



INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY
Antioxidant Activity and In Vitro Inhibition Property of *Mitrocarpus hirtus* Leaf Extracts Against *Tinea capitis* (Scalp Ringworm).

*H.D. Kabiru, K.B. Ahmad, O.S. Idris and S.R. Ahuoiza
 Department of Chemistry, Federal University Lokoja, Kogi State.

*Corresponding Author: hassan.kabiru@fulokoja.edu.ng

Received on: October, 2022

Revised and Accepted on: November, 2022

Published on: December, 2022

ABSTRACT

Tinea capitis or scalp ringworm is a fungal skin disorder commonly found in children below 12-year-old. The management of ringworm by mean of synthetic drugs continue to face setback as result of antibiotic resistance being exhibited by the disease-causing fungi. *Mitrocarpus hirtus* (L) is a weed plant found on gardens, farms and fields with wide geographical distributions. Pharmacological studies of the plant show its potent antifungal potentials as it is used traditionally for the treatment of skin diseases. The aim of this preliminary study is to examine the antioxidant activities and inhibitory properties of various solvent extracts of *M. hirtus* leaves against *Tinea capitis* by standard procedures. The in vitro antioxidant activities show that all extracts possess moderate radical scavenging properties with IC₅₀ values of 159.12±0.48µg/mL, 832.48±64.53µg/mL, 547.68±12.3553µg/mL and 168.41±5.0 µg/mL for Aqueous Residue, n-hexane, chloroform and ethylacetate extracts respectively. Considerable inhibitory effect on the growth of *Tinea capitis* was observed from the zone of inhibitions of the extracts with ethylacetate fraction exhibiting highest inhibition as the concentration increases. This study shows the potential of *M. hirtus* to inhibit the growth of *tinea capitis*. An extensive research is recommended in order to isolate the compounds responsible for the observed activities of the plant.

Keywords: *Tenia capitis*, *Mitrocarpus hirtus*, Antioxidants, Dermatophytosis

1.0 INTRODUCTION

Skin diseases are common health problems that affect a vast number of people around the world. They are thought to be caused by a variety of pathogens, the most common of which being bacterial and fungal species. *Clostridium perfringens*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes* are among the bacteria that commonly cause skin infections, while *Trichophyton rubrum*, *Candida albicans*, *Trichophyton tonsurans*, *Candida krusei*, and *Candida glabrata* are examples of fungus often and clinically related with similar effect (Asong *et al.*, 2019).

Dermatophytosis is a disease of global significant caused by pathogenic fungi known as *dermatophytes*, which affects both animals and humans. These fungi are classified into three genera: namely *Trichophyton*, *Microsporum*, and *Epidermophyton* (Begum *et al.*, 2020).

Dermatophytes are the most common cause of skin infections, which are becoming more common in developing countries. Clinical presentations of these microorganisms include *Tinea pedis*, *Tinea corporis*, *Tinea cruris*, *Tinea capitis* and other pathologic clinical symptoms (Mysore *et al.*, 2019). *Dermatophytosis* is considered a highly contagious human infectious disease, it is generally chronic in nature and poses irreversible damage to the skin, hair, and nails if not properly treated (Begum *et al.*, 2020).

Tinea capitis (scalp ringworm) is one of the most common dermatophyte infections caused by various species of *Trichophyton* and *Microsporum*. While the causative agents of *tinea capitis* vary by region, *Trichophyton schoenleinii*, *Trichophyton rubrum*, *Microsporum audouinii*, *Trichophyton mentagrophytes*, and *Trichophyton tonsurans* are the most common species proven to be the major causes of the disease in Nigeria (Dogo *et al.*, 2016). *c* (Ali *et al.*, 2007).

With approximately one billion cases of dermatophytes related diseases worldwide, the management of *tinea capitis* is facing serious setback due to drug resistance exhibit by fungi towards the existing antifungal drugs (Marquez & Quave, 2020). In an effort to combat the threat of various forms of multi-drug resistance, medicinal plants are being studied in order to obtain bioactive constituents to verify and investigate for novel antimicrobial potentials (Mulat et al., 2019).

