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PRELIMENARY PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF ETHANOL EXTRACT OF *MITRACARPUS SCABER ZUCC*

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ABSTRACT

Medicinal plants are plants which have a recognized medical use. They range from the plants that are used in the production of mainstream pharmaceutical products to plant used in herbal medicine preparation.To formulate the crude extract of mitracarpus scaber Zucc as syrup. The antimicrobial activity of the formulation was assessed using agar-plates and the concentrations of the extract varying from 100mg/hole, 50mg/hole, 25mg/hole and 12mg/hole to determine the minimum inhibitory concentration against bacterial and fungal organisms such as Staphylococcus aureus, Streptococcus spp, Shigella spp, Corynebacterium spp, Pseudomonas aeruginosa, Salmonella spp, and Candida albinans. Candida albicans was resistance to the extract at the concentration of 12mg/hole and the growth of others organisms was inhibited by the formulations at all concentration. Conclusively, these findings should be suitable for inclusion in the proposed pharmacopoeia of Nigerian medical plants.

Keywords; Antimicrobial activity,Extract, syrup, Bacteria and Fungal

INTRODUCTION

1.0 MEDICINAL PLANT

Medicinal plants are plants which have a recognized medical use. They range from plants which are used in the production of mainstream pharmaceutical products to plants used in herbal medicine preparations, (Dutta,2008). Herbal medicine is one of the oldest forms of medical treatment in human history, and could be considered one of the forerunners of the modern pharmaceutical trade. Medical plants can be found growing in numerous settings all over the world, (Benjamin et al., 1986).

Some medicinal plants are wild crafted, meaning that they are harvested in the wild by people who are skilled at plant identification. Sometimes plants cannot be cultivated, making wild crafting the only way to obtain them, and some people believed that wild plants have more medicinal properties. Wild crafting can also be done to gather medicinal plants for home use, with people seeking out plants to Use in their own medicinal preparations, (Fluck, 1976).

Other medicinal plants may be cultivated. One of the advantages of cultivation is that it allows for greater control over growing conditions, which can result in a more prediction and consistent crop.cultivation also after for mass production,which makes plants more commercially viable, as the can be processed in large numbers and price low that people will be able to afford them (Google, 2010).

People who work with medicinal plants can process them in a variety of ways. Many plants contain pharmacologically active compounds which can access by making teas, tisanes, and other preparations, (Abere, 2007).

The history of studying and working with medicinal plants is quite long. Chemists are always interested in studying medicinal plants which have not been researched before, to identify which compounds in the plants are active and to see how those

compound work. Usually; the goal is to develop a synthetic version of the compound which can be easily produced in a laboratory and packaged in pharmaceutical preparations. Chemists may also be interested in historic medical treatments, examining plants to see whether or not preparations used historically would have worked, and if they would have, how they would have worked, (Google, 2010).

1.1 MITRACARPUS SCABER

The family Rubiaceae, popularly known as madder family belongs to the Gentianales order, recently called Rubiales order. The family consists of about 500 general and 6,000 species distributed all over the world. Some of them are tropical trees and shrubs (erect, struggling or twining) while few members are herbs (erect or decumbent), (Evans, 2002).

Mitracarpus scaber is a perennial annual herb of about 30cm tall or much smaller and possess rough leaves (Abere et al., 2007).

In Nigeria, it is known as obuobwa in Igbo language, Gogamasi (Gududal) in Hausa language, Irawo ile in Yoruba language and Rumcwor in Kuteb language, (Gbile,1984).

The plant family, Rubiaceae, which parades a long list of plants of medicinal importance, has mitracarpus scaber as example, (Gill 1992). It is claimed that it possesses antimicrobial activities when crude extracts from the plant is used, (Irobi, 1993). According to Ahonkkai et al., formulated the crude extracted from the leaves as soap solution but it was observed that the availability of the antimicrobial principle was hindered by the active principle by the soap. This study, therefore, aims at establishing a simple formulation that does not hinder the availability of the active principle from the product.

