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Research Article

Antimicrobial activity of dried pulverized, solvent extracts and Black soap samples of *Mitracarpus villosus* against some nosocomial infection causing microorganisms

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Abstract

Nosocomial infections are assuming epidemic proportions especially in rural communities in poor developing economies where usual antibiotics are not available or affordable or resistance to treatment antibiotics are on the increase. Alternative preventatives are being investigated especially as the breaking of the integrity of the skin barrier is usually responsible for turning infections by opportunistic microbes into life threatening diseases. Herbal solutions appear to be the most readily available options in such places. Herbs and herbal extracts are known to have various effects on microorganisms and thus their efficacy as drugs for preventing and treatment of different skin conditions is increasingly becoming the subject of evaluation procedures. Same herbal plant extracted using different solvents are observed to have varying effect on same microorganisms. The efficiency of herbs as preventatives and treatments, depend on the vehicle of delivery of the active ingredient to the diseased area. Therefore various treatment models have been designed to ensure better delivery of the active constituents of the herbs and thus increase the possibility of treatment success. We have in this study compared the antimicrobial properties of Gentamycin with that obtained with different concentrations of dried and pulverized *Mitracarpus villosus* extracted with hot water (HW) and methanol (Me), these extracts admixed with produced Black soap, dried pulverized *Mitracarpus* and dried pulverized *Mitracarpus* admixed with black soap against *Candida albicans* (Ca)(MTCC 227), *Staphylococcus aureus* (Sa)(ATTC 2785), *Proteus mirabilis* (Pm)(ATTC 21784) and *Pseudomonas aeruginosa* (Pa)(ATTC 27856) using Agar Diffusion Method.

Results show that in general hot water extract, methanol extract and dried and pulverized *Mitracarpus villosus* were all less effective than the samples admixed with black soap. The efficiency of the herb was in the order hot water extract< hot water extract soap < methanol extract < methanol extract soap <dried and pulverized *Mitracarpus villosus* < dried, pulverized *Mitracarpus villosus* soap _ control drug.

In conclusion soap appears to be acting synergistically with the various samples to increase their antimicrobial activity. The dried and pulverized sample admixed with black soap had approximately the same activity (24 ± 4.5mm) against *Staphylococcus aureus* as the control drug (Gentamycin) at the same concentration (10,000µg/ml). The dried and pulverized *Mitracarpus villosus* admixed with black soap shows the potential of being an alternative preventative and treatment herb for

Staphylococcus aureus, *candida albicans* and *proteus mirabilis* caused nosocomial infections when its toxicity has been evaluated and it is found to be safe or as safe as the control.

INTRODUCTION

Healing with herbs, or medicinal plants, is as old as mankind. Ever since ancient times, people looked for drugs in nature to cure diseases. Medicinal plants use at the beginnings, were instinctive as in the case with animals [1]. The awareness of medicinal plants usage is a result of the many years of struggles against illnesses which led man to search for drugs in bark, seeds, fruit bodies and other parts of plants. Contemporary science has acknowledged the efficacy of medicinal plants and has included in modern pharmacotherapy a range of drugs of plant origin, which have been known and used by ancient civilization throughout the millennia [2]. Historically all medicinal preparations were derived from plants, whether in the simple form of the raw plant materials or in the refined form of crude extracts, mixtures and so on [3].

World Health Organisation [WHO] has defined medicinal plants as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs [4]. The importance of medicinal plants and traditional health systems in solving the

health care problems of the world is gaining increasing attention. Most of the developing countries have adopted traditional medical practice as an integral part of their culture and this renewed interest is on account of the following assumptions by the indigenous population.

1. Plants are natural and therefore less toxic and safer than synthetic drugs
2. Medicinal plants are readily available than manufactured drugs.
3. Medicinal plants infusion can be made at home and are therefore less expensive than industrially manufactured drugs [5]

Over 300,000 medicinal plants have been discovered in various parts of the African continent and many more are without doubt available in the whole world [6]. The bioactive constituents of plants responsible for their healing properties are mucilage, gums, glycosides, tannins, alkaloids, essential oils, pectin and so on. These constituents change depending on time of collection, season, climate type, age and region of harvest of the medicinal plant.

Nosocomial infections have increased in recent times as more and more people exist together in institutions. The prevalence of these infections is higher in rural based institutions in poorly developed economies [7]. Some of the microorganisms that have been indicated in the spread of these infections are *Candida albicans*, *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are all organisms that do not usually cause serious infections in healthy individuals and are thus opportunistic pathogens [8]. However, when the immunity systems of individuals are compromised or in situations of ill health or accidents resulting in the breakdown of the integrity of the skin mantle, infections by these organisms have been known to cause life threatening diseases [9]. As most developing countries and some developed countries are resorting to the use of herbal cures, the determination of the antimicrobial properties of medicinal plants has become imperative.