Mitracarpus hirtus (L) is a weed plant belonging to the *Rubiaceae* family and is grown widely on gardens, farms and fields. It is commonly distributed in tropical and subtropical regions of America, Asia and Africa (B/kudu et al., 2018). In Nigeria, the plant is used traditionally to treat skin diseases such as ringworm, rashes, itch and eczema (Suleiman, 2015). Pharmacological studies of *M. hirtus* and other members of the family such as *M. scaber*, *M. villosus*, and *M. frigidus* revealed their potential as Antifungal, Anti-diabetic, Anti-diarrheal, Anticancer and Anti-inflammatory agents (Ekalu, 2021). Phytochemical screening of various parts of the plant indicated the presence of tannins, alkaloids, steroids, flavonoids, saponins, terpenoids and cardiac glycosides (B/kudu et al., 2018).

However, scientific information about efficacy of *M. hirtus* against *tinea capitis* has not been documented to the best of our knowledge. The purpose of this study was to investigate the antioxidant and antifungal activity of *Mitracarpus hirtus* (L) against *tinea capitis* (scalp ringworm).

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Fresh *M. hirtus* plant was collected from an outskirt of Lokoja and was taken to the Herbarium Unit of Department of Botany, Federal University Lokoja where it was identified and authenticated.

2.2 Sample Preparation

The aerial part of *Mitracarpus hirtus* was carefully separated from the main plant and dried under shed for two weeks. The dried sample was pulverized using laboratory mortar and pestle into a fine

powder. The powdered sample was kept in an air tight container until needed for analysis.

2.3 Extraction

500 g powdered sample of *M. hirtus* was macerated using 2000mL of 70% methanol for seven days at room temperature with occasional agitation. The sample was filtered using Whatman filter paper No.1 to obtain crude extract. The crude extract was concentrated in a dry incubator oven at 500°C and the dried extract was weighed and stored.

2.4 Fractionation

The hydro methanol crude extract of *M. hirtus* was suspended in water and fractionated using liquid-liquid extraction with chloroform, n-hexane and ethyl acetate to obtain chloroform (MCL), n-hexane (MNH) and ethyl acetate (MEA) fractions as well as aqueous residue (MHA).

2.5 Determination of Antioxidant Properties of *M. hirtus*

DPPH radical scavenging activity of the various fractions of *M. hirtus* was determined following the procedure of Oyedemi *et al* (2010) with minor modification. 0.135 mM of DPPH was prepared in methanol to obtain a stock solution. The plant extracts were prepared by dissolving 0.025-0.5 mg of each extract in 1.0 ml of methanol. Standard antioxidant drug (ascorbic acid) was prepared the same way as the extracts. 1.0 ml of the DPPH solution was mixed with 1.0 ml of each of extracts and standard ascorbic acid. The reaction mixtures were vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixtures was measured at 517 nm using UV spectrophotometer. The DPPH scavenging activity of the plant extracts was calculated according to the equation:

$$\text{DPPH radical scavenging activity} = \{(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})\} \times 100$$

Where Abs control is absorbance of the control and Abs sample is absorbance of the sample.

2.6 Disease Sample Collection and Examination

Disease sample was collected from volunteer patients with ringworm at Adankolo, Lokoja, Kogi State. Scrapings from the infected areas were collected in sterilized plastic bags and taken to

microbiology laboratory of the Department of Microbiology, Federal University Lokoja for further examination and analysis.

2.7 Preparation of *T. capitatus* Culture

After critical examination and screening to remove scales and broken hair. The samples were placed on Sabouraud slants dextrose agar for fungal growth and the cultures were kept at a temperature of 28°C in a B.O.D incubator. Biochemical tests (e.g. catalase test and urease test) were conducted on the culture after 7 days to confirm the presence of *tine capitatus*.

2.8 Determination of Antifungal Properties of *M. hirtus*

Agar well diffusion method was used to determine the antifungal activities of the extracts of *M. hirtus* as described by Gonelimali et al., (2018) with minor modification. Sterile sabouraud dextrose agar media was poured into sterile Petri dish and then inoculated with the test organism and mixed well. After solidification, a standard cork borer of 6 mm diameter was used to cut a well into agar plates containing inoculums. Thereafter, the

solution of known concentration of the various extracts of the plant were added to the respective wells. The plates were then incubated at 37°C for 24 hours after which the plates were observed for zones of inhibition and the zones were measured after the incubation period and result recorded in millimeter.

