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Antimicrobial activity of extract and topical cream formulation of *Mitracapus villosus* (Rubiaceae)

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Abstract
Crushed leaves of *Mitracapus villosus* is used in traditional medicine practice for the treatment of skin diseases and its ethnomedicinal use has been established. This study therefore investigated the antimicrobial activities of the crude extract and the cream formulation of *Mitracapus villosus* aerial parts (leaves, inflorescences and stem) by agar diffusion against four reference bacteria *(Staphylococcus aureus, Proteus vulgaris, Escherichia coli and Klebsiella sp.)* and reference fungi *(Candida albicans)*. The minimum inhibitory concentration (MIC) obtained for the crude extract was 4 % both for *C. albicans* and *Staph. aureus* and 20 % for *Klebsiella sp*. While *E. coli* and *Proteus vulgaris* showed a high degree of resistance to concentrations > 20 %. The MIC for the cream formulations were 5 % for both *C. albicans* and *Staph. aureus*, while *Klebsiella sp* did not show any inhibition even at concentrations > 20 %. The zones of inhibition ranged from 3-22 mm at 4-50 % w/v for the extract (i.e., *C. albicans*, *Staph. aureus* and *Klebsiella sp*) and that for the cream was 3 – 13 mm (i.e *C. albicans* and *Staph. aureus*). However, the other organisms did not display any zone of inhibition at all. The results indicate that *Mitracapus villosus* extract can be formulated into creams for topical application in treating infections caused by susceptible organisms.

Keywords: *Mitracapus villosus*, topical cream, extraction, antimicrobial activity

INTRODUCTION

Medicinal plants are the oldest known health care products. Their importance is still growing although it varies depending on the ethnological, medical and historical background of each Country. Phytomedicines have been formulated for both topical and systemic uses. These include tablets, syrups, creams, lotions and ointments.

Mitracapus villosus (Rubiaceae) is an annual herb found in tropical farmlands. It is widely employed in traditional medicine in West Africa in the treatment of headaches, toothache, amenorrhoea, dyspepsia, hepatic disease and leprosy (Bisignano 2000). The crushed leaves of the plant are used as dressings for fresh cuts, wounds, and ulcers (Gills 1992). In southern Nigeria, the plant is used (crushed leaves) in the treatment of ring worm infection in the head (*Teanea capitis*) and eczema on the face. Several authors have reported on the use of this plant in the treatment of various skin diseases including infectious dermatitis, eczema and scabies (Bisignano 2000, Gills 1992, Moulis et al; 1992 and Sanogo 1996). Moulis 1992 and other workers demonstrated a potent antifungal activity against *Candida albicans*

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and *trichophytum soudanese*. Sanogo (1996) showed that *Mitracapus villosus* exhibited broad antifungal and antibacterial activities against standard strains and clinical bacterial isolates of *Staph aureus* and *Candida albicans* responsible for common skin infections. The leaves and stem are widely used in its locality for the treatment of skin diseases such as eczema hence it is popularly known as eczema leaf. In the Nigerian drug market, topical formulations of herbal products are a rare commodity. This is so because significant effort has not been made by researchers and corporate bodies to promote the use of herbal formulation in our locality. This study therefore attempts to formulate cream of crude extract of *Mitracapus villosus* and evaluate its antimicrobial activity.

EXPERIMENTAL

Preparation of plant material: Aerial parts (i.e., leaves, inflorescence and stem) of *Mitracapus villosus* were collected from fields within the University of Benin. Botanical identification and authentication was done by the Forestry Research Institute of Nigeria (F.R.I.N), Ibadan, Nigeria, and samples were deposited in the same Institute. The plant parts were dried and pulverized. Chemicals used include cetomacrogol and ethanol obtained from BDH Chemicals Ltd, UK. Nutrient agar and Sabouraud dextrose agar (Oxoid Ltd, UK) were employed in this study for the cultivation of bacteria. All the micro-organisms used in this work were collected from the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Extraction procedure: The powdered plant (25 g) was extracted using 200 ml of ethanol by maceration for 48 hours and the extracts were filtered and dried *in vacuo* by a rotary evaporator (BÜCHI R110, Germany).

Formulation of cream. Cetomacrogol emulsifying ointment BP was used (BP, 1980). Cetomacrogol emulsifying wax (300 g) and white soft paraffin (500 g) were melted in a beaker over a hot water bath. Liquid paraffin, 200 g (240 ml) was added. The mixture was stirred and allowed to cool. The cream was formed by mixing 70 ml of the aqueous extract with 30 g of cetomacrogol emulsifying ointment (BP, 1980) at 70 ^{o}C and the mixture was allowed to cool with occasional stirring. The procedure was repeated using different concentrations (0-50 %w/v) of *Mitracapus villosus* aqueous extract.

