**In vitro** Antifungal Efficacies of Ethyl Acetate Fractions of *Mitracarpus villosus* from Abuja, Nigeria

M. I. Aboh¹,²*, B. O. Olayinka², G. O. Adeshina², P. Oladosu¹ and K. Ibrahim¹

¹Department of Microbiology, Human Virology and Biotechnology, National Institute for Pharmaceutical Research and Development, Idu. P.M.B. 21 Garki, Abuja, Nigeria.
²Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

**Authors’ contributions**

This work was carried out in collaboration between all authors. Author MIA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors BOO, GOA, PO and KI managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/BMRJ/2015/15051

Received 3rd November 2014
Accepted 1st January 2015
Published 16th March 2015

**ABSTRACT**

Introduction: The use of medicinal plants in the treatment of diseases is as old as man. The development of synthetic (orthodox) drugs led to a decline in the use of herbs however in the past few decades, there has been an increase in the use of medicinal plants, especially in developing countries. Several reports have shown that herbal medicines are well tolerated when compared with synthetic drugs. Over eighty percent of the population in Africa, most especially West Africa, has been reported to depend on medicinal plants for the treatment of infections and diseases.

Aims: The main objective of this study is to assess the anti-fungal potentials of the ethyl acetate extract and fractions of the aerial parts of *Mitracarpus villosus* (Sw.) DC from Abuja, Nigeria.

Methods: The powdered plant was extracted successively and exhaustively with hexane, ethyl acetate, ethanol and water. Thirty fractions were obtained from the extract using the bioassay-
1. INTRODUCTION

Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions [1]. More than 80% of the population in developing countries has been reported to depend on plants for their medical needs [2]. Research have shown that plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties [3].

In recent times, there has been a worldwide increase in the incidence of fungal infections, as well as a rise in the resistance of some species of fungi to different fungicidal agents used in medicinal practice [4]. The incidence of fungal infections is thus increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. This increase is directly related to the growing population of immunocompromised individuals especially children resulting from changes in medical practice such as the use of intensive chemotherapy and immunosuppressive drugs [5].

*Mitracarpus villosus* (S.W) D.C belongs to the family Rubiaceae. It grows as an erect perennial annual plant with height up to 60 cm. It can be found on old and abandoned farmlands where it grows as a weed with a wide distribution ranging from forest to savanna zones in tropical climates. Phytochemical evaluation of the leaves of this plant has been reported to show the presence of alkaloids, tannins, cardiac glycosides, saponins, soluble carbohydrate, flavonoids, reducing sugar, cyanide, glycoside, steroid and terpenoid [6]. In Tropical Africa, fresh extracts of *M. villosus* have been employed in traditional medicine for the treatment of sore throat. In West Africa, natives use extract of the plant for management and cure of several disease conditions which include headaches, toothaches, amenorrhoea, hepatic diseases, gastrointestinal disturbance (like dyspepsia), sexually transmitted diseases, leprosy [7]. The juice from the fresh plant is used to treat skin infections like ringworm and eczema, stoppage of bleeding and as first aid treatment for fresh cuts, wounds and ulcer. The aerial parts of *M. villosus* have also been formulated into lotions and skin ointments for the treatment of skin infections [8]. In Nigeria, the extracts of the juice from the fresh aerial parts of the plant is applied on the skin surface to treat skin diseases and heal wounds [9]. There is limited documentation on the antifungal potential of the ethyl acetate extract of *Mitracarpus villosus*. The main objective of this study is to evaluate the anti-fungal activities of the ethyl acetate extract of the aerial parts of *Mitracarpus villosus* (Sw.) DC from Abuja, Nigeria.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Fresh aerial parts of *Mitracarpus villosus* was collected between June and September 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.

2.2 Drying of Plant Material

The aerial parts fresh plant was air dried at 25°C for 10 days. The completely dried aerial parts were crushed to coarse powder by grinding with wooden mortar and pestle.
2.3 Extraction of Plant Material

Successive extraction was employed for the extraction of active component of the plant. Simply, the dried powdered aerial parts of the plant was placed in a Soxhlet extractor (Quick Fit- England) and the plant material extracted successively and exhaustively each time with each of the various solvents in the order of increasing polarity i.e. hexane, ethyl acetate and ethanol respectively. Lastly, the marc was macerated in water and all the extract concentrated, dried and weighed [10].

