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Preliminary studies on the antifungal activities of the successive extracts of *Mitracarpus villosus* (Sw.) Dc aerial parts obtained in Abuja, Nigeria

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Aim: A worldwide increase in the incidence of fungal infections has been observed in the past few decades, with a rise in the resistance of some fungal species to different fungicidal agents used in medicinal practice. This study aims at evaluating the antifungal properties of *Mitracarpus villosus.*
Methodology and results: The aerial parts of *M. villosus* was subjected to successive extraction with hexane, ethyl acetate and ethanol. The powdered plant was screened for phyto-compounds. Antifungal activities of the ethyl acetate extracts against clinical isolates of *Candida albicans*, *C. krusei*, *Trichophyton verrucosum*, *T. mentagrophytes*, *Aspergillus fumigatus* and *A. niger* were investigated using agar diffusion, broth dilution and micro broth dilution methods. The antibiotic susceptibility profiles of the fungal isolates to fluconazole and ketoconazole were also determined. Phytochemical screening of *Mitracarpus villosus* revealed the presence of tannins, saponins, flavonoids, terpenes, phenols and resins. The results of the zones of inhibition showed that the ethyl acetate extract of *Mitracarpus villosus* produced the strongest antifungal activity with diameter zones of inhibition ranging from 18.67-21.67 mm at an exposure concentration of 12.5 mg/mL. The minimum inhibitory concentration and minimum fungicidal concentration values of the ethyl acetate extract against all the fungal isolates tested were 0.5-2.0 mg/mL and 2.0-8.0 mg/mL respectively.
Conclusion, significance and impact study: The results confirm the claims of traditional healers in the use of the plant. This plant holds great promise for development into phytomedicine for the treatment of fungal infections in the near future.

Keywords: phyto-compounds, crude extracts, susceptibility

INTRODUCTION

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being (Avijgan *et al.*, 2010). Early humans recognized their dependence in nature in both health and illness. Led by instinct test and experience, primitive people treated illness by using plant, animal parts and minerals that were not part of their diet (Anwanni *et al.*, 2005).

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported to be well tolerated when compared with synthetic drugs (Iniaghe *et al*., 2009). Also, there has been little or no report of any form of microbial resistance during the administration and use of herbal medicines. More importantly in Africa, particularly West Africa, new drugs are often beyond the reach of the poor such that up to 80% of the population uses medicinal plants as remedy against infections and diseases (Ezekiel *et al.*, 2009). The high prevalence of fungal infections worldwide which is further complicated in immunocompromised states as well as the increasing rate of resistance of most pathogenic fungi to existing orthodox antifungals, justifies the need for this study

The plant *Mitracarpus villosus* (S.W) D.C belongs to the family Rubiaceae. *M. villosus* is an erect perennial annual herb up to 60 cm high. It grows as a weed on old and abandoned farmlands and is distributed widely from forest to savanna zones of the tropics. In various parts of tropical Africa, it is traditionally used for treatment of sore throat. It has been used in traditional medicine practices in West Africa for the treatment of headaches, toothaches, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases as well as leprosy (Dalziel, 1937). It has also been used to treat ringworm and eczema, fresh cuts, wounds and ulcer. The aerial portion of this plant has also been used to make lotion and skin ointment used for skin diseases and infections (Bisignano *et al*., 2000). In

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Nigeria, the extracted juice from aerial parts is topically applied against skin diseases and on wounds (Abere *et al*., 2007).

The main objective of this study is to evaluate the anti-fungal activities of the successive extracts of the aerial parts of *M. villosus* (Sw.) DC obtained in Abuja, Nigeria.

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh aerial parts of *M. villosus* was collected from the National Institute for Pharmaceutical Research and Development (NIPRD) garden. The plant was identified and authenticated in the herbarium of the Department of Medicinal Plant Research and Traditional Medicine, NIPRD, Abuja, Nigeria. The sample of the plant was deposited in the herbarium for reference purpose with Voucher specimen No. NIPRD/ H/ 4208.

Preparation of crude plant material

M. villosus aerial parts were dried at 25 °C for 10 days. The completely dried aerial parts were crushed to coarse powder by grinding with wooden mortar and pestle.

Preliminary extraction of plant material

Using a Soxhlet extractor (Quick Fit- England), the powdered aerial parts was extracted successively and exhaustively each time with each of the various solvents starting from the less polar solvent to the more polar solvent. The solvents used were hexane, ethyl acetate and ethanol respectively. Finally the marc was macerated in water for 24 h. After each extraction, the extract was concentrated, dried and weighed.