3.0 RESULTS AND DISCUSSION

Antioxidant activity of *M. hirtus* was determined using DPPH in vitro assay and the result was presented in fig 1. The result obtained shows the relationship between the increasing of DPPH radical scavenging activities and concentration of various extracts of *M. hirtus*. In vitro DPPH assay has been used extensively to test for the radical scavenging activity of plant extracts. DPPH[•] is a deep violet radical specie with maximum absorption of 517 nm. When antioxidants in the extracts react with DPPH they release electron or hydrogen atom thereby reducing the radical to light yellow, colourless or bleached product, resulting in decrease in absorption (Adesanoye & Farombi, 2014).

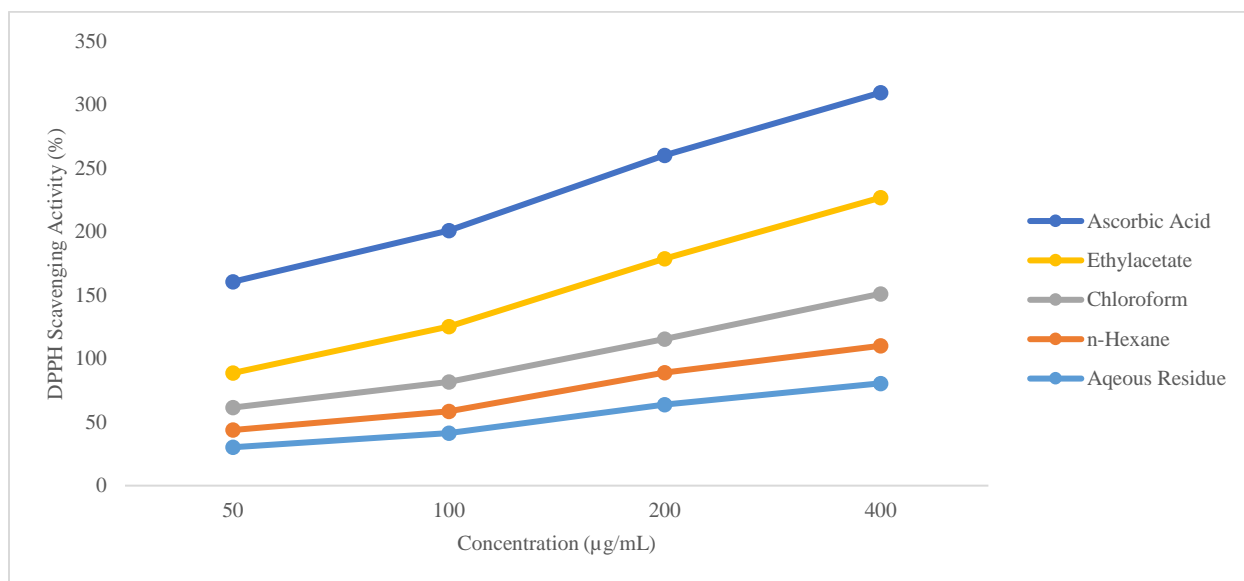


Fig 1: DPPH Scavenging Activity of *M. hirtus* Extracts

Table 1: IC₅₀ Values of *M. hirtus* Extracts and Standard Ascorbic Acid

Extract	IC ₅₀ (µg/mL)
Aqueous Residue	159.12±0.48
n-hexane	832.48±64.53
Chloroform	547.68±12.35
Ethylacetate	168.41±5.0
Std. Ascorbic Acid	-779.99±64.6

Values are expressed as Mean ± S.D from triplicate readings

The list of IC₅₀ values of various extracts of *M. hirtus* was presented in table 2. IC₅₀ is the concentration of extracts that causes 50% inhibition of DPPH, with the low IC₅₀ value indicating strong ability of the extract to act as DPPH scavenger (Merghem & Dahamna, 2020). From the result, the standard ascorbic acid has lowest IC₅₀ value of -779.99±64.6µg/mL and n-hexane extract with highest value of 832.48±64.53µg/mL. The moderate values of 159.12±0.48µg/mL, 168.41±5.0µg/mL and

547.68±12.35µg/mL were observed in aqueous residue, ethylacetate and chloroform extracts respectively.

According to Jadid et al. (2019), extract with IC₅₀ values of 10-50µg/mL, 50-100µg/ml and >100µg/mL are considered to exhibit strong, moderate and weak antioxidant activities respectively. The result of this work implies the poor antioxidant activity of the extracts compared to that of the standard ascorbic acid.