2.4 Fractionation of Ethyl Acetate Extract

The fractionation of ethyl acetate extract was done using the bioassay-guided fractionation by employing the accelerated gradient chromatographic (AGC) technique [11]. Silica gel G (E-Merck, Germany) was used as an absorbent. Gradient elution was effected using hexane and ethyl acetate sequentially with increasing polarity. The column was successively eluted with hexane (100%), hexane–ethyl acetate (90:10,80:20,70:30,60:40,50:50,40:60, 30:70,20:80,10:90%), ethyl acetate (100%), ethyl acetate- ethanol (90:10,80:20). The fractions were collected and combined on the basis of their thin layer chromatographic (TLC) profiles.

2.5 Antifungal Assay of Extracts of M. villosus

2.5.1 Preparation of antifungal agents

Stock solutions of fluconazole powder (Sigma Aldrich, Cat No. F8929) and ketoconazole powder (Sigma Aldrich, Cat No. K1003) was prepared in dimethyl sulfoxide (DMSO, BDH, Germany). Sabouraud dextrose broth was used to dilute the stock solutions to their required concentrations.

2.5.2 Fungi used

The fungi used for the study were clinical isolates of Candida albicans, Candida krusei, Aspergillus fumigatus, Aspergillus niger, Trichophyton mentagrophytes and Trichophyton verrucosum obtained from The Department of Microbiology and Biotechnology, National Institute for Pharmaceutical research and Development (NIPRD), Abuja, Nigeria. Trichophyton verrucosum was collected from the Dermatophylosis Center, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

2.5.3 Cultivation and standardization of test fungi

Eighteen-hour culture of the test Candida spp. on Sabouraud dextrose agar (SDA) was standardized according to National Committee for Clinical Laboratory Standards [12]. Colonies of the pure culture of the fungi on solid medium was gradually added to normal saline and its turbidity compared to 0.5 McFarland standard of which is approximately 1.5 × 10⁷ cfu/ml. This was finally diluted with SDB to a population of 1.5 × 10² cfu/ml. Fungal spores Trichophyton spp. and Aspergillus spp., were harvested from SDA slant cultures (7-10 day old) by flooding with 10 ml sterile normal saline containing 3% w/v Tween 80. Sterile glass beads were used to dislodge the spores [13]. Standardization of the fungal spore suspension to 1.0 × 10⁶ spores / ml was carried out using a single-beam spectrophotometer (Spectronic 20D; Milton Roy Company, Pacisa, Madrid, Spain) at a wave length of 530 nm (OD530) and adjusted to 80 – 85% transmittance (Aspergillus spp.) and 70 -72% (Trichophyton spp.). Quantification was done by spreading 100µL of suspension on Sabouraud dextrose agar plate and incubated at 37°C for 18 h and 30°C for 72 hours for yeast and moulds respectively [14]. The cultures were checked for purity based on their morphological characteristics on media, morphology on staining and biochemical tests. The fungi were maintained on Sabouraud dextrose agar (SDA) at 4°C until required for use [14].

2.5.4 Susceptibility of the organisms to ethyl acetate extract of M. villosus

One milliliter of standardized culture of each test organism was spread on Sabouraud dextrose agar (excess aseptically drained) and the plates allowed to dry at 37°C temperature in a sterilized incubator. The susceptibility of the organisms to the plant extracts was carried out using the agar diffusion cup plate method [15]. Simply, a sterile cork borer (6 mm) was used to bore holes in the agar plates and the bottoms of the wells sealed with the molten Sabouraud dextrose agar. With the aid of a micropipette, 0.1 ml each of gradient concentrations (mg/ml) of the crude ethyl acetate extracts was dispensed into the holes. Sterile distilled water was used as control. The plates were allowed to stand at room temperature for one hour in order to allow extract diffuse into the agar before incubation at 37°C for 18 hours.
(yeast) and 30°C for 72 hours up to 5 days (dermatophytes and moulds). The zones of inhibition produced by the extract on the test organisms were measured using a well-calibrated meter ruler to the nearest millimeter. The experiment was carried out in triplicates.

