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Report

In vitro Antimicrobial Activity of the Extract of *Mitracarpus scaber* Leaves Formulated as Syrup

TA Abere[1*], AO Onyekweli[2], and GC Ukoh[3]

[1]Department of Pharmacognosy. [2]Department of Pharmaceutics and Pharmaceutical Technology. [3]Department of Pharmaceutical Microbiology. Faculty of Pharmacy, University of Benin, PMB 1154, Benin-City, Nigeria.

Abstract

Purpose: *To formulate the crude extract of the leaves of Mitracarpus scarber "Zucc" as a syrup.*
Method: *The antimicrobial activity of the formulation was assessed using agar plates and concentrations of the extract varying between 25mg/ml and 300mg/ml to determine the minimum inhibitory concentration at 37^{o}C against bacterial and fungal organisms namely, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Sarcina lutea, Candida albicans and Klebsiella pneumoniae.*
Result: *The growth of Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Candida albicans was inhibited by the formulation at a minimum inhibitory concentration of 75mg/ml. Pseudomonas aeruginosa, and Sarcina lutea resisted all the concentrations of the formulation used. The presence of sucrose in the formulation rendered the formulation pleasant to taste.*
Conclusion: *The extract from the leaves of Mitracarpus scarber "Zucc" can be formulated into a pleasantly tasting oral dosage form despite its bitter taste.*

Keywords: *Antimicrobial, Mitracarpus scaber, syrup, formulation, minimum inhibitory concentration.*

Correspondence E-mail : eseabere@yahoo.com

INTRODUCTION

The yearly Herbal Medicine Trade Fair in Nigeria and the increasing publicity and patronage this attracts, irrespective of the social, educational or religious background of the people are indicative of acceptance of herbal medical practice[1]. The setting up of Traditional Medicine Board by some State Governments in our country and the on-going registration of herbal products by National Agency for Food, Drug Administration and Control (NAFDAC) are equally indicative of recognition by the Government.

Some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been suitably prepared and standardized[2]. Recently the World Health Organization (WHO) has recommended the use of Arthemisinin derivatives derived from *Artemisia annua* (Composite), a Chinese herb, as a first line drug in the treatment of malaria[3, 4]. This is as a result of WHO's recognition that 80% of world population use herbal medicine for some aspect of Primary Health Care[5].

The plant family, *Rubiaceae*, which parades a long list of plants of medicinal importance, has *Mitracarpus scarber "Zucc"* as example[6, 7]. It is claimed that it possesses antimicrobial activities when crude extracts from the plant is used[7-10]. Ahonkhai *et al*[11] formulated the crude extracts from the leaves as soap solution but it was observed that the availability of the antimicrobial principle was hindered by the soap. This was attributed to uptake of the active principle by the soap. This suggests that a new formulation is needed. This study, therefore, aims at establishing a simple formulation that does not hinder the availability of the active principles from the product. Considering that this extract is ingested in the treatment of sore throat and other upper respiratory diseases, URD,[12] despite its bitter taste, this study also aims at formulating the crude extract into a pleasant oral dosage form.

MATERIALS AND METHODS

Laboratory grades of Petroleum ether and methanol from British Drug Houses (BDH) as well as Granulated Sugar purchased from the open market were used. The following media, Blood Agar (Oxoid 271), Nutrient Agar (Oxoid) and Sabouraud Agar (Oxoid) were equally provided for microbial studies.

The plant was collected locally in Benin-City, Nigeria, identified and authenticated as *Mitracarpus scarber "Zucc",* family Rubiaceae, by the plant curator of the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy University of Benin, Benin-City, Nigeria, where a voucher specimen of the plant is deposited. It was sun dried and powdered using Moulinex mill after which it was stored in a dry and well-stoppered bottle.

The organisms used were clinical isolates of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Candida albicans and Sarcina lutea* (from the University of Benin Teaching Hospital, Benin-City), *Escherichia coli* (NCTC 10418) and *Staphylococcus aureus* (NCTC 6751) typed cultures.

Preparation of the Crude extract

1.5kg of the powdered plant material in each batch was exhaustively extracted by soxhlet extraction method using either Petroleum ether, (A), or Methanol, (B), or 70%$^{v}/_{v}$ Methanol in H_2O, (C). The solvent used in each batch was recovered under pressure until dry extracts were obtained and then stored separately in amber colored bottles labeled as A, B and C.

