African Journal of Pharmaceutical Research & Development

Vol. 3 No.1 pp.38-42 (September, 2011)

AntiFungal Activity and Stability of a Cream Formulation of *Mitracarpus villosus* (Rubiaceae) Extract

Arhewoh M.I., *Eichie F.E. and Iwuagwu U.N.

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City 300001, Nigeria

ABSTRACT

The antifungal activity of cream formulation of *Mitracapusvillosus* crude extract has been reported. The stability of the cream was also studied by determining the effect of storage under ambient temperature conditions on pH and antifungal activity. Extract of *Mitracapusvillosus* aerial parts (leaves, inflorescences and stem) was obtained by maceration in ethanol and cream formulation prepared using cetomacrogol ointment BP. The extract and cream were investigated for antifungal activity by the agar diffusion assay against three reference fungi *(Microsporumaudouinii, Tineamentagrophyte and Candida albicans)*. The minimum inhibitory concentrations (MIC) obtained for the crude extract was 5%w/v. The zones of inhibition of the formulated cream at 5 and 20%w/v ranged from 2 – 6 and 7.5 – 12 mm, respectively. The pH of the *M. villosus*cream was slightly acidic ranging from 4.5 to 5.9 while the cream base (without the extract) was basic with pH > 7. There was no significant change ($p>0.05$) in the antifungal activity of the cream after storage for 12 weeks. The results indicate that *Mitracapusvillosus* extract can be formulated into creams for topical application in treating infections caused by these fungi. Furthermore, the cream was stable over a 12 weeks period of storage.

Key words: *Mitracapusvillosus*, topical cream, extraction, antifungal activity, stability.

INTRODUCTION

*Mitracarpusvillosus*Zucc.is an annual, erect, 4-angled pubescent stems that can grow up to 60cm tall. It is a common weed in upland areas from the forest to the savanna zones. It is widespread in tropical Asia and Africa.Seven compounds isolated from the *Mitracapus* methanol extract were identified as gallic acid, 4-methoxyacetophenone, 3,4,5-trimethoxy acetophenone, 3,4,5 trimethoxybenzoic acid, Kaempferol- 3-O-rutinoside, rutin, and psoralen [1]. The presence of coumarin-like compounds [2] has been linked to the antifungal activity of the plant. Moreover, other authors have reported the antifungal activity of pentalongin, a naphthoquinoid pigment isolated from the fresh aerial parts of *M. scaber* [3].

M. villosus is widely employed in traditional medicine in West Africa for headache, toothache, amenorrhea, dyspepsia, hepatic diseases and, leprosy [4]. The crushed leaves of the plant are used as dressing for fresh cuts, wounds and, ulcers [5]. In southern Nigeria, the plant is used (crushed leaves) in the treatment of ringworm infection on the head (*teneacapitis*) and eczema on the skin. Earlier studies by Sanogo et al [6] and, Irobi and Daramola [7] on aerial powdered parts of *M. villosus* plant has shown that aqueous and ethanolic extracts of *M. villosus* has great antifungal and antibacterial activities. We recently reported the antibacterial activity of the aqueous cream formulation of extracts of *M. villosus*in which the MIC of the cream formulation was documented for the different bacterial organisms tested (Eichie et al, 2011).

* Corresponding author:Phone: +234-80- 36347181

Since one of its major uses in ethno medicine is in the topical application against fungal infection, this study therefore explored the effect of the cream formulation against selected strains of fungi including yeasts and moulds. Furthermore, a major problem associated with herbal dosage forms is their stability. The effect of storage was carried out under ambient temperature conditions.

MATERIALS

MirtracapusVillosus

*M. villosus (Rubiacea*e) was obtained from a farm land in Ekosodin behind University of Benin, Benin City. Botanical identification and authentication was done by the Forestry Research Institute of Nigeria (F.R.I.N), Ibadan, Nigeria, with voucher numbers assigned to the sample and deposited in the same Institute. The aerial part of the plant (stem, leaves and inflorescence) was air-dried for one week following which they were pulverized and passed through a 2 mm aperture sieve. The powders were packed in air-tight polyethylene bags prior to analysis.

Chemicals and Culture media

All the organisms used in this work were collected from the Pharmaceutical microbiology Laboratory, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Saboraud – dextrose Agar was obtained from, International Diagnostic Group Plc, Cetomacrogol wax, white soft paraffin, and liquid paraffin were obtained from HalewoodChemcials Ltd, England. Ethanol (99 %) (Riedel–de Haen^{R}) was obtained from Sigma-Aldrich Germany. All other chemicals used where of reagent grade.

METHODS

Extraction of*M. villosus*.

The powdered plant (10 g) was extracted with 100 ml of absolute alcohol by maceration for 4 days. The greenish extract obtained was passed through a Whatman filter paper No.1 (Whatman U.K) and the filtrate concentrated using a vacuum evaporator (Buch R110 Germany) at 50 ^{0}C.