Phytochemical screening of *M. villosus*

The phytochemical screening for the presence or absence of secondary metabolites in *M. villosus* crude extracts of the fresh aerial part and dried aerial part was performed using the methods described by Harborne (1998) Evans (2002) and Sofowora (1993). The following secondary metabolites were ascertained: alkaloids, tannins, saponins, cardiac glycosides, anthraquinones, phlobatannins, terpenes, sterols, resins, balsams, flavonoids, phenols and volatile oil. The methods used by Harborne (1998) were used to determine the presence of alkaloids, cardiac glycosides and phlobatannins. The presence of saponins was detected using the method of Sofowora (1993), while tannins and anthraquinones were screened for using the method of Trease and Evans (1989).

Antifungal assay of extracts of *M. villosus*

Preparation of antifungal agents

Stock solutions of fluconazole powder (Sigma Aldrich, Cat No. F8929) and ketoconazole powder (Sigma Aldrich, Cat No. K1003) was prepared by dissolving appropriate quantity of the antifungal agent in dimethyl sulfoxide (DMSO, BDH, Germany) and later diluted to their require concentrations with broth.

Fungi used

The fungi used were clinical isolates from the Department of Microbiology and Biotechnology, NIPRD, Abuja. These include *Candida albicans*, *C. krusei*, *Aspergillus fumigatus*, *A. niger*, *Trichophyton mentagrophytes* and *T. verrucosum*. *T. verrucosum* was collected from the Department of Dermatophytosis, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

Cultivation and standardization of test fungi

Eighteen-hour broth culture of the test *Candida* spp. was suspended into sterile Sabouraud dextrose liquid medium. It was standardized according to National Committee for Clinical Laboratory Standards Institute (CLSI, 2002) by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 cfu/mL. However, for *Trichophyton* spp. and *Aspergillus* spp., fungal spores were harvested from 7 days old SDA slant cultures by washing with 10 mL sterile normal saline containing 3% w/v Tween 80 with aid of sterile glass beads to help in dispersing the spores (Olowosulu *et al.*, 2005). Thereafter, the spore suspension was standardized to 1.0×10^6 spores/mL by using a single-beam spectrophotometer (Spectronic 20D; Milton Roy Company, Pacisa, Madrid, Spain) at 530 nm (OD530) of the suspensions and adjusted to 80-85 % transmittance (*Aspergillus* spp.) and 70-72 % (*Trichophyton* spp.). All adjusted suspensions were quantified by spreading 100 µL on Sabouraud dextrose agar plate and incubated at 37 °C for 18 h for yeast and 30 °C for 72 h for dermatophytes and moulds (Aberkane *et al*., 2002). All cultures were checked for purity (by morphological growth on media, staining and biochemical tests) and maintained on Sabouraud dextrose agar (SDA) for the fungal test organisms respectively at 4 °C (in the refrigerator) until required for use.

Antifungal screening of the ethyl acetate extract of *M. villosus*

Eighteen hours overnight cultures of *Candida* spp. and innoculum suspensions of the moulds and dermatophytes prepared from fresh, mature (3- to 5-day-old) cultures (in some cases an extended incubation was required for proper sporulation of the isolate) in Sabouraud dextrose liquid medium were standardized to produce inoculum size of 10^6 cfu/mL. One millilitre of the diluted culture of

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each test organism was used to flood Sabouraud dextrose agar media and excess aseptically drained. The plates were allowed to dry at 37 °C in a sterilized incubator. Adopting the agar diffusion cup plate method (Olowosulu *et al.*, 2005), a sterile cup borer (6 mm) was used to bore holes in the agar plates. The bottoms of the wells (holes) were sealed with the appropriate molten Sabouraud dextrose agar. Using micropipette, 0.1 mL each of 200 mg/mL concentration of the solvent extracts of the crude plants and extracted secondary metabolites of the plant were dispensed into the holes. Distilled water and the solvents used in diluting the extracts were used as control. These were allowed to diffuse into the agar at 25 °C for 1 h before incubation at 37 °C for 18 h (yeast) and 30 °C for 72 h up to 5 days (dermatophytes and moulds). The zones of inhibition of the test organisms were measured to the nearest millimeter, using a well-calibrated meter ruler. The experiment was carried out in triplicates.