2.5.5 Determination of minimum inhibitory concentration (MIC) of the extract and fractions

The minimum inhibitory concentration (MIC) of the extract and fractions was tested using the serial broth micro dilution method [16]. Round bottom 96-well microtitre plates were used for the assay. Fifty microliters of the working solutions of extracts and fractions was added into the wells in row 1 and 2 of each column. The last eleven wells from rows 2 to 12 were filled with 50μl of sterilized Saboraud dextrose broth and an identical two-fold serial dilution were made from wells in rows 2 to the rows 11. The last wells in row 12 served as drug-free controls. Sterile SDB was used as negative control while ketoconazole and fluconazole served as positive controls. Lastly, 50μl of standardized fungal inoculum (10⁶ cfuml⁻¹) were added in all the wells from column A to H and mixed thoroughly to give final concentrations. Tests were done in triplicates. The innoculated microplates were sealed with parafilm and the plates incubated at 37°C for 18 hours (yeast) and 30°C for 48 hours and up to 7 days (dermatophytes and moulds). MIC was defined as the first well with no visible growth after 24 hours.

2.5.6 Determination of minimum fungicidal concentration (MFC) of the extract and fractions

The in vitro minimum fungicidal concentration was done by transferring fifty microfighters from the wells without any visible growth after MIC determination into fresh wells containing Saboraud dextrose broth and incubated at 37°C for 18 hours (yeast) and 30°C for 48 hours and up to 7 days (dermatophytes and moulds). The lowest concentration resulting in no growth on subculture was taken as the minimum fungicidal concentration [17].

2.6 Statistical Analysis

The results obtained from the experiment were expressed as mean±standard deviation. One way ANOVA (Smith’s Statistical Package version 2.80) at p=0.05 was adopted in the analysis of the findings.

3. RESULTS AND DISCUSSION

A total of 30 fractions were collected from the elution of the ethyl acetate extract. Identical fractions were combined based on thin layer chromatographic values giving 6 fractions M1 – M6 (Table 1). The result of preliminary antifungal activities showed that the ethyl acetate extract inhibited the growth of the test fungi with varying degree of inhibition which increased with increase in the concentration of the agent (Table 2). The results of the minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) is shown in Tables 3 and 4 respectively. The effect of ethyl acetate extract and fractions was strongest on T. verrucosum. Trichophyton verrucosum a zoophilic dermatophyte is known to be the predominant causative agent of cattle ringworm infection. It is also known to affect (with lower prevalence), sheep, goats and other ruminants. Trichophyton verrucosum has also been implicated in, several human outbreaks by direct contact with infected animals or indirect contact with infectious propagules in the environment [18].

Table 1. Combination of ethyl acetate fractions of M. villosus

| Sample | Fraction combination |
|--------|---------------------|
| M1     | 1-6                 |
| M2     | 7 – 9               |
| M3     | 10 – 12             |
| M4     | 13 – 14             |
| M5     | 15 – 25             |
| M6     | 26 – 30             |

Plants contain bioactive constituents as protective substances against bacteria, fungi, viruses and pests [19]. The mode of action of ethyl acetate extract could be related to their ability to alter membrane properties leading to cell death [20]. All the test fungi were found to be susceptible to ethyl acetate extracts and fractions of M. villosus (Table 2), which is in line with the works done in the past [21] which reported the potent antifungal activity of M. villosus. However, there were differences in the inhibition of growth among the compounds against different strains of fungi. The ethyl acetate extract and fractions were shown to be fungistatic at lower concentrations and fungicidal at higher concentrations. The inhibitory action of M. villosus against Candida spp. has been linked to the presence of acetophenone derivatives [9].
Table 2. Antifungal susceptibility testing of the ethyl acetate extract of *M. villosus*

| Organisms | 200 mg/ml | 100 mg/ml | 50 mg/ml | 25 mg/ml | 12.50 mg/ml |
|-----------|-----------|-----------|----------|----------|-------------|
| Ca1       | 27.67±0.33 | 26.67±0.33 | 22.67±0.33 | 22.00±0.00 | 21.67±0.33 |
| Ca2       | 28.67±0.33 | 28.00±0.00 | 25.00±0.00 | 24.00±0.00 | 23.67±0.33 |
| Ca3       | 31.33±0.33 | 31.00±0.00 | 27.00±0.00 | 24.67±0.33 | 24.00±0.00 |
| Ca4       | 31.00±0.00 | 30.67±0.33 | 27.00±1.00 | 24.67±0.33 | 23.67±0.33 |
| CK        | 28.67±0.33 | 26.67±0.33 | 26.67±0.33 | 21.67±0.33 | 21.00±0.00 |
| AF        | 24.33±0.33 | 22.00±0.00 | 20.67±0.33 | 19.67±0.33 | 19.67±0.33 |
| AN        | 25.67±0.33 | 24.67±0.33 | 21.67±0.33 | 20.67±0.33 | 20.67±0.33 |
| TM        | 27.67±0.33 | 26.67±0.33 | 24.67±0.33 | 23.00±0.00 | 22.00±0.00 |
| TV        | 28.00±0.00 | 26.67±0.33 | 25.00±0.00 | 24.00±0.00 | 22.67±0.33 |