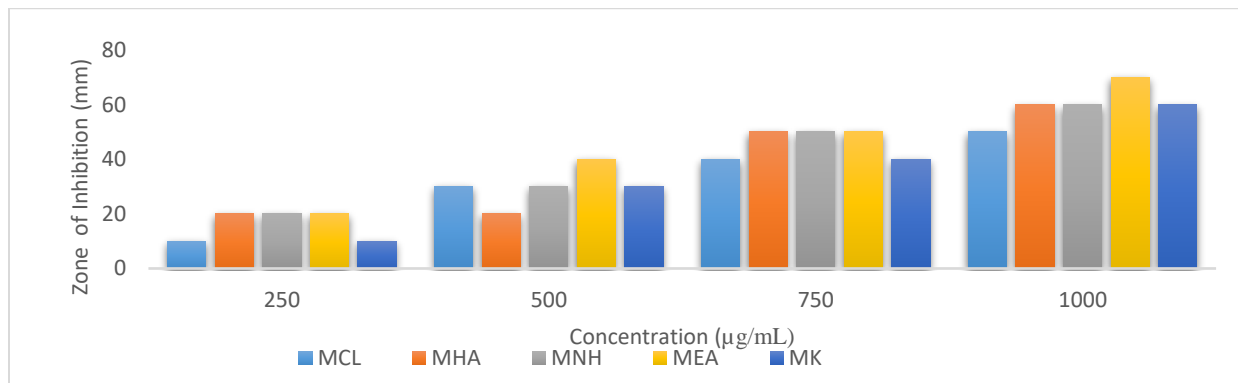


Fig 2: Inhibitory Activities of *Mitrocarpus hirtus* Leaf Extracts Against *Tinea capitis*

The in vitro inhibitory activities of the various solvent extracts of *M. hirtus* are shown in figure 2. The result indicates degree of inhibitory effect of different concentrations of *M. hirtus* extracts on the growth of *T. capitis*. Moderate activity of the extracts was obtained at low concentration while higher activity similar to that of the standard antifungal activity was observed at higher concentration. Salhi et al. (2017) describe the antifungal effects of plant extracts to be attributed to the various phytochemicals presence in the plants. B/kudu et al. (2018) reported good antifungal activities of the ethanol extracts of the aerial part of *M. hirtus* with MIC of 70 µg/ml, 60

µg/ml, and 50 µg/ml against common dermatophytes *Microsporum* sp, *Trichophyton* sp, and *E. floccosum*. Preliminary investigation on *M. hirtus* by Idris et al., (2015) show the plant is a good source of phytochemicals such as tannins, alkaloids, steroids, flavonoids, saponins, terpenoids, phlobatannins and cardiac glycosides and these may be responsible for the observed antifungal activities of the extracts against *T. capitis* (Salhi et al., 2017).

4.0 CONCLUSION

This study has shown the various extracts of arial parts the *M. hirtus* to exhibit good antioxidant and

antifungal activities against *T. capitis*. The results obtained from our in vitro studies can serve as a lead for more comprehensive ex vivo experiments in order to develop a clinically effective drugs from this plant for the management of ringworm and other skin related disorders. *M. hirtus* is a very common and widely growing plant which has been used traditionally by locals in the treatment of skin diseases such as eczema. Extensive research on this plant in the field of dermatology is highly recommended to effectively explore its potential applications as an alternative management of skin related diseases.

