

Evaluation of Antifungal Activity of Ethanolic Crude extract of *M. Hirtus* Plant against Dermatophytes

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ABSTRACT

Introduction: Plants are considered among the important sources of bioactive compound, especially in traditional medicine that has been used for centuries. Although ethno botanical surveys have reported wide use of *Mitracarpus hirtus* in traditional medicine, but there is hardly scientific literature on fungal efficacy regarding to *M. hirtus*.

Objective: the purpose of this study was to determine antifungal activity of *M. hirtus* against dermatophytes.

Methodology: The herb was collected from the bush and dried under normal room temperature, and maceration process was use for the extraction. The Agar well diffusion method was used for Antifungal activity, as well as tube dilution method for the MIC.

Result: The *M. hirtus* whole plant showed antifungal activity with mean values zone of inhibition at different concentration, and wide zone of inhibition was obtain at 1500mg/ml (22mm) for *E. floccosum* (20mm) for *Trichophyton* sp, and (19mm) *Microsporum* sp. MIC of *Microsporum* was obtain at 70µg/ml, 60µg/ml of *Trichophyton* sp, and *E. floccosum* 50µg/ml. The conclusion from this study is that *M. hirtus* plant extract has antifungal activity against *E. floccosum*, *Trichophyton* sp, and *Microsporum* sp almost similar with commercial fungicidal drugs (Terbinafine).

Keywords: *Mitracarpus hirtus*, Dermatophytes.

INTRODUCTION

There are Approximately 1.5 million different species of fungi on Earth, but only 300 cause sickness to the humans. [1]

Candidiasis and Mucomycosis or dermatophytosis are the most frequently the types of fungal affecting the human. [1] Fungi live in the open air, in soil, on plants and trees, as well as on many indoor surfaces and on human skin. Most of fungi are not dangerous, but some species can be harmful to health, particularly when the immune system is weakened, usually by drugs or disorders. [1]

Basically the types of fungal infection can be opportunistic or primary, and can affect many areas of the body (systemic) or only one area (localized). Opportunistic fungal infections usually occur due to the weakened immune system, such as those with Acquired Immunodeficiency syndrome (AIDs). [1] It can be very aggressive, spreading quickly to other organs and often leading to death. Examples include Candidiasis, Mucormycosis or dermatophytosis. However some Primary fungal infections can occur in people with a normal immune system, sometimes with serious consequences. These include Histoplasmosis, Blastomycosis, Coccidioidomycosis, Paracoccidioidomycosis. [2]

Globally the prevalence of dermatomycoses have increased in recent years and yet the modern drugs used against dermatophytosis, have a number of limitation which includes side effects, limited efficacy and increase of drug resistance in human pathogens. [3] These short comings have created a need to search for newer, safer, and more effective fungal

agents either from the plants or synthetically.

Plants containing bioactive compounds which considered the source of modern and traditional medicine. Long-term history indigenous plants have offer suitable raw materials for many industries such as pharmaceutical, cosmetic, perfumery and food. [4] The presence of various life sustaining constituents in plants have stimulate scientists to examine various plants with a view of determining their potential on antimicrobial effects. [5] *Mitracarpus hirtus* L., belongs to the Rubiaceae family, and is commonly distributed throughout gardens, farms and fields in tropical and subtropical regions such as United States of America, India, Malaysia, Myanmar Thailand, west and east African countries. [6] Ethno botanical surveys where show that the wide used of *M. hirtus* for the treatment of fungal infections, skin disease such as rashes, itching, eczema, ringworm, toothache, venereal diseases, by applying the leaf sap, rubbing leaves on skin or taken orally. [6]

However in terms of toxicity, scientific studies, *M. hirtus* plant has LD₅₀ greater than 5000mg/ml and no serious changes were obtain on hematological, biochemical and histopathological parameters on wistar rats. [7] Regarding the antifungal activity there is lack of scientific validation to probe the antifungal effect of *M. hirtus*, and traditionally people have been using this plant on fungal infections. The purpose of this study was to investigate the efficacy of this plant extract against selected dermatophytoses as the scientific assessment of the claim for therapeutic efficacy.

MATERIALS AND METHODS

Plant materials, collection and identification

The plant was collected from the bush in Kanyamabona village area of Ishaka Bushenyi Municipality in Uganda. The collected plant was taken to herbalist as well as Botanist at the Department of Biology &

Science Laboratory Technology at Mbarara University of Sciences and Technology (MUST) Uganda, for identification.