Preparation of Syrup and Simple solutions

The B. P. method [13] was adopted to prepare simple syrup, which was used as diluent to prepare concentrations of the crude extracts, (A, B and C) varying between 25mg/ml and 300mg/ml from stock solution, 500mg/ml. For Simple solution, distilled

Table 1: Sensitivity of Extract Samples (A, B & C) at 75mg/ml on Test Organisms

Test Organisms	#Zone of Inhibition (Mean)	
	Simple Solution	Extract in Syrup
**C. albicans*	22mm	22mm
E. coli (NCTC 10418)	21mm	21mm
S. aureus (NCTC 6751)	28mm	28mm
**E. coli*	20mm	20mm
**K. pneumoniae*	23mm	22mm
**Ps. aeruginosa*	nil	Nil
**S. aureus*	27mm	26mm
****Sarcina lutea***	nil	Nil

**:Clinical isolates.*
#: Mean zone diameters from two replicates are recorded

water was used as diluent to prepare the same concentrations.

Antimicrobial activity of the Extract

All the extracts were first screened for antimicrobial activity by the Well plate method [14]. Nutrient and Sabouraud agar plates were seeded with overnight cultures of the test organisms namely *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 6751) and clinical isolates of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Candida albicans and Sarcina lutea.* Wells, 15mm wide, were cut in the agar plates with cork borer and 1.5mls of the different extracts were pipetted and carefully added to the wells. The Nutrient agar plates were incubated right side up at 37^{o}C for 24 hrs while the Sabouraud agar plate was incubated at room temperature for 48 hrs. The zones of inhibition were then measured and the mean of two replicates recorded.

Minimum Inhibitory Concentration of Extract in Syrup

The minimum inhibitory concentration was determined by Agar dilution method [15]. A 1gm/ml and 500mg/ml dilution of each of the extract were made in water. From these stocks, graded concentrations of the extracts in syrup were made to achieve 25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml, 200mg/ml and 300mg/ml final concentrations in 20mls of both Nutrient and Sabouraud agar plates. These plates were spotted with 0.1ml overnight cultures of the test organisms. The Nutrient agar plates were incubated right side up at 37 ^{o}C for 24 hours while the Sabouraud agar plates were incubated at room temperature for 48 hours. The zones of inhibition were measured from two replicates and the mean recorded.

RESULTS

In the Table, the antimicrobial activity of the water extract (simple solution) of *Mitracarpus scarber "Zucc"* was demonstrated. All the samples inhibited the growth of most of the organisms. It also shows the sensitivity of the syrup samples prepared using the different extraction methods. It therefore indicates that the method of extraction did not affect the sensitivity of the active principles of the extract present in the formulation. Also formulating the extract as syrup did not alter their sensitivity. The minimum inhibitory concentration (MIC) of the extract in syrup was also determined. Irrespective of the method of extraction of the crude extract, all the syrup formulations were

active at 75mg/ml against all organisms used except *Pseudomonas aeruginosa* and *Sarcina lutea* which grew even at a high concentration of 300mg/ml. It is equally interesting to note that *Candida albicans* was inhibited at 75mg/ml.

DISCUSSION

Reports have shown that the extract of *Mitracarpus scarber "Zucc"* has antibacterial and antifungal activities[8, 11, and 16]. This work equally has confirmed such findings. Although Ahonkhai, *et. al* [11] stated that application of heat in the extraction method may have affected the potency of the antifungal principle(s), this work has shown that application of heat to the various extracts did not affect the activity of the extract to both bacterial and fungal organisms in the different formulations. Rather the inhibition to growth of the organisms by the extracts can be attributed to the potency of active principles as well as the strain and possibly, weakness in strength of organism used. Previous work [11] demonstrated that the extract had low activity over *Pseudomonas aeruginosa* despite the fact that this organism is known to be a very recalcitrant gram-negative organism. This work equally confirms that. This further confirms that heating may not be responsible for the insensitivity shown.

Formulating this extract, as Syrup did not hinder the diffusion of the active principle(s) in the formulation hence the zones of inhibition at 75mg/ml recorded for both the simple solution and the syrup were the same. Sucrose, a sweetening agent and main content of syrup, has been used to mask the bitter taste of the extract and so, a pleasantly tasting oral dosage form of the extract was easily formulated. Extract of *Mitracarpus scarber "Zucc"* can, therefore, be formulated as simple syrup.

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