PRELIMINARY STUDIES ON PHYTOCHEMICAL AND ANTIMICROBIAL INVESTIGATION OF PLANTS (IRAWO-ILE) MITRACARPUS VILLOSUS, EUPHORBIA HIRTA AND SPERMACOCE OCYMOIDES.

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ABSTRACT

Mitracarpus villosus, Euphorbia hirta and Spermacoce ocymoides are three plants that are called by the same local name (Irawo-Ile) in South-western part of Nigeria. These plants were investigated for some of their components and antimicrobial activities of their leave extracts against bacteria and fungi.

Phytochemical analysis revealed that the three plants contain saponins, tannins and volatile oils. They do not contain steroids. Infrared and ultraviolet-visible (U.V) analysis suggested that the plants contain Benzene ring, hydroxyl group and aniline in their structure.

It was found from antimicrobial analysis that the plants extract exhibited antimicrobial activities at a concentration of 25mg/ml. The zone of inhibition of the plants extract against fungi and bacteria ranges between 10 – 35mm with minimum inhibitory concentration (MIC) ranges between 25 – 50mg/ml for fungi while that of bacteria ranges between 25 – 100mg/ml with S. Ocymoides less effective against bacteria. The ethanolic extracts of both dried and fresh leaves of the plants show more antimicrobial activity against the tested organisms than n-hexane extracts of the plants.

Keywords: *Mitracarpus villosus, Euphorbia hirta , Spermacoce ocymoides, Phytochemical and antimicrobial activities*

1. INTRODUCTION

Mitracarpus villosus, Euphorbia hirta and Spermacoce ocymoides ❩Fig 1❑ are called Irawo-ile in south-western, Nigeria because of their similar antifungal and antibacterial properties. Irawo-ile is called African Borreria or Green Borreria in English. They belong to family known as Rubiaceae. Mitracarpus villosus fresh leaves are green in colour with the characteristic mild odour, bitter and peppery taste [1]. The matured leaf size is 3.5cm (length) and 0.8-1.3cm (breath). Euphorbia hirta stem is slender and cylindrical often reddish or white covered with yellowish bristly hair especially in the younger parts. Its leaves are oppositely arranged lanceolate and up to 5cm long, they are greenish or reddish underneath asymmetrical and of rounded base in the axis which appears very dense. Spermacoce ocymoides leaf is partly fused at base, small, puberulous, margin not hairy. The fresh leaves are green in colour, semi-leathery and about 13cm long, 0.21cm wide, linear- lanceolate or ovate to elliptic [2,3]. These plants grow to about a height of 40-50cm. The plants are useful in curing gonorrhea, dysentery and skin diseases [4]

Much study has not been carried out on these plants. Jegede et al, 2005[1] investigated the pharmacognostic properties of the leaves of mitracarpus villosus.

Mostly, the importance of these plants are not known and so it is normally cut off as waste material as a result of this, we embark on this study to consider some of the components and anti-microbial effects of the leave of these plants on some micro-organisms.

Euphorbia hirta

Spermacoce ocymoides

Mitracarpus villosus

Figure 1: Picture of Euphorbia hirta, Spermacoce ocymoides and Mitracarpus villosus

2. MATERIAL AND METHODS

2.1 Sampling

The three plants samples; Mitracarpus villosus, Euphorbia hirta and Spermacoce ocymoides were collected in July, 2008 at Ogbomoso, Oyo state, Nigeria. The plants were identified at Botany department, University of Ibadan and Department of Pure and Applied Biology, LAUTECH, Ogbomoso. The samples were air dried for ten days, ground into powder and stored in a colourless plastic bottle.

2.2 EXTRACTION

Three solvents were used for these extractions namely ethanol, n-hexane and water. 20.0g each of the powdered samples were weighed into three labeled conical flask containing 200ml of ethanol each. The mixtures were left for four days and then filtered. The filtrates were put in the round bottom flask, heated with water bath and connected to coil condenser through which cold water flows to condense the vapour from the solvent of the extract which is collected in a receiving flask. This was done in order to concentrate the extracts.

For n-hexane, the samples were soaked for two days instead of four days.

With distilled water extraction: 20.0g each of fresh leafs of the plants were weighed and rinsed with water. The rinsed samples were ground; more water was added for extraction and then filtered.