Determination of minimum inhibitory concentration (MIC) of the extract

The minimum inhibitory concentration (MIC) of the extract and reference antibiotics to the test fungi determined by using serial broth microdilution method (Shanmugapriya *et al.*, 2012). This assay was performed using round bottom 96-well clear microtitre plates. The wells in row 1 of each column were left blank and the last eleven wells from rows 2 to 12 were filled with 50 µL of sterilized Saboraud dextrose broth. Working solution of plant extracts were added to the wells in row 1 of each column and wells in row 2 of each column and an identical two-fold serial dilution were made from wells in rows 2 to the rows 10. The last wells in row 12 served as drug-free controls. An appropriate solvent blanks were included as negative control. Lastly, 50 µL of standardized fungal inoculum (10^6 cfu/mL) were added in all the wells from column A to H and mixed thoroughly to give final concentrations. Tests were done in triplicates. The cultured microplates were sealed with parafilm and incubated at 37 °C for 24 h for bacterial and yeast species. The plates were incubated at 37 °C for 18 h (yeast) and 30 °C for 48 h and up to 5 days (dermatophytes and moulds). MIC was defined as the first well with no visible growth after 24 h.

Determination of minimum fungicidal concentration (MFC) of the extract

Fifty microliters of the wells that did not show any visible growth after M.I.C determination were inoculated in fresh wells containing Saboraud dextrose broth and incubated at 37 °C for 18 h (yeast) and 30 °C for 48 h and up to 7 days (dermatophytes and moulds). Minimum fungicidal concentration (MFC) were determined as the lowest concentration resulting in no growth on subculture (Hafidh *et al.*, 2011).

Statistical analysis

Results obtained were expressed as mean±standard deviation and analysed using one way ANOVA (Smith's Statistical Package version 2.80) at $p<0.05$.

RESULTS

The phytochemical screening of the powdered plant part revealed the presence of tannins, saponin, flavonoids, terpenes, phenols and resins while alkaloid, sugars, carbohydrates, balsams and anthraquinones were not detected (Table 1). Generally, the ethyl acetate extracts produced the highest zones of inhibition against all the fungal isolates (*Candida* spp., *Aspergillus* spp. and *Trichophyton* spp.). This was followed by the hexane extracts with antifungal activity against the yeasts only (*C. albicans* and *C. krusei*). The ethyl acetate extract of *M. villosus* produced strong antifungal activity inhibiting the growth of *C. albicans* (diameter zone of inhibition > 18 mm) at a concentration of 50 mg/mL which was comparable with fluconazole ($p>0.05$) The hexane extracts on the other hand, produced an intermediate antifungal activity (diameter zone of inhibition > 10 mm) while the ethanol and water extracts were inactive against *C. albicans*. The growths of *Aspergillus* sp. and *T. mentagrophytes* were also inhibited by the ethyl acetate extract of *M. villosus* (diameter zone of inhibition > 18 mm) at a concentration of 50 mg/mL which was comparable with ketoconazole ($p>0.05$).

Table 1: Phytochemical constituents of the aerial parts of *M. villosus.*

Secondary metabolites	Inference
Carbohydrates	-
Terpenes	+
Sterols	-
Saponins	+
Tannins	+
Anthraquinones	-
Balsams	-
Resins	+
Alkaloids	-
Flavonoids	+
Phenols	+
Volatile oil	-

+, present; -, absent

The hexane, ethanol and water extracts however, were inactive against these organisms. The ethyl acetate and ethanol extracts of *M. villosus* also inhibited the growth of *Trichophyton verrucosum* while the water and hexane extracts were inactive against *T. verrucosum.* However the diameter zone of inhibition produced by the ethanol extract was significantly lower ($p<0.05$) than the ethyl acetate extract (Table 2). An increase in the concentration of the ethyl acetate extracts (3.125-50 mg/mL) led to an increase in antifungal activities against all the tested fungi, as shown by an increase in the diameter of zones of

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inhibition ranging from 18.0-26.0 mm (Tables 3 and 4). The minimum inhibitory concentration and minimum fungicidal concentration values of the ethyl acetate extract against all the fungal isolates tested were 0.5-2.0 mg/mL and 2.0-8.0 mg/mL respectively (Tables 5 and 6). The MIC and MFC values of the ethyl acetate extract was significantly higher than the standard drugs used (p<0.05).

Table 2: Susceptibility of the test fungi to the solvent extracts of *M. villosus* at concentration of 50 mg/mL.

