnsouda SK: Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal, 6: 71-79. 2016.

Chanda S, Baravalia Y: Screening of some plant extracts against some skin diseases caused by oxidative stress and microorganisms. Afr J Biotechnol, 9: 3210-3217. 2010.

Cheesbrough M: Biochemical tests to identify bacteria. In S. Davies, S. Hardy, E. Bridson and M. Tofiq (Eds.), *District Laboratory Practice in Tropical Countries* (2nd ed.). New York: Cambridge University Press. pp. 62-70. 2006.

Clinical Laboratory Standards Institute: Reference method for broth dilution antifungal susceptibility testing of yeasts: Approved standard M27-A2. Wayne (PA). 2002.

Dalziel JM: The Useful Plants of West Tropical Africa pp. 401. 2004.

Egharevba RKA, Ikhatua MI: Ethno- medical uses of plants in the treatment of various skin diseases in ovia North East, Edo State, Nigeria. Res J Agric Biol Sci, 4(1): 58-64. 2008.

Ekpendu TO, Akah PA, Adesomoju AA Okogun JI: Anti-inflammatory and antimicrobial activities of *Mitracarpus scaber* extracts. Pharm Biol, 32 (2):191-196. 2006.

Fineboy U, Nyangwae U, Mbashak R: Prelimenary phytochemical and antimicrobial studies of ethanol extract of *Mitracarpus scaber* Zucc. Glob Sci J, 7(8): 499-514. 2019.

Gbile ZO: (2004). *Vernacular names of Nigerian Plants (Yoruba).* Nigeria: Forestry Research Institute of Nigeria, Ibadan p.87. 2004.

Gracelin DHS: De-Britto AJ, Rathna Kumar: PBJ. Qualitative and quantitative analysis of phytochemicals in five species. Int Pharm Pharm Sci, 5(1): 67. 2013.

Koohsari H, Ghaemi EA, Sadegh SM, Jahedi M, Zahiri M: The investigation of antibacterial activity of selected native plants from North of Iran. J Med Life, 8(2): 38-42. 2015.

Moussa MB, Rabiou H, Inoussa M, Bakasso Y, Mahamane A: Bio-indicateurs de la fertilité des sols et la perception paysanne à l'ouest du Niger. J Appl Biosci, 89(1): 8281-8290. 2015.

Nwodu UU, Chigor VN, Okoh AY: *In-vivo* evaluation of the antiviral activity of *Cajanus cajan* on measles virus. Arch Virol, 156: 1551-1557. 2011.

Oghenejobo M, Uvieghara KE, Omughele E: Antimicrobial activities and phytochemical screening of the leaf extract of *Mitracarpus scaber*. Sch Acad J Biosci, 1(6): 227-230. 2013.

Okunade AL, Clark AM, Hufford CD, Oguntimein BO: *Planta Medicinale*, 65: 447- 448.2005.

Onawunmi OE, Adelowo FE, Ipadeola AO, Edewo TI, Ayoola PB, Odunola OA: Preliminary studies on phytochemical and antimicrobial investigation of plants (Ira-wole) *Mitracarpus villosus*, *Euphobia hirta* and *Spermacoce ocymoides*. Int J Res Rev Appl Sci, 10(1): 78-81. 2012.

Ouadja B, Anani K, Djeri B, Ameyapoh YO, Karou DS: Evaluation of the phytochemical composition,antimicrobial and anti-radical activities of *Mitracarpus scaber* (Rubiaceae). J Med Plants Res, 12(28): 439-499. 2018.

Razik BM, Hasan HA, Murtadha MK: The study of antibacterial activity of *Plantago major* and *Ceretonia silique*. Iraqi Postgrad Med, 11(1): 130-135. 2012.

Thamaraiselvi PL, Jayanthi P: Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. Asian J Plant Sci Res, 2(2): 115-122. 2012.

Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H: Phytochemical screening and extraction: A review. Int Pharm Sci, 1(1): 98-106. 2011.

Ubani CS, Oje OA, Ihekogwo FNP, Eze EA, Okafor CL Parameters on antibacterial susceptibility of *Mitracarpus Villosus* ethanol extracts; using samples from South East and South-Southern regions of Nigeria. Glob Adv Res J Microbiol, 1(7): 120-125. 2012.

Zahin M, Aqil F, Khan MSA, Ahmad J: Ethnomedicinal plants derived antibacterials and their prospects. In D. Chattopadhyay (Ed.), *Ethnomedicine: A Source of Complementary Therapeutics* pp. 149-178. 2010. Research Signpost 37/661 (2), Fort P.O. Trivandrum-695 023 Kerala, India