Mitracarpus villosus is a plant often regarded as weed and is of the family *Rubiaceae*. Its common names in Nigeria include irawole (Yoruba), obuobwa (Ibo), and gududal (Sokoto Fulani). It is variously used in these areas for treatment of skin diseases, in particular eczematous eruptions. In other African countries like Senegal, it is used in the treatment of sore throat, leprosy and antidote against arrow poison. In Mali, the plant is used traditionally for headache, toothache, amenorrhea, dyspepsia, hepatic disease, veneral diseases and leprosy [10]. Chemo-microscopic investigation of the leaves of this plant was carried out by some researchers [11]. They found that it contained lignin, oil, calcium oxalate, and starch. They also reported that its ethanolic crude extract was active against *Staphylococcus aureus* and some other microorganisms. Other scientists reported that *Mitracarpus villosus* leaves have sedative properties and may contain psychoactive principles [12] and possess anti-venomous properties [13]. There were also some reports on the antimicrobial activity of phyto compounds from *Mitracarpus villosus* that found it active against *Candida albicans* and some other microorganisms [14,15].

Various antibiotics have been used as treatment regime against nosocomial infections. These drugs include the azole series against *Candida* with Ketoconazole being the most prominent [16], 2% Mupirocin against *Staphylococcus aureus* [17], aminoglycosides like Gentamycin against *Pseudomonas aeruginosa* and *Proteus mirabilis* [18]. Though drugs like Ketoconazole and Gentamycin which is a broad spectrum antibiotic are used in spite of their toxicity [19], there have been increasing reports of resistance of these organisms to the administered drugs [18].

These important development, necessitate the search for alternative treatment regime. We have embarked on a study of the antimicrobial and antifungal effects of *Mitracarpus villosus* with a view to determining its effects on *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus mirabilis* which are all causative agents in nosocomial infections so that if found effective it can be introduced as alternative preventive and treatment regime against these infections.

EXPERIMENTAL

Sample collection, identification and preparation

The plant sample was collected and identified at the Botanical Garden of the University of Ibadan, Ibadan, Nigeria. The plant was air dried and then pulverized. The microorganisms for the study were obtained from Dr O.O. Oladosu of the Department of Chemistry, University of Ibadan, and Ibadan.

Extraction of bioactive material

Hot water extract was obtained by blending 200g of pulverized sample plant with 1 litre of hot distilled water for minutes. The blended material was then filtered under gravity with Whatmann No 1 filter paper and the water removed by rotary evaporator to obtain the extract.

The methanol extract was obtained by blending 200g of the pulverized plant sample with 1 litre of analytical grade methanol in an explosion proof blender for 5 minutes. The blended material was filtered under reduced pressure using a vacuum pump. The excess solvent in the extract was removed with the aid of a rotary evaporator.

Preparation of solutions of sample extracts and control drug

2g of sample extract was carefully solubilized in 200ml Dimethyl sulphoxide (DMSO) to give a stock solution of 10,000µg/ml. The stock solution was then serially diluted to obtain 1000µg/ml; 100µg/ml and 10 µg/ml sample extract solutions.

10,000µg/ml control drug's (Gentamycin) stock solution was prepared by adding 0.25ml of 40mg/ml injection solution of Gentamycin to 0.75ml of DMSO

Preparation of black soap

Palm kernel oil and extracted *Theobroma cacao* pods ash lye was used to prepare soap in a fully boiled preparatory method.

Preparation of test samples

The soap was then admixed with various percentages (5%,

10%, 20%, and 25%) of dried and pulverized *Mitracarpus villosus* plant samples to produce test samples.

Determination of microbial activity

The microbial activity of all test samples, were determined using the Agar disc diffusion method by reading and recording the zones of inhibition of the microorganisms. The minimum inhibitory concentrations were also determined.

RESULTS AND DISCUSSION

The results of the microbial assay are presented on Tables (1-4). Gentamycin the drug used as the positive control in this research work is a broad spectrum antibiotic of the aminoglycoside class and inhibits the growth of both gram positive and gram negative bacteria especially *Pseudomonas* and *Staphylococcus* microbes. It was active against all the microorganisms with the maximum activity being 25mm inhibition zone and minimum 16mm (Table 1 – Table 4). It was most active against *Staphylococcus aureus* followed by *Proteus mirabilis* and then *Candida albicans* and lowest against *Pseudomonas aeruginosa*.