Organisms	Zone of inhibition (mm)					
	HEX	ETA	ET	W	FCZ (50 µg/mL)	KTZ (50 µg/mL)
Ca1	11.0±0.57^{a}	25.0±0.0^{b}	IA	IA	25±0.57^{b}	NA
Ca2	10.67±0.33^{a}	25.0±0.57^{b}	IA	IA	25.67±0.33^{b}	NA
Ca3	10.67±0.33^{a}	25.0±0.0^{b}	IA	IA	26.67±0.33^{c}	NA
Ca4	11.33±0.57^{a}	25.0±0.0^{b}	IA	IA	26.33±0.33^{c}	NA
Ck	10.67±0.33^{a}	25.0±0.0^{c}	IA	IA	20.0±2.0^{b}	NA
AF	IA	20.67±0.33^{a}	IA	IA	NA	25.67±0.33^{b}
AN	IA	24.33±0.33^{a}	IA	IA	NA	26.33±0.33^{b}
TM	IA	25.67±0.33^{a}	IA	IA	NA	25.33±0.33^{a}
TV	IA	26.0±0.0^{b}	18.67±0.33^{a}	IA	NA	27.33±0.33^{c}

Values are mean inhibition zone (mm)±SD of three replicates
Values with different superscripts on the same row are significantly different (p<0.05)
HEX, Hexane; ETA, Ethylacetate; ET, Ethanol; W, Water; FCZ, Fluconazole; KTZ, Ketoconazole; NA , Not applicable; Ca, *Candida albicans*; CK, *Candida krusei*; AN, *Aspergillus niger*, AF, *Aspergillus fumigates*; TM, *Trichophyton mentagrophytes*; TV, *Trichophyton verucossum*; IA, Inactive

Table 3 : Antifungal susceptibility testing of ethyl acetate extracts of *Mitracarpus villosus* against yeast.

Organisms	Zone of inhibition (mm)					
	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL	Fluconazole (50 µg/mL)
Candida albicans	25±0.0^{d}	22.0±0.57^{c}	20±0.0^{b}	18.67±0.33^{a}	18.0±0.0^{a}	25±0.57^{d}
Candida krusei	25±0.0^{c}	20.0±0.0^{b}	18.67±0.33^{a}	18.33±0.33^{a}	18.67±0.33^{a}	20.0±2.0^{b}

Values are mean inhibition zone (mm)±S.D of three replicates
Values with different superscripts on the same row are significantly different (p<0.05)

Table 4: Antifungal susceptibility testing of ethyl acetate extracts of *Mitracarpus villosus* against filamentous fungi.

Organisms	Zone of inhibition (mm)					
	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL	Ketoconazole (50 µg/mL)
AF	20.67±0.33^{d}	19.67±0.33^{c}	19.0±0.57^{c}	18.67±0.33^{b}	17.0±0.0^{a}	25.67±0.33^{e}
AN	24.33±0.33^{d}	23.67±0.88^{c}	20±0.0^{c}	18.33±0.88^{b}	17.67±0.67^{a}	26.33±0.33^{e}
TM	25.67±0.33^{d}	24.67±0.33^{c}	22.0±0.0^{b}	20.67±0.33^{a}	20.0±0.0^{a}	25.33±0.33^{d}
TV	26.0±0.0^{d}	25.67±0.33^{c}	23.67±0.33^{b}	21.67±0.33^{a}	21.0±0.0^{a}	27.33±0.33^{e}

Values are mean inhibition zone (mm)±S.D of three replicates
Values with different superscripts on the same row are significantly different (p<0.05)
AN, *Aspergillus niger*; AF, *Aspergillus fumigates*; TM, *Trichophyton mentagrophytes*; TV, *Trichophyton verucossum*

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Table 5: Minimum inhibitory concentration and minimum fungicidal concentration of ethyl acetate extract of *M. villosus* against yeast.

Organism	Minimum inhibitory concentration		Minimum fungicidal concentration	
	Extract	Fluconazole	Extract	Fluconazole
Candida albicans	1.0 ±0.33[a]	0.025 ±0.0[b]	4.0 ±0.33[a]	0.025 ±0.0[b]
Candida krusei	2.0 ±0.33[a]	0.2 ±0.33[b]	8.0 ±0.0[a]	2.0 ±0.33[b]

Values are mean inhibition zone (mm)±S.D of three replicates
Values with different superscripts on the same row are significantly different (p<0.05)

Table 6: Minimum inhibitory concentration and minimum fungicidal concentration of ethyl acetate extract of *M. villosus* against filamentous fungi.