Values are mean inhibition zone (mm) ± SD of three replicates; values with different superscripts on the same row are significantly different (P=0.05). Ca, Candida albicans; CK, Candida krusei; AN, Aspergillus niger; AF, Aspergillus fumigatus; TM, Trichophyton mentagrophytes; TV, Trichophyton verrucosum

Table 3. Minimum inhibitory concentration of ethyl acetate extract and fractions of *Mitracarpus villosus*

| ORG   | Minimum inhibitory concentration (µg/mL) |
|-------|-----------------------------------------|
|       | ETA | M1 | M2 | M3 | M4 | M5 | M6 | FCZ | KCZ |
| Ca1   | 1000.00±0.33 | 2000.00±0.57 | 1000.00±0.00 | 1000.00±0.00 | 500.00±0.57 | 2000.00±0.00 | 2000.00±0.00 | 25.00±0.00 | NA   |
| Ca2   | 1000.00±0.57 | 2000.00±0.00 | 1000.00±0.00 | 1000.00±0.00 | 2000.00±0.33 | 500.00±0.00 | 2000.00±0.57 | 25.00±0.33 | NA   |
| Ca3   | 500.00±0.00 | 1000.00±0.00 | 1000.00±0.33 | 2000.00±0.00 | 2000.00±0.00 | 250.00±0.00 | 1000.00±0.00 | 25.00±0.00 | NA   |
| Ca4   | 500.00±0.00 | 2000.00±0.00 | 500.00±0.00 | 500.00±0.33 | 2000.00±0.00 | 500.00±0.00 | 500.00±0.00 | 25.00±0.33 | NA   |
| CK    | 2000.00±0.33 | 4000.00±0.33 | 500.00±0.00 | 500.00±0.00 | 1000.00±0.33 | 2000.00±0.00 | 2000.00±0.00 | 25.00±0.33 | NA   |
| AF    | 2000.00±0.00 | 2000.00±0.00 | 2000.00±0.33 | 2000.00±0.00 | 4000.00±0.00 | 4000.00±0.00 | 4000.00±0.00 | 25.00±0.33 | NA   |
| AN    | 1000.00±0.57 | 2000.00±0.33 | 500.00±0.00 | 1000.00±0.00 | 2000.00±0.00 | 4000.00±0.00 | 4000.00±0.00 | 25.00±0.57 | NA   |
| TM    | 2000.00±0.00 | 1000.00±0.00 | 1000.00±0.00 | 1000.00±0.00 | 1000.00±0.00 | 500.00±0.00 | 500.00±0.00 | 8.00±0.33 | NA   |
| TV    | 500.00±0.33 | 250.00±0.00 | 250.00±0.00 | 250.00±0.00 | 250.00±0.00 | 500.00±0.00 | 500.00±0.00 | 4.00±0.00 | NA   |

Values are mean inhibition zone (mm) ± SD of three replicates; values with different superscripts on the same row are significantly different (P=0.05)

FCZ, Fluconazole; KTZ, Ketoconazole; NA, Not applicable; ORG, organisms; Ca, Candida albicans; CK, Candida krusei; AN, Aspergillus niger; AF, Aspergillus fumigatus; TM, Trichophyton mentagrophytes; TV, Trichophyton verrucosum
Table 4. Minimum fungicidal concentration of ethyl acetate extract, secondary metabolites and fractions of *Mitracarpus villosus*