The hot water extract was mainly active at 10,000µg/ml against the organisms. Its activity was only about 40% of that of the control. Ad-mixing the extract with soap increased its activity as it became active against the microorganisms between 100 -10,000µg/ml with activity increasing with increase in concentration and lowering its minimum inhibitory concentration to 100µg/ml. The exception was *Pseudomonas aeruginosa*, where it was only active between 1000 – 10,000µg/ml with minimum inhibitory concentration of 1000µg/ml. The activity also increased to a maximum of 56% of that of the positive control.

The Methanol extract was active against all the organisms at all the concentrations with activity in the order Sa > Pm > Ca > Pa. The maximum activity of this extract was obtained at 10,000µg/ml and was about 60% of the positive control's activity. The minimum activity here was the same with that of the maximum for hot water extract. With the inculcation of the Methanol extract into the black soap, the maximum activity of the extract was enhanced to 68% of the activity of the positive control.

The dried and pulverized *Mitracarpus villosus* was active at all concentrations against *Staphylococcus aureus and Proteus mirabilis*. As soon as the dried and pulverized *Mitracarpus villosus*

Table 1: Microbial activity of *Mitracarpus villosus* against *Candida albicans* in zones of inhibition.

Sample	Concentrations of medicinal plant			
	10,000 µ g/ml	1000 µ g/ml	100 µ g/ml	10 µ g/ml
PC	24 ± 5.0mm	20 ± 2.5mm	18 ± 2.6mm	18 ± 3.5mm
PS	-	-	-	-
HW	10 ± 4.0mm	10 ± 2.1mm	-	-
HWS	14 ± 5.0mm	10 ± 5.0mm	7 ± 5.0mm	-
ME	12 ± 3.6mm	11 ± 1.0mm	10 ± 4.0mm	9 ± 5.6mm
MES	17 ± 6.0mm	15 ± 3.3mm	10 ± 4.5mm	10 ± 6.2mm
SPS 5%	17 5.5mm	-	-	-
SPS 10%	18 ± 4.4mm	-	-	-
SPS15%	18 ± 5.0mm	-	-	-
SPS 20%	19 ± 6.0mm	-	-	-
SPS 25%	20 ± 4.7mm	-	-	-

Each value is the mean of three measurements ± st.d
Abbreviations: SPS: Soap + Pulverized Mitracarpus of various Percentages; PC-Positive Control = Gentamycin; PC-Dried and pulverized Mitracarpus; HW: Hot Water Extract; HWS-Hot Water Extract + Soap, ME: Methanol Extract; MES: Methanol Extract + Soap

Table 2: Microbial activity of *Mitracarpus villosus* against *Pseudomonas aeruginosa* in zones of inhibition.

Samples	Concentration of medicinal plant			
	10,000 µ g/ml	1000 µ g/ml	100 µ g/ml	10 µ g/ml
PC	20 ± 3.5mm	20 ± 2.7mm	19 ± 2.5mm	19 ± 5.5mm
PS	-	-	-	-
HW	10 ± 4.0mm	-	-	-
HWS	14 ± 3.7mm	11 ± 5.0mm	-	-
ME	12 ± 4.2mm	10 ± 5.7mm	10 ± 3.0mm	9 ± 5.0mm
MES	14 ± 5.0mm	11 ± 3.5mm	11 ± 4.5mm	10 ± 4.5mm
SPS5%	12 ± 5.5mm	-	-	-
SPS10%	12 ± 2.7mm	9 ± 4.5mm	-	-
SPS15%	13 ± 6.0mm	10 ± 2.5mm	-	-
SPS20%	14 ± 2.5mm	10 ± 3.0mm	6 ± 4.0mm	-
SPS25%	14 ± 5.0mm	11 ± 3.7mm	9 ± 3.5mm	-

Each value is the mean of three measurements ± st.d
Abbreviations: SPS: Soap + Pulverized Mitracarpus of various Percentages; PC-Positive Control = Gentamycin; PC-Dried and pulverized Mitracarpus; HW: Hot Water Extract; HWS-Hot Water Extract + Soap, ME: Methanol Extract; MES: Methanol Extract+soap

Table 3: Microbial activity of *Mitracarpus villosus* against *Proteus mirabilis* in zones of inhibition.