Infrared, Ultra-Violet, phytochemical and antimicrobial screenings were carried out on the filtrates.

2.3 Phytochemical Screening

Phytochemical screening for some constituents of the plant extracts was done using standard qualitative methods as described by various authors (Trease et al., 1989 and Edeoga, 2005)[5,6]. The plants were screened for Saponins, Tannins, Volatile oil and steroid as follows:-

Saponins Test

5ml of each of the extracts were vigorously shaken for two minutes with 10ml of water in a test tube. Frothing which persisted on warming was taken as an evidence for the presence of Saponins.

Emulsion Test for Saponins

5 drops of Olive oil was added to $3cm^3$ of the extract in a test-tube and the mixture was shaken vigorously. A stable emulsion which formed indicates the presence of saponins

Test for Tannis

To a small quantity of the plant extracts, were added 4ml of water and a drop of ferric chloride. Green precipitate indicates the presence of tannins.

Test for Volatile oil

The extract of each of the plants were dissolved in 90% alcohol and drops of ferric chloride were added. Green colouration, indicate the presence of volatile oils.

Test for steroids: Salkowski Test

5 drops of concentrated H_2SO_4 was added to $1cm^3$ of the ethanolic extract of each plant. Absence of red colouration indicates absence of steroids in the extracts.

Infrared Spectroscopy analysis: Small portion of the extracts were used for the IR analysis on Nicolet Avatar 330FT-IR by thermo Electron Corporation using KBr disc

Ultraviolet-Visible-analysis: This analysis was done using Genesys 10 scanning machine with the extracts

2.4. Preparation of the antimicrobial discs

Filter papers were punched to produce paper discs. The paper discs were then sterilized in an autoclave at 150^{o}C for 2hours.

The isolate used for the determination of the anti microbial property of the extracts were taken from nutrients agar slants and subcultures on nutrients agar plates. The plates were incubated in an incubator at 37^{o}C for 18hours to get young cultures of the isolates.

After the sub culturing, the young cultures were then inoculated on fresh nutrient agar plates using sterile cotton swabs. With a sterile forceps, the extract soaked-paper discs were introduced on each inoculated plates, the plates were incubated at 37^{o}C in an incubator for 18hours. At the end of the incubation period (18hours), the plates were brought out and the zones of inhibitions around each disc were noted and measured in millimeter (mm)

The extracts from fresh *Mitracapus villosus* was also applied to the scrape part of a body that has eczema (a fungi causing skin disorder). The scrape eczema part was also culture on nutrient agar and found to grow. The extracts of *M. villosus* was found to inhibit its growth.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The result of phytochemical analysis for the extracts of the three plants is shown in Table 1. It can be seen from the table that the plants extracts contain saponin, tannin, volatile oils. Spermacoce ocymoides does not contain tannin. They do not contain steroid. The presence of tannin and volatile oil show that the plants can be used as purgative and herbal medicine respectively. (Gills, 1992)[7].

3.2 Results for Antimicrobial Test

The crude extract of Mitracarpus villosus, Euphorbia hirta, and Spermacoce ocymoides were obtained using ethanol and n-hexane on both fresh and dried leaves of the plants and were tested for their in-vitro antibacterial and antifungal activities using agar diffusion techniques.

Ethanolic extracts of both fresh and dried leaves produced definite antibacterial and antifungal activities against; Staphylococcus aureus, Candida albicans, Aspergillus niger and Fusarium flocciferum. Ethanolic extract of dried Mitracarpus villosus is the most effective against C. albican followed by E. hirta (Table 2) .E. hirta is the most effective inhibitor of Aspergillus niger,Fusarium flocciferum and Staphylococcus aureus. The same trend is followed by n-hexane extract except that the M. villosus is the most effective inhibitor of Staphlococcus aureus. Extracts from dried samples of the plants is more effective compared to the extracts from fresh samples of the plants (Table 3)

Also, n-hexane extracts of both fresh and dried leaves show some antibacterial and antifungal activities against Staphylococcus aureus, Candida albicans and Aspergillus niger while the fresh leaves in n-hexane of the three plants does not posses any definite action against Fusarium flocciferum.