Organism	Minimum Inhibitory Concentration		Minimum Fungicidal Concentration	
	Extract	Ketoconazole	Extract	Ketoconazole
Aspergillus fumigatus	2.0 ±0.33[a]	0.025 ±0.0[b]	4.0 ±0.33[a]	0.05 ±0.0[b]
Aspergillus niger	1.0 ±0.33[a]	0.025 ±0.0[b]	4.0 ±0.0[a]	0.05 ±0.0[b]
Trichophyton mentagrophytes	2.0 ±0.0[a]	0.008 ±0.33[b]	4.0 ±0.33[a]	0.016 ±0.0[b]
Trichophyton verrucosum	0.5 ±0.0[a]	0.004 ±0.0[b]	2.0 ±0.0[a]	0.016 ±0.33[b]

Values are mean inhibition zone (mm)±S.D of three replicates
Values with different superscripts on the same row are significantly different (p<0.05)

DISCUSSION

Plants have been known to posses bioactive constituents as protective substances against bacteria, fungi, viruses and pests (Marjorie, 1999). Results from this study, revealed that the degree of antifungal activities of the test plant varied from one test organism to another. The ethyl acetate extract of *M. villosus* was shown to produce the strongest antifungal activity against all the fungi tested. The hexane extract on the other hand, produced inhibitory activities on the yeasts only. However there was a significant difference between the zones of inhibition produced by ethyl acetate extract and the hexane extracts of the plant (p<0.05) The gradual increase in the zones of inhibition with increase in concentration of the extract show that the inhibitory action of the extract on the fungi tested is dependent on the amount of drug used (Tables 3 to 4). Generally, the ethyl acetate extract had the strongest antifungal activity on *Trichophyton verrucosum* and *T. mentagrophytes*. *T. verrucosum* is the usual zoophilic dermatophyte involved in cattle ringworm. It also affects, but with lower prevalence, sheep, goat and other ruminants. Aside from animal involvement, several human outbreaks of *T. verrucosum* infection have been reported so far by direct contact with infected animals or indirect contact with infectious propagules in the environment (Nasrin *et al.*, 2007). Northern Nigerian is known for cattle rearing, thus infection with this organism in humans is very common. With further study, this extract holds great promise in combating infections caused by *T. verrucosum*. The ethyl acetate extract of *M. villosus* also inhibited the growth of *T. mentagrophytes,* a dermatophyte implicated in the spread of ring worm from animals to humans. The ethyl acetate extract of *M. villosus* also possessed strong antifungal activities against *Aspergillus* spp. and *Candida* spp. With the increasing rate of candidiasis infection worsened by the high rate of HIV/AIDS infection, this plant holds great promise for development into phytomedicine for the treatment of candidiasis in the near future. *A. fumigatus* is the most frequent cause of invasive fungal infection in immunosuppressed individuals, which include patients receiving immunosuppressive therapy for autoimmune or neoplastic disease, organ transplant recipients, and AIDS patients (Ben-Ami *et al.*, 2010). The zones of inhibition of the ethyl acetate extract against all the fungi tested at 50 mg/mL was comparable to the standard drugs (fluconazole and ketoconazole) used. The antifungal activity of the extract can be linked to the presence of secondary metabolites which have been shown to possess bioactive properties Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins which are responsible for the biological properties of such plants (Thamaraiselvi *et al.*, 2012). Researchers have reported the presence of tannins, flavonoids, saponins and phenols from extracts of *M. villosus* (Edeoga *et al*., 2005; Abere *et al.*, 2007; Onawunmi *et al.*, 2012; Ubani *et al.*, 2012).

CONCLUSION

In conclusion the ethyl acetate extract of *M. villosus* have shown to possess good antifungal properties. The findings of this study have shown the need for further investigation to establish the economic viability of exploiting *M. villosus* plant to address fungal infections.