Preparation of Cetomacrogol OintmentB.P

Cetomacrogol Ointment B.P was first prepared using 50 g of cetomacrogol emulsifying wax, 30 g of white soft paraffin and 20 g of liquid paraffin [9]. They were melted together in a porcelain dish over a hot water bath, and later cooled.

Preparation of *M. Villosus* cream

The formula used for the preparation of the cream is shown in Table 1. A quantity of cetomacrogol ointment B.P. (6 g) was melted over a hot water bath on a porcelain dish to 70 ^{0}C. 1g of *M. villosus* extract was dissolved in 14 ml purified water and 0.1 ml of phenol was added. The solution was brought to the same temperature as the water bath before mixing with the oily phase. The mixture was stirred occasionally over a cool water bath to cool. The 20 g cream (5 %^{w}/$_{w}$) was transferred quantitatively into a wide mouthed jar. The production of this 20 g cream was repeated using 4 g of *M. villosus* extract (high concentration) and 0 % w/w cream, which was used as the control.

Table 1: Formula for the preparation of *M. villosus* cream

MATERIAL	QUANTITY
Cetomacrogol ointment B.P.	6 g
M. villosus extract	1 g
Phenol solution (2 %)	0.1 ml
Purified water	14 ml

Assessment of antifungal activity of *M.*v*illosus* crude extract and the cream

The agar diffusion method was employed to access the antifungal activity of *M. villosus* cream and ethanolic extract. In this procedure, four 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 organism, respectively, by flooding the surface of the set agar plates with a suspension of the organisms in the subcultured nutrient broth and the excess was discarded. Different wells were bored (with the aid of a sterile cork borer) into the surface of the dextrose agar plate. The bottom of each well was sealed with a drop of molten agar. A sterile syringe was used to introduce 0.2 ml of the different concentrations of the *M. villosus* extract and the cream into the wells. The agar plates were left for about 30 min in order to allow for diffusion of the test sample into the agar. The plates were then incubated for 7-days at 28-30^{o}C. After incubation, the width of the zones of inhibition on the plate were recorded and used as a measure of activity. This procedure was repeated trice for each concentration and the mean results and standard deviations were obtained. The creams were stored in tightly sealed wide mouthed jars in the laboratory and the pH and

antifungal activities were determined every four weeks over a period of 12 weeks.

RESULTS AND DISCUSSION

Characteristic features of the *M. villosus* extract.

The powdered aerial parts (550 g) of the *M. villosus* plant which was green in colour yielded 20.5g after extracting with alcohol and concentrating with a vacuum evaporator to obtain a yield of 3.7%. The sticky greenish black substance obtained had a peculiar pungent odour. The extract was soluble in water and dimethyl sulphoxide (DMSO). The phytochemical properties have been reported in earlier studies [3, 7, 8].

Preliminary screening of antifungal activity of the extract

Prior to the preparation of the cream, it was necessary to carry out a preliminary test on the *M. villosus* extract to determine the minimum inhibitoryconcentration (MIC). The MIC is the simplest measure of antimicrobial activity of the lowest concentration required to inhibit the growth of a microorganism. The determination of the MIC was also necessary to enable us determine the concentration of the plant extract that will be used for the cream formulation.

Table 2: Antifungal activity of the *M. villosus*extract.

Micro-organism	Sensitivity of Extract
M. Audouinii	+++
T. Mentagiophytes	+++
C. Albicans	+

+ = indicate level of sensitivity to the extract.

From Table 2, the dermatophytes (moulds) which are responsible for infection on the skin, hair, nails of living host were more susceptible than the *Candida Albicans* (yeasts) which cause mainly deep mycotic infections. This is supported by results from earlier studies [3, 7].

Table 3: Zone of inhibition of the M. villosus extract

Micro–organism	zone of inhibition (mm)			
Concn. (%,$^{w}/_{v}$)	1	2	4	5
M. Audouinii	-	-	-	12.5 ± 2.7
T. Mentagophytes	-	-	-	17 ± 3.1
C. Albicans	-	-	-	12 ± 1

The appearance of zone of inhibition surrounding the disc indicates sensitivity of the microorganisms to the *M. villosus* extract. From Table 3, the MIC of all the organisms was obtained at 5%w/v of the *M. villosus* extract. The inhibitory concentrations obtained from the preliminary investigation on the antimicrobial activity of the extract in the above section were formulated into topical creams. The result obtained from the evaluation of the cream is shown in Table 4.

Characteristic features of the *M.villosus* cream.

The *M. villosus* cream prepared also retained the pungent odour and the dark green colour of the extract. There may be need to include a perfume to mask the pungent odour and improve the aesthetic values of the cream.