The zone of inhibition produced by both the ethanolic extracts and n-hexane extracts of the dried and fresh leaves of the plants on S. aureus ranges from 10 – 30mmm while that of ethanolic and n-hexane extracts of dried leaves ranges 10 – 35mm (Table 2). The minimum inhibitory concentrations (MIC) of the extracts are in the range 25mg/ml – 50mg/ml while the minimum bacteriocidal concentrations (MBC) is in the range 25mg/ml – 100mg/ml. The crude extracts of Mitracarpus villosus which was applied directly on skin disorder, eczema was found to cure the disease within three days. It can be seen that the plants will be an effective cure for fungi skin disorder.

3.3 Infrared Analysis of the Plants Extract:

The infrared spectroscopy of the samples show major peaks at 1635.62 , 1635.54 and 1636.06 cm^{-1} for M.villosus, E. hirta and S. ocymoides respectively which is typical of compound that contain carbonyl group (C=O), C=N. Absorption at 3565.31, 3573.70 and 3568.15cm^{-1} are also observed for the three plants extract which is typical of O-H, N-H stretching frequency. Absorption at 2068.68cm^{-1} is common to the three plants samples; this shows the presence of C≡C, C≡N or S=C=N. The three plants have similar activities due to nearly identical functional group obtain from their extracts.

3.4 Ultraviolet (UV) Analysis : The three plants, M. villosus , E. hirta and S. ocymoides absorb at 229 & 322nm , 217nm and 265nm respectively. There are many compounds with different conjugation that fall into these categories i.e para-nitroaniline, chloroaldehyde, chlorobenzene, phenol and butadiene. These three plants will contain one or more of this group of compounds.

Table 1: Summary of Phytochemical analysis Results

Extracts	Saponins	Tannins	Volatile oils	Steriods
Mitracarpus villosus	+	+	+	-
Euphorbia hirta	+	+	+	-
Spermacoce ocymoides	+	-	+	-

Keys: + = Present - = Absent

Table 2: Comparative antimicrobial activities of Dried samples of M. villosus, E. hirta and S. ocymoides

Plant extracts in ethanol	M.villosus			E. hirta			S . Ocymoides		
Concentration mg/ml	25	50	100	25	50	100	25	50	100
Organisms	Zones of inhibition (mm)								
C. albicans	35	35	35	20	20	30	10	12	20
Aspergillus niger	12	16.25	20	25	30	35	15	20	25
Fusarium flocciferum	10	12	15	20	25	18	20	15	20
Staphylococcus aureus	12.5	15	20	19	20	30	11	15	20
Plants extract in n-hexane	M.villosus			E. hirta			S . Ocymoides		
Organisms	Zones of inhibition (mm)								
C. albicans	18	20	30	25	25	30	20	30	30
Aspergillus niger	10	12	25	30	30	30	25	30	30
Fusarium flocciferum	15	18	25	25	35	35	25	30	30.5
Staphylococcus aureus	20	25	30	15	15	18	15	20	20

Table 3: Comparative antimicrobial activities of Fresh samples of M.Villosus, E. hirta and S. ocymoides

Plant extracts in ethanol	M.villosus			E. hirta			S . Ocymoides		
Concentration mg/ml	25	50	100	25	50	100	25	50	100
Organisms	Zones of inhibition (mm)								
C. albicans	10	20	25	18	10	10	20	20	25
Aspergillus niger	18	20	25	20	20	30	20	30	35
Fusarium flocciferum	30	25	10	10	12	15	20	25	30
Staphylococcus aureus	11.5	15	15	15	15	20	10	12	15
Plants extract in n-hexane	M.villosus			E. hirta			S . Ocymoides		
Organisms	Zones of inhibition (mm)								
C. albicans	18	20	25	15	18	15	10	12	15
Aspergillus niger	18	20	20	10	18	18	NA	15	20
Fusarium flocciferum	NA	NA	NA	NA	NA	NA	NA	NA	NA
Staphylococcus aureus	20	25	30	10	10	10	18	10	10

4. CONCLUSION

Phytochemical analyses reveal that the three plants contain similar constituents which are useful for medicinal purposes. Infrared and ultra-violet spectrophotometric analysis suggested the presence of carbonyl, chlorobenzene , phenol within the extracts. More analysis such as tin-layer chromatography, GC-MS, H-NMR C^{13}- NMR, needed to be done to ascertain the actual component of the plants. The three plants extract can be used as antifungal as well as antibacterial.

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