Samples	Concentration of medicinal plant			
	10,000 µ g/ml	1000 µ g/ml	100 µ g/ml	10 µ g/ml
PC	23 ± 5.5mm	22 ± 3.7mm	20 ± 1.0mm	20 ± 2.5mm
PS	19 ± 3.0mm	17 ± 3.5mm	15 ± 1.5mm	12 ± 4.5mm
HW	10 ± 3.5mm	10 ± 4.0mm		-
-				
HWS	13 ± 4.7mm	10 ± 3.8mm	8 ± 4.5mm	8 ± 2.3mm
ME	15 ± 4.5mm	12 ± 4.5mm	10 ± 3.8mm	9 ± 1.8mm
MES	17 ± 2.5mm	14 ± 2.0mm	12 ± 2.5mm	10 ± 2.0mm
SPS5%	17 ± 3.0mm	-	-	-
SPS10%	19 ± 1.0mm	-	-	-
SPS15%	19 ± 5.0mm	10 ± 3.5mm	-	-
SPS20%	20 ± 5.5mm	15 ± 4.5mm	-	-
SPS25%	20 ± 5.5mm	15 ± 4.7mm	13 ± 2.5mm	12 ± 4.0mm

Each value is the mean of three measurements ± st.d
Abbreviations: SPS: Soap + Pulverized Mitracarpus of various Percentages; PC-Positive Control = Gentamycin; PC-Dried and pulverized Mitracarpus; HW: Hot Water Extract; HWS-Hot Water Extract + Soap, ME: Methanol Extract; MES: Methanol Extract + Soap

Table 4: Microbial activity of *Mitracarpus villosus* against *Staphylococcus aureus* in zones of inhibition.

Samples	Concentration of medicinal plant			
	10,000 µ g/ml	1000 µ g/ml	100 µ g/ml	10 µ g/ml
PC	25 ± 7.5mm	24 ± 5.0mm	19 ± 5.0mm	16 ± 1.0mm
PS	10 ± 1.0mm	9 ± 5.3mm	8 ± 2.0mm	6 ± 6.0mm
HW	10 ± 4.6mm	-	-	-
HWS	13 ± 3.6mm	10 ± 5.5mm	10 ± 2.6mm	7 ± 5.0mm
ME	15 ± 3.5mm	15 ± 4.0mm	12 ± 2.5mm	10 ± 4.5mm
MES	17 ± 5.5mm	14 ± 3.5mm	10 ± 3.6mm	7 ± 3.0mm
SPS5%	18 ± 2.5mm	-	-	-
SPS10%	18 ± 1.5mm	-	-	-
SPS15%	19 ± 3.6mm	-	-	-
SPS20%	20 ± 4.0mm	15 ± 3.6mm	13 ± 3.5mm	10 ± 2.5mm
SPS25%	24 ± 4.5mm	20 ± 2.5mm	18 ± 4.0mm	15 ± 2.5mm

Each value is the mean of three measurements ± st.d
Abbreviations: SPS: Soap + Pulverized Mitracarpus of various Percentages; PC-Positive Control = Gentamycin; PC-Dried and pulverized Mitracarpus; HW: Hot Water Extract; HWS-Hot Water Extract + Soap, ME: Methanol Extract; MES: Methanol Extract+soap

was mixed with soap its activity was enhanced. It became active against all the microorganisms especially at 10% - 25% of the mixture. Its activity varied from 48% - 96% of that of the positive control and the activity was dried and pulverized *Mitracarpus villosus* concentration dependent.

At 10,000µg/ml and 20% and 25% pulverized sample addition, the herbal soap was active at all concentrations from 10µg/ml – 10,000µg/ml with the lowest activity being about 40% of that of the control. At 10,000µg/ml its activity is between 56% - 96% of the control which is remarkable. These samples were most active against *Staphylococcus aureus* followed by *Proteus mirabilis*, then *Candida albicans* and finally *Pseudomonas aeruginosa*. It is in the herbal soap's activity against *Staphylococcus aureus* that the influence of the kernel soap was more pronounced. The herb was originally only active against this organism at about 40% of that of the control drug at 10,000µg/ml. Its activity increased to 96% when it was mixed with the soap. This synergism is remarkable and represents 240% increase in activity and a confirmation of its ability to treat infections by.

Staphylococcus aureus

The control drug gentamycin is known to have some allergic effects. No allergic responses have been reported in the use of *Mitracarpus villosus.* The herb samples could therefore be used as an alternative in the treatment of skin related diseases to protect the integrity of the skin mantle and thus prevent life threatening cases of the nosocomial infections. However, the toxicity of the herb should be determined.

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