Table 4: Antifungal activity of the cream

Micro–organism	zone of inhibition (mm) (mean ± SD)		
Cream Concentration ($\%^{w}/_{w}$)	0	5	20
M. Audouinii	-	6.4 ± 0.9	12.1 ± 0.7
T. Mentagophytes	-	4.5 ± 0.1	10.5 ± 0.7
C. Albicans	-	2.3 ± 0.2	7.5 ± 0.9

The *M. villosus* extract was formulated into a cream at concentrations of 1 and 4 times the MIC of the extract. Both creams at concentrations 5 and 20%$^{w}/_{w}$, respectively, were subjected to antifungal test to determine if the prepared creams will still retain the initial activity of the crude *M. villosus* extract. From the results in Table 4, the formulation of this extract as cream did not significantly hinder the diffusion of the active principle in the formulation. The reduction in the zone of inhibition of the cream may be attributed to the partitioning of the extract between the aqueous and organic components of the cream since its solubility in the aqueous and organic environment will be different. The 20% w/v (4MIC) of the cream showed a higher zone of inhibition which was not directly proportional to the concentration of the extract in the cream.

Table 5: The mean pH values of *M. villosus cream*

Concentration (%w/w)	pH values (mean ± SD)			
Storage period (weeks)	1	4	8	12
0	7.13 ± 0.07	7.18 ± 0.19	7.23 ± 0.91	7.70 ± 0.95
5	4.72 ± 0.01	4.63 ± 0.04	5.31 ± 0.09	4.58 ± 0.16
20	4.95 ± 0.03	4.91 ± 0.03	5.85 ± 0.07	5.37 ± 0.09

Effect of storage on the stability of *M. villosus*cream

The result of storage at ambient temperature is shown in table 5. The control, which was prepared excluding the active, gave a pH at the range of 7.13 - 7.70. The pH of the 5%w/w and 20%w/w of the *M. villosus* cream was slightly acidic. This is probably as a result of the *M. villosus* extract, which on previous quantitative evaluation suggests that the extract contains gallic acid, which is well known for its antimicrobial properties [3]. The change in the pH over the 12 week was not significant ($p > 0.05$). Also, the peeling effect noticed on use of the *M. villosus* plant as a local herb has been linked to the slight acidic nature of the extract. In order to ascertain the microbial stability of the cream, the antifungal activity was carried out at the 12th week (Table 6).

The result obtained showed retention of initial activity of the cream with no significant change as with the result obtained in table 4.

Table 6: Antifungal activity of the cream after storage at ambient temperature for 12 weeks

Micro–organism	zone of inhibition (mm)		
Concentration (w/w)	0	5	20
M. Audouinii	-	5.1 ± 0.5	12.2 ± 1.7
T. Mentagophytes	-	4.5 ± 0.9	9.5 ± 1.7
C. Albicans	-	1.5 ± 0.1	5.5 ± 1.1

CONCLUSION

M. villosus extract formulated into cream has been shown in this study to be effective against *Candida albicans* and more importantly, the dermatophytes, which are major causes of nail, hair, and skin infections in man [7]. However, the cream formulation still have challenges to be overcome such as masking the colour, odour, and the cost of extracting the active from natural sources. Further purification of extract to get specific active constituents may solve the problem of colour and odour, while it may also make it possible to identify the active constituent and synthesize it in the laboratory through cheaper sources, which will minimize the cost of harvesting and extraction. Another possibility could be to test the effect of aqueous extraction.

ACKNOWLEDGEMENT

The authors are grateful to the laboratory staff of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin for all the assistance they rendered during this work.

REFERENCES

1. Anderson TF and Voorhees JJ. PsoralenPhotochemotherapy of Cutaneous disorders. Ann. Rev. Pharmacol and Toxicol. 20; 1980: 235- 241.
2. `Benjamin TV and Hugbo PC. An approach to thes study of medicinal plants, antimicrobial activities with reference to M*itracarpusscabe*r In: Sofowora (E.d), The State of Medicinal Plant research in Nigeria. University of Ife press, Ile Ife, Nigeria. 1986; pp. 243-251.
3. Moulis C, Peliser J, Bamba D, Fourastel. Pentalogin, antifungal naphtoquinoid pigment from *Mitracarpusscaber*. Second international congress on Ethnopharmacology, Uppsala, Sweden. Stockholm. The Swedish Academy of Pharmaceutical Sciences. 1992.
4. Bisignano G. Antimicrobial activity of *Mitracarpusscaber* extract and isolated constituents. Letters in Applied Microbiol. 30; 2000: 105-107

5. Gill LS. Ethnomedical Uses of Plants in Nigeria. University Press, Benin City, Nigeria. 1992. p 162.

6. Sanogo R. Selective antimicrobial activities of *Mitracarpusscaberzucc.*against*Candida* and S*taphylococcus spp*. Phytomedicine. 2; 1996: 265-268.
7. IrobiON, and Daramola SO. Antifungal activities of crude extracts of *MitracarpusVillosus (Rubiaceac*). J Ethnopharmacol. 44; 1993: 137- 140.
8. Eichie FE, Arhewoh MI, Isesele JE, Okoh EO. Antimicrobial activity of extract and topical cream formulation of m*itracapusvillosus* (Rubiaceae). J. Pharm. Biores. 2011; In press.
British Pharmacopoeia.Her Majesty Stationery Office. Vol.2; 2003: London pp. 1905, 2010.