Storage, drying and pulverization

The collected samples were screened, washed, and dried in a shade to avoid direct sunshine that could degrade some of the compounds in the plants. [7] They were displayed on a dry cement floor in an isolated room and changed the position daily to prevent spoil until complete dryness. The dried samples were grinded using the mortar and pestle according procedures described by Paola and collaborators, [8] and fine powder was obtain by sieving. After weighing, the powder was packed in clean labeled bottles and stored a 20°C until use.

Plant Extraction

Extraction was performed by macerating air-dried, powdered *Mitracarpus hirtus* 500g with 10000ml of 70% ethanol at room temperature for 48hr, and was occasionally shaken. [7] In the laboratory, the crude hydroalcoholic extract was filtered using Whatman filter paper No. 1, and concentrated in dry incubator oven (50°C). The dry residue was stored at 4°C, and, at the time of use, was re-suspended in distilled water. [7]

Determination of extract yield (% yield)

According to Paola (8) the yield (% w/w) from all the dried extracts was calculated as:

Yield (%) = (W1 x 100)/W2 where W2 is the weight of the dry extract after getting out the solvent, and W1 is the weight of the plant powder.

Preparation of stock solution

Preparation of the stock solution was done by dissolving four grams of the extract in ten milliliters of distilled water to give a stock solution of concentration 400 mg/ml. [7] This stock solution was prepared at the time of fungal sensitivity test as well as MIC.

To determine the antifungal activity of *M. hirtus* ethanolic crude extract

Test organism and Preparation of inocula

Wild type clinical isolate of *E. floccosum*, *Trichophyton* sp and *Microsporum* sp were obtain from the Kampala International University Microbiology laboratory, and revitalized at 27°C on Potato Dextrose Agar (PDA) (OXOID, Hampshire, England) for 14 days. [9] Spores of revitalized fungus were collected from cultures on agar plates after 7days of incubation as described by Broekaert *et al.*, [10] The sporangial suspension concentration was estimated with regards to the conidium and spores forming fungi, and the micro dilution standardized by Clinical and Laboratory Standards Institute (9) which involves an inoculum of spores adjusted spectrophotometrically to 2.5×10^5 CFU/ml at wave length of 530nm of 0.11 O.D. [11] The fungal spore suspension was stored in 20% glycerol at -4°C to avoid contamination and growth.

Determination of antifungal activity

Antifungal activity of the *M. hirtus* plant extract was determined by Agar well Diffusion method. [12] The PDA medium was prepared according to manufacturer's instructions (39g/L). The test fungal culture was evenly spread over the medium by sterile cotton swabs. Wells (6 mm) were made in the medium using sterile cork borer. A volume of 200µl of extracts was transferred into each well, incubated at 30°C for 48 – 72 hr, and the plates were then observed for the formation of clear zones around the wells indicating the presence of antifungal activity. [13] The zone of

inhibition was measured using caliber (all inhibition minus 6mm of the well) and recorded. Ethanol (70%) and Terbina fine obtained from pharmacy (Manufactured by Amoun Pharmaceutical Co. Labour City, Cairo, Egypt) 200µl each were used as the negative and positive control respectively.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) *M. hirtus* extract was determined using tube dilution method. [14] The fungal spore inoculum of 100µl at 2.5×10^5 dilution was inoculated into test tubes with (1800µl) nutrient broth in eight different test tube, and the plant extract was serially diluted, ranging from 100mg/ml to 800mg/ml. [14] A volume of 100µL of each extract dilution was mixed in each inoculated test tube, incubated at 30°C for 48-72hr, and then examined for visual turbidity. [12] The results of the extracts were compared with a standard, positive control (Terbinafine 10µg/mL).

RESULTS

Table (1) summarizes the activity of ethanolic extract of *M. hirtus* extract, which showed various degrees of inhibitory activity towards tested pathogenic fungi. The results obtained from this study recorded low activity of the extract on *Microsporum* (17mm zone inhibition) at higher concentration (1500mg/ml), as compared with *E. floccosum* with highest zone of inhibition (22mm).

Table 1: Fungi mean zone of inhibition (mm) of *M. hirtus* extract against selected Dermatophytes compared to Terbina fine inhibition.

Test organisms	Concentration of the plant extract (mg/mL)			(µg/mL)	Ethanol(%alcohol
	500	1000	1500	20	96
<i>E. floccosum</i>	5±5.00	20±3.21	22±4.50	20±5.03	0±00
<i>Trichophyton</i> sp	3±3.01	15±1.1	19±3.03	22±33	0±00
<i>Microsporum</i> sp	3±3.00	13±2.3	17±4.0	19±2.9	0±00

Presented as mean ±Standard deviation

The MIC values of *M. hirtus* plant extract showed high to moderate (80–100% inhibition) antimycotic activity against all the test dermatophytes, which varied between 50µg/ml (*E. floccosum*), 60µg/ml

(*Trichophyton*) to 70µg/ml (*Microsporum*); Terbinafine (reference antifungal) gave MIC values between 10 µg/ml and 20µg/ml.