REFERENCES

- Adesanoye, O. A., & Farombi, E. O. (2014). *In Vitro Antioxidant Properties of Methanolic Leaf Extract of Vernonia Amygdalina Del.* 29(December), 91–101.
- Ali, S., Graham, T. A. D., & Forgie, S. E. D. (2007). The assessment and management of tinea capitis in children. *Pediatric Emergency Care*, 23(9), 662–668.
<https://doi.org/10.1097/PEC.0b013e31814efe06>
- Asong, J. A., Amoo, S. O., McGaw, L. J., Nkadameng, S. M., Aremu, A. O., & Otang-Mbeng, W. (2019). Antimicrobial Activity, Antioxidant Potential, Cytotoxicity and Phytochemical Profiling of Four Plants Locally Used against Skin Diseases. *Plants 2019, Vol. 8, Page 350*, 8(9), 350.
<https://doi.org/10.3390/PLANTS8090350>
- B/kudu, A. A., Odda, J., Aliero, A. A., & Oloro, J. (2018). Evaluation of Antifungal Activity of Ethanolic Crude extract of *M. Hirtus* Plant against Dermatophytes. *Galore International Journal of Health Sciences and Research*, 3(1), 18–23.
- Begum, J., Mir, N. A., Lingaraju, M. C., Buyamayum, B., & Dev, K. (2020). Recent advances in the diagnosis of dermatophytosis. *Journal of Basic Microbiology*, 60(4), 293–303.
<https://doi.org/10.1002/JOBM.201900675>
- Dogo, J., Afegbua, S. L., & Dung, E. C. (2016). Prevalence of Tinea Capitis among School Children in Nok Community of Kaduna State, Nigeria. *Journal of Pathogens*, 2016, 1–6.
<https://doi.org/10.1155/2016/9601717>
- Ekalu, A. (2021). Medicinal uses, phytochemistry, and pharmacological activities of *Mitracarpus* species (Rubiaceae): A review. *Scientific African*, 11.
<https://doi.org/10.1016/j.sciaf.2020.e00692>
- Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology*, 9(JUL), 1639.
<https://doi.org/10.3389/FMICB.2018.01639/BIBTEX>
- Idris, M., Nkafamiya, I., Akinterinwa, A., & Japari, J. (2015). Preliminary Studies on Some Medicinal Plants in Girei, Adamawa State of Nigeria. *British Journal of Pharmaceutical Research*, 6(3), 203–213.
<https://doi.org/10.9734/bjpr/2015/16205>
- Imanirampa, L., & Alele, P. E. (2016). *Antifungal activity of Cleome gynandra L. aerial parts for topical treatment of Tinea capitis: an in vitro evaluation.* <https://doi.org/10.1186/s12906-016-1187-9>
- Jadid, N., Hidayati, D., Hartanti, S. R., & Arasyi, B. (2019). *Antioxidant activities of different solvent extracts of Piper retrofractum Vahl . using DPPH assay* Antioxidant Activities of Different Solvent Extracts of *Piper retrofractum Vahl . using DPPH Assay.* 020019(June 2017).
<https://doi.org/10.1063/1.4985410>
- Marquez, L., & Quave, C. L. (2020). Prevalence and therapeutic challenges of fungal drug resistance: role for plants in drug discovery. *Antibiotics*, 9(4), 1–9.
<https://doi.org/10.3390/antibiotics9040150>
- Merghem, M., & Dahamna, S. (2020). In-Vitro Antioxidant Activity and Total Phenolic Content of *Ruta montana L.* Extracts. *Journal of Drug Delivery and Therapeutics*, 10(2), 69–75.
<https://doi.org/10.22270/jddt.v10i2.3919>
- Mulat, M., Pandita, A., & Khan, F. (2019). Medicinal Plant Compounds for Combating the Multi-drug Resistant Pathogenic Bacteria: A Review. *Current Pharmaceutical Biotechnology*, 20(3), 183–196.
<https://doi.org/10.2174/1872210513666190308133429>
- Mysore, V., Parthasaradhi, A., Kharkar, R., Ghoshal, A., Ganjoo, A., Ravichandran, G., Saraswat, A., Shah, Y., Singh, M., Remadevi, T., & Matte, P. (2019). Expert consensus on the

- management of androgenetic alopecia in India. *International Journal of Trichology*, 11(3), 101–106. https://doi.org/10.4103/ijt.ijt_24_19
- Salhi, N., Mohammed Saghir, S. A., Terzi, V., Brahmi, I., Ghedairi, N., & Bissati, S. (2017). Antifungal Activity of Aqueous Extracts of Some Dominant Algerian Medicinal Plants. *BioMed Research International*, 2017. <https://doi.org/10.1155/2017/7526291>
- Suleiman. (2015). Efficacy of Leaf Powders of *Senna obtusifolia* (L.) and *Mitracarpus hirtus* (L.) DC. on the Survival of *Sitophilus zeamais* Motschulsky [Coleoptera: Curculionidae] on Sorghum Grains During Storage. *Entomology and Applied Science Letters*, 2(1), 34–38. <http://www.easletters.com/issues.html>