| ORG | ETA     | M1          | M2          | M3          | M4          | M5          | M6          | FCZ       | KCZ       |
|-----|---------|-------------|-------------|-------------|-------------|-------------|-------------|-----------|-----------|
| Ca1 | 4000.00±0.33<sup>a</sup> | 8000.00±0.00<sup>a</sup> | 2000.00±0.33<sup>a</sup> | 4000.00±0.33<sup>a</sup> | 2000.00±0.00<sup>a</sup> | 4000.00±0.57<sup>a</sup> | 4000.00±0.00<sup>a</sup> | 25.00±0.33<sup>a</sup> | NA        |
| Ca2 | 4000.00±0.00<sup>b</sup> | 4000.00±0.33<sup>b</sup> | 500.00±0.00<sup>b</sup>  | 4000.00±0.00<sup>b</sup> | 4000.00±0.57<sup>a</sup> | 4000.00±0.33<sup>b</sup> | 8000.00±0.33<sup>b</sup> | 25.00±0.00<sup>a</sup> | NA        |
| Ca3 | 4000.00±0.33<sup>c</sup> | 8000.00±0.00<sup>c</sup> | 4000.00±0.00<sup>c</sup> | 4000.00±0.00<sup>c</sup> | 4000.00±0.00<sup>c</sup> | 1000.00±0.00<sup>c</sup> | 4000.00±0.00<sup>c</sup> | 25.00±0.57<sup>a</sup> | NA        |
| Ca4 | 4000.00±0.00<sup>d</sup> | 8000.00±0.00<sup>d</sup> | 2000.00±0.33<sup>d</sup> | 4000.00±0.57<sup>d</sup> | 8000.00±0.33<sup>d</sup> | 8000.00±0.57<sup>d</sup> | 16000.00±0.00<sup>d</sup> | 25.00±0.00<sup>d</sup> | NA        |
| Ck  | 8000.00±0.37<sup>e</sup> | 8000.00±0.37<sup>e</sup> | 1000.00±0.00<sup>e</sup> | 4000.00±0.00<sup>e</sup> | 8000.00±0.00<sup>e</sup> | 8000.00±0.00<sup>e</sup> | 4000.00±0.57<sup>e</sup> | 2000.00±0.00<sup>e</sup> | NA        |
| AF  | 4000.00±0.33<sup>f</sup> | 4000.00±0.33<sup>f</sup> | 4000.00±0.33<sup>f</sup> | 4000.00±0.00<sup>f</sup> | 8000.00±0.33<sup>g</sup> | 8000.00±0.57<sup>f</sup> | 8000.00±0.00<sup>f</sup> | NA        | 50.00±0.33<sup>f</sup> |
| AN  | 4000.00±0.00<sup>g</sup> | 4000.00±0.33<sup>g</sup> | 1000.00±0.00<sup>g</sup> | 2000.00±0.57<sup>g</sup> | 4000.00±0.33<sup>g</sup> | 8000.00±0.00<sup>g</sup> | 8000.00±0.00<sup>g</sup> | NA        | 50.00±0.33<sup>g</sup> |
| TM  | 4000.00±0.33<sup>h</sup> | 2000.00±0.00<sup>h</sup> | 2000.00±0.57<sup>h</sup> | 2000.00±0.00<sup>h</sup> | 2000.00±0.33<sup>h</sup> | 1000.00±0.00<sup>h</sup> | 1000.00±0.33<sup>h</sup> | NA        | 16.00±0.33<sup>h</sup> |
| TV  | 2000.00±0.00<sup>i</sup> | 500.00±0.00<sup>i</sup>  | 500.00±0.00<sup>i</sup>  | 500.00±0.00<sup>i</sup>  | 500.00±0.00<sup>i</sup>  | 500.00±0.00<sup>i</sup>  | 1000.00±0.00<sup>i</sup> | 1000.00±0.33<sup>i</sup> | NA        |

Values are mean inhibition zone (mm)±SD of three replicates.

Values with different superscripts on the same row are significantly different (P=0.05).

FCZ, Fluconazole; KTZ, Ketoconazole; NA, Not applicable; ORG, organisms; Ca, Candida albicans; CK, Candida krusei; AN, Aspergillus niger; AF, Aspergillus fumigatus; TM, Trichophyton mentagrophytes; TV, Trichophyton verrucosum.
The alarming increase in the rate of candidiasis infection which has been worsened by the high infection rate of HIV/AIDS, necessitate research into plants with great promise for development into phytomedicine for the treatment of candidiasis in the near future. Recently *Aspergillus fumigatus* have been tagged as the most frequent causative agent of invasive fungal infection in immunocompromised people especially, those receiving immunosuppressive therapy for autoimmune or neoplastic disease, organ transplant recipients, and AIDS patients [22]. With the strong antifungal activity shown by the fractions *M. villosus* against this organism, this plant shows promising characteristics for the use in the treatment and management of invasive fungal infections. Generally, the anti fungal activity of the ethyl acetate extract and fractions of *M. villosus* was strong and comparable to that of ketoconazole and fluconazole, which has been linked to the combined action of the secondary metabolites present in the extract [20].