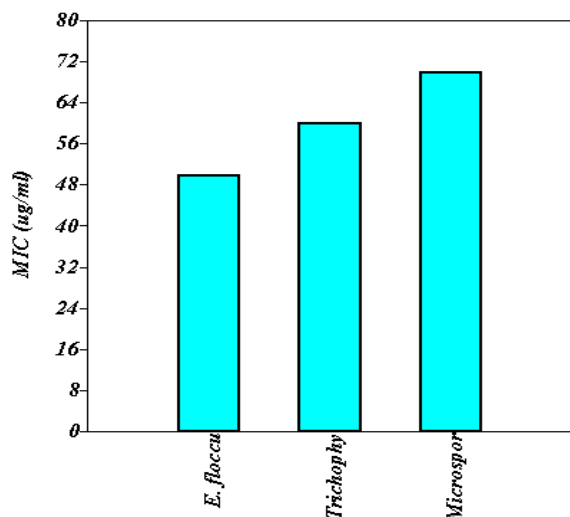


Figure 1: Result of minimum inhibitory concentration (MIC) of *M. hirtus* extract against dermatophytes

DISCUSSION

The traditional use of plants extract as medicines offer the source for which essential plant may be useful for specific infections and non-infectious disease conditions. Traditionally, many plant extracts, such as Eucalyptus, tea tree, clove and viscose, have been used as topical antiseptics, as well as antimicrobial properties due to the present of their bioactive compounds. [15] This provide a base to investigate scientifically those plants and others which have been used in traditional medicines as potential sources of novel antimicrobial compounds. [16] *Mitracarpus hirtus* is a plant species that has numerous bioactive compounds with different pharmacological activity, which may a lot of benefit to human. According to Saad *et al*, [17] they reported that the apprehension of consumers for lack of scientifically valid evidence has favorite to perform studies concerning the efficacy of plant species which are used by the public as natural drugs.

The results in table 1 indicated that the ethanolic crude extract of *M. hirtus* plants revealed antifungal activity against selected dermatophytos organisms (*E. floccosum*, *Trichophyto sp* and *microsporium sp*). The extracts showed various degrees of inhibitory effects towards all the tested pathogenic fungi, with lower

activity on *Microsporium sp* (zone of inhibition 19mm) as compared with other test organisms. The extract indicated no activity at the lower concentration (250mg/ml), but at higher concentrations (1000-1500mg/ml), the plant showed antifungal activity almost similar with commercial fungicide (Terbinafine) used as the positive control (20mm).The activity of this plant extract may be due to the presence of main bioactive substance of alkaloids that have antimicrobial properties. [18]

The results of Minimum inhibitory concentration (MIC) were obtain as 50 μ g/ml, 60 and 70 for *E. floccosum*, *Trichophyton sp* and *Microsporium sp* respectively(Fig. 1), after 8 test tubes containing fungal organisms and the extract were incubated for 72hrs. MICs of less than 100 μ g/ml suggest good antimicrobial activity. [19] This indicated that the plant extract has demosterated good activity against all the selected dermatophytoses.The mechanism of such inhibitory activity of this plant may be occurring due to the fact that the plant extracts contain bioactive substances which cause breakage of cytoplasmic membrane of the fungal cell leading to leakage or damage of the intracellular components, [20] or may be the extracts have interaction with lipid bilayer in fungal cell membranes (outer and inner membrane). or maybe the extract potentiate the effect through water osmotic pressure which cause cell to swell more and lead to death; or may be the extracts has an effect on the protein synthetic path way of the fungal cell leading to inhibition of DNA synthesis, as described by. [21] The results were in correspondance with some previous work done by Khalil, [22] on some plants extract against *Trichophyton* and *Njatena*, [23] on essential oil from the leaves of *Ageratum houstonianum* against Dermatophytos.

Further studies are required to identify the specific chemical of the bioactive compounds in this plant responsible for the observed antifungal activities. There is also a need to study the

in-vivo antifungal activity of this plant using laboratory animals, for reassurance in medical used.

CONCLUSION

The results from this study showed that *M. hirtus* plant extracts exhibit antifungal effects against *E. floccosum*, *Trichophyton* sp and *Microsporum* sp almost similar to commercial fungicide (Terbinafine), used as the positive control. Highest activity of *M. hirtus* extract was recorded on *E. floccosum* and *Trichophyton* sp followed by *Microsporum* sp.

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