Antimicrobial activity. The agar diffusion or punch hole method was employed to assess the antimicrobial activity of *Mitracapus villosus* extract and cream. In the procedure, five Petri dishes were filled to 5 mm depth with molten sterile agar and allowed to set. The Petri dishes containing the nutrient agar were each inoculated with the test organisms respectively by flooding the surface of the set agar plates with a suspension of the organisms in the subculture nutrient broth and the excess was discarded. A sterile cork borer was used to punch holes into the agar plates. The bottom of each well was sealed with a drop of molten agar. A sterile syringe was used to introduce 0.2 ml each of samples of plant extract and cream into the wells. The agar plates were left for 30 min in order to allow for diffusion of the test samples into the agar. The plates were then incubated at 37 ^{o}C for 24 h for the bacteria and 7 days for the fungi, and the zones of inhibition were determined. This procedure was repeated using different concentrations (0-50 %w/w) of *Mitracapus villosus* pure extract and cream in order to establish the minimum inhibitory concentrations (MIC).

Table 1: Minimum inhibitory concentration values for the crude extract and cream formulation

Microorganisms	Minimum inhibitory concentrations	
	Crude extract (% w/v)	Cream formulation (% w/v)
Candida albicans	4	5
Staph aureus	4	5
Klebsiella sp.	20	-
E. coli	-	-
Proteus vulgaris	-	-

Note: - represents zero MIC values.

Table 2: Zones of inhibition produced by crude extracts at different concentrations

Conc (%w/v)	Zone of inhibition for the micro-organisms (mm)				
	Candida albicans	*Staph. aureus*	*Kleb. sp.*	*E. coli*	*Proteus vulgaris*
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	13	3	-	-	-
5	14	5	-	-	-
10	18	9	-	-	-
20	19	15	6	-	-
50	22	19	9	-	-

Table 3: Zones of inhibition produced by cream formulations of *Mitracapus villosus*

	Concentrations (% w/v)			
	0	1.25	2.5	5
Microorganisms	Zones of inhibition (mm)			
Candida albicans	-	-	-	13
Staph aureus	-	-	-	3
Klebsiella sp.	-	-	-	-

RESULTS AND DISCUSSION

The ethanolic extract appeared viscous and dark green in colour after drying. It had a peculiar aromatic odour. This extract was soluble in water and dimethyl sulphoxide (DMSO). The results of the minimum inhibitory concentration (MIC) of the extract as well as the cream formulation are shown in Table 1. An organism was considered sensitive only if inhibited by a concentration of the drug that can be achieved at the site of infection. *Candida albicans* and *Staphylococcus aureus* had the least MIC values of 4%w/v for the extract and 5%w/v for the cream, while *Klebsiella spp* had the highest with 20%w/v for the extract only and did not show any MIC for the cream formulation. *Escherichia coli* and *Proteus vulgaris* did not show any MIC value at all. It can be seen that the cream formulation did not significantly reduce the activity of the extract.

The results obtained from the studies on the antimicrobial activity of the crude extract are presented in table 2. The diameter of growth inhibition zone was used to evaluate the sensitivity of the microorganisms to the extract. It can be seen from the table that *Candida albicans* was the most sensitive to the activity of the crude followed by *Staph. aureus* and finally *Klebsiella. Escherichia coli* and *Proteus vulgaris* did not show any sensitivity to the extract irrespective of the concentrations used. Furthermore, increasing the concentrations from 10 to 20%^{w}/v did not give a proportionate increase in antimicrobial activity. This showed that the highest antimicrobial activity was observed with

fungi while bacteria had the least activity. The Gram positive organisms were more susceptible to the extract while the Gram negative organisms were least susceptible. This is probably as a result of the cell wall available in Gram negative organisms which provide extra protection for them. However, most human Gram negative organisms are enterobacterieceae. Gram positive bacteria and fungi are mainly responsible for skin infections. This is similar to the earlier reports from previous studies (Sanogo 1996, Irobi and Daramola 1993 and 1994).

The zones of inhibition produced by cream formulations of *Mitracapus villosus* are shown in Table 3. Since the crude extract did not show any activity against *E. coli* and *Proteus spp.* it was therefore considered not necessary to include these organisms in this aspect of the antimicrobial activity screening of the cream formulation. From the results, *Candida albicans* and *Staph. aureus* showed a mild sensitivity to the cream while *Klebsiella* did not show any activity. This low sensitivity of these organisms to the cream may be attributed to further dilution of the extract by the cream base as well as possible partitioning of the active principles in the extract between the aqueous and organic phases of the cream. This implies that a higher concentration of the extract will be required in formulating the cream to obtain an effective concentration that will render a beneficial therapeutic effect.

Conclusion. *Mitracapus vilosus* extract formed a stable cream when introduced as an active in aqueous cream BP. The antimicrobial activity of the extract was retained in the cream formulation. The cream can be applied topically for treatment of skin infections caused by *Candida albicans and Staphylicoccus aureus.*

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