REFERENCES

Abere, T. A., Onwukaeme, D. N. and Eboka, C. J. (2007). Pharmacognostic evaluation of the leaves of *Mitracarpus scaber* Zucc (Rubiaceae). *Tropical Journal of Pharmaceutical Research* **6 (4), 849-851.**

Aberkane, A., Cuenca-Estrella, M., Gomez-Lopez, A., Petrikkou, E., Mellado, E., Monzón, A.,

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Rodriguez-Tudela, J. and Eurofung L. N. (2002). Comparative evaluation of two different methods of inoculum preparation for antifungal susceptibility testing of filamentous fungi. *Journal of Antimicrobial Chemotherapy* **50, 19-22.**

Anwannil, H. G. and Alta, R. (2005). Trends in Entropharmacology. *Journal of Entropharmacology* **100, 43-49.**

Avijgan, M., Mahboubi, M., Darabi, M., Saadat, M., Sarikhani, S. and Nazilla, K. (2010). Overview on *Echinophora platyloba,* a synergistic antifungal agent candidate. *Journal of Yeast and Fungal Research* **1(5), 88-94.**

Ben-Ami, R., Lewis, R. E. and Kontoyiannis, D. P. (2010). Enemy of the (immunosuppressed) state: An update on the pathogenesis of *Aspergillus fumigatus* infection. *British Journal of Haematology* **150(4), 406-417.**

Bisignano, G., Sanogo, R., Marino, A., Aquino, R., Angelo, V. D., German-Ograve, R., De Pasquale, M. P. and Pizza, C. (2000). Antimicrobial activity of *Mitracarpus scaber* extract and isolated constituents. *Letter of Applied Microbiology* **30(2), 105-108.**

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

Dalziel, J. M. (1937). The useful plants of West Tropical Africa. **pp. 401.**

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005).Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4 (7), 685-688.**

Evans, W. C. (2002). Trease and Evans Pharmacognosy. WB Saunders Ltd. London. **pp. 32-547.**

Ezekiel, C. N., Anokwuru, C. P., Nsofor, E., Odusanya, O. A. and Adebanjo, O. (2009). Antimicrobial activity of the methanolic and crude alkaloid extracts of *Acalypha wilkesiana* cv. *macafeeana* copper leaf. *Research Journal of Microbiology* **4, 269-277.**

Hafidh, R., Ahmed S. Abdulamir, L., Se-Vern, F., Abu, B., Faridah, A., Fatemeh, J. and Zamberi, S. (2011). Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. *The Open Microbiology Journal* **5, 96-106.**

Harborne, J. B. (1998). Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd. London. **pp. 49-188.**

Iniaghe, O. M., Malomo, S. O. and Adebayo, J. O. (2009). Proximate composition and phytochemical constituents of leaves of some *Acalypha* species. *Pakistan Journal of Nutrition* **8, 256-258.**

Marjorie, M. C. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12(4), 564-582.**

Nasrin, A., Eskandar, M. and Abdolghani, A. (2007). Characterization of an anti-dermatophyte cream from *Zataria multiflora* Boiss*. Iranian Journal of Pharmaceutical Sciences* **3(2), 77-84.**

Olowosulu, A. K., Ibrahim, Y. K. E. and Bhatia, P. G. (2005). Studies on the antimicrobial properties of formulated creams and ointments containing *Baphia nitida* heartwood extract. *Journal of Pharmacy and Bioresources* **2(2), 124-130.**

Onawunmi O. E., Adelowo F. E., Ipadeola A. O., Edewo T. I., Ayoola, P. B. and Odunola O. A. (2012). Preliminary studies on phytochemical and antimicrobial investigation of plants (ira-wole) *Mitracarpus villosus*, *Euphobia hirta* and *Spermacoce ocymoides*. *International Journal of Research and Reviews in Applied Sciences* **10(1), 78-81.**

Shanmugapriya, P., Suthagar, P., Lee, W. C., Roziahanim, M. and Surash, R. (2012). Determination of minimum inhibitory concentration of *Euphorbia hirta* (L.) extracts by tetrazolium microplate assay. *Journal of Natural Products* **5,68-76**

Sofowora, A (1993) Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. **pp. 289.**

Thamaraiselvi, P. L. and Jayanthi P. (2012) Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. *Asian Journal of Plant Science and Research* **2 (2), 115-122.**

Trease, G. E. and Evans, W. C. (1989). Phytochemical screening. *Pharmacognsy*. 11th edn. Brailliar Tiridel Can. Macmillian Publishers, London, England. **pp 235-238.**

Ubani, C. S., Oje, O. A., Ihekogwo, F. N. P., Eze, E. A., and Okafor, C. L. (2012). Parameters on antibacterial susceptibility of *Mitracarpus villosus* ethanol extracts; using samples from South East and South-Southern regions of Nigeria. *Global Advanced Research Journal of Microbiology* **1(7), 120-125.**

ISSN (print): 1823-8262, ISSN (online): 2231-7538