4. CONCLUSION

The ethyl acetate extract and fractions of *M. villosus* possess antifungal activity against a broad spectrum of fungi ranging from yeast to dermatophytes. This medicinal plant holds great promise for the development of novel antifungal drug.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Borris RP. Natural products research: Perspectives from a major pharmaceutical company. J. Ethnopharmacol. 1996;51:29–38.
2. Balick MJ, Arvigo R, Romero L. The development of an ethnobiomedical forest reserve in Belize: Its role in the preservation of biological and cultural diversity. Conserva. Bio. 1994;8:316-317.
3. Cowan MM. Plant products as antimicrobial agents. Clin. Micro. Rev. 1999;12(4):564-82.
4. Abad MJ, Ansuategui M, Bermejo P. Active antifungal substances from natural sources. Arch. for Org. Chem. 2007;7:116-145.
5. Akansha J, Shubham J, Swati R. Emerging fungal infections among children: A review on its clinical manifestations, diagnosis, and prevention. J. Pharm Bioalli. Sci. 2010;2(4):314–320.
6. 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. Glo Adv. Res. J. Microbio. 2012;1(7):120-125.
7. Dalziel JM. The useful plants of west tropical Africa. 3rd ed. Crown agents for Oversea Government and Administration. Millbank, London.1956;455.
8. Bisignano G, Sanogo R, Marino A, Aquino R, Angelo VD, German-Ograve R, et al. Antimicrobial activity of *Mitracarpus scaber* extract and isolated constituents. Let. of Appl. Microbio. 2000;30(2):105-108.
9. Abere TA, Onwukaeme DN, Eboka CJ. Pharmacognostic evaluation of the leaves of *Mitracarpus scaber* Zucc (Rubiaceae). Trop. J. Pharm. I Res. 2007;6(4):849-851.
10. Aboh MI, Olayinka BO, Adeshina GO, Oladosu P. Antifungal Activities of Phyto Compounds from *Mitracarpus villosus* (Sw.) DC from Abuja, Nigeria. J. Microbio. Res. 2014, 4(2): 86-91.
11. Adeshina OG, Onaolapo JA, Ehinmidu JO, Odama LE. Phytochemical and antimicrobial studies of the ethyl acetate extract of *Alchornea cordifolia* leaf found in Abuja, Nigeria Journal of Med. Plants Res. 2010;4(8):649-658.
12. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts: Approved standard M27-A2. Wayne (PA): CLSI; 2002.
13. Olowosulu AK, Ibrahim YKE, Bhatia PG. Studies on the antimicrobial properties of formulated creams and ointments containing *Baphia nitida* heartwood extract. J. Pharm. and Biore. 2005;2(2);124-130.
14. Aberkane A, Cuenca-Estrella M, Gomez-Lopez A, Petrikkou E, Mellado E, Monzon A, et al. Comparative evaluation of two different methods of inoculum preparation.
for antifungal susceptibility testing of filamentous fungi. J. Antimicro. Chem. 2002;50:19–22.

15. Shanmugapriya P, Suthagar P, Lee C, Roziahanim M, Surash R. Determination of minimum inhibitory concentration of Euphorbia hirta (L.) extracts by tetrazolium microplate assay. J. Nat. Pro. 2012;5:68-76.

16. Hafidh R, Ahmed S, Abdulamir L, Se-Vern F, Abu B, Faridah A, et al. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. The Open Microbio. J. 2011;5:96-106.

17. Nasrin A, Eskandar M, Abdolghani A. Characterization of an anti-dermatophyte cream from Zataria multiflora Boiss. Iran. J. Pharm. Sci. 2007;3(2):77-84.

18. Marjorie MC. Plant products as antimicrobial agents. Clin. Microbio. Rev. 1999;12(4):564-582.

19. Aqil H, Al-Charchafchi F, Ghazzawi D. Biochemical, antibacterial and antifungal activity of extracts from Achillea fragrantissima and evaluation of volatile oil composition. Der Pharm. Sinica. 2012;3(3):349-356.

20. Irobi ON, Daramola SO. Bactericidal properties of crude extracts of Mitracarpus villosus. J. Ethnopharm. 1994;42(1):39-43.

21. Aboh MI, Olayinka BO, Adesina GO, Ibrahim K. Preliminary studies on the antifungal activities of the successive extracts of Mitracarpus villosus (Sw.) Dc aerial parts obtained in Abuja, Nigeria. Mal. J. of Microbio. 2014;10(2):133-138.

22. Ben-Ami R, Lewis RE, Kontoyiannis DP. Enemy of the (immunosuppressed) state: An update on the pathogenesis of Aspergillus fumigatus infection. Bri. J. Haem. 2010;150(4):406–417.