Considering that this extract is infested in the treatment of sore throat and upper respiratory diseases, URD, (Ahonkhai et al., 1999). Despite its bitter taste, this study also aims at formulating the crude extract into a pleasant oral dosage form.

1.2 MEDICINAL IMPORTANCE OF MITRACARPUS SCABER

The extracts of mitracarpus scaber is widely used in traditional medicine practices in West Africa for the treatment of headaches,

toothaches, amennorrhoea, dyspepsia, hepatic diseases, veneral diseases as well as leprosy. It is claimed that the plant has both antibacterial and antifungal activities, (Gill, 1992).

In Senegal, the plane is used for the treatment of sore throat and also for leprosy in the same way as cola cordifolia, and in Nigeria, the juice from the crushed plant is known to be applied topically for the treatment of skin diseases such as ringworm, lice, itching, craw-craw and other fungi wounds and ulcers, (Abere, 2007).

According to Jokuk, it is used as an ingredient in fish poison by some pagan tribe.

The ethanol extract and isolated constituents of the aerial parts of mitracarpus scaber were reported to exhibit both antibacterial and antimycotic activities (Bisignano et al. 2000). The ethanolic extract was subsequently fractionated and monitored by Bioassy leading to the isolation of seven compound screened for antibacterial and antimycotic activities. The crude extract also compared favorably with 0.5% Hibitance at c0ncentration of 30% W/V and 100% W/V against staphyloccus aurous, Pseudomonas aeruginosa, candida albicans, Trichophyton rubrum and Trichopyton tonsurans, through when a 35% w/v of the extract was incorporated into a liquid soap formulation, the antimicrobial activity was reduced,

(Ahonkhai et al., 1999). Extracts from the leaves of mitracarpus scavber have been successfully formulated into a pleasantly tasting oral dosage form despite its taste for the treatment of sore throat and upper respiratory disease (Abere et al., 2007).

Evaluation of the effects of mitracarpus scaber on carbon tetrachloride induced acute liver damage in rat showed significant hepatotection both in vitro and in vivo (GERMANO et al., 2007).

Some drugs of plant origin in conventional medicinal practice are not pure compounds but direct extracts or plant materials that have been suitably prepared and standardized (WHO, 2001). The World Health Organization (WHO) has recommended the use of arthemisinin derivatives from Aremisia annual (composite), A Chinese herb with established pharmacognonostic data, as a first line drug in the treatment of malaria.

Establishment of the pharmacognostic profile of the leaves of mitracarpus scaber will assist in standardization, which can guarantee quality, purity and identification of samples.

MATERIALS AND METHODS

Mitracarpus scaber (Rubiaceae) plants were collected in kpambo, Ussa local Government Area , Taraba State in Nigeria and identified by Prof. S.S.Sanusi, Centre for trans-sahara studies University of Maiduguri, Voucher specimens are preserved for future reference.

APPARATUS

Mortar and pestle ,Weighing balance ,Beakers,Round bottom flask (3litre),Measuring cylinder (200ml) ,Condenser,Water bath ,Electromantle,Funnel,Linen (muslin cloth) ,Tray,Oven

Light source ,REAGENTS,90% Ethanol (1800ml) ,10% Distilled water (200ml)

3.1 PREPARATION OF CRUDE EXTRACT

250g of the powdered sample was measure and poured into the round bottom flask. 90 percent ethanol and 10 percent distilled water was measure using measuring cylinder and was poured into the sample in the flask, then mix with the sample and reflux for 4 hours. After refluxing it was allow to cooled and was filter using linen, funnel and beaker. The solution was pour into the tray and was put into the oven at temperature of 40 ^{0}C for 3 days.

3.2 PREPARATION OF TEST SOLUTIONS

The stock solution of the ethanol extract was used as diluent to prepare concentrations of the crude extract (A, B, C and D) varying between 5g/5ml, 5g/10ml, 2.5g/10ml and 1.25g/10ml from the stock solution, distilled water was used as diluents to prepare the concentration.

3.3 ANTIMCROBIAL ACTIVITY OF THE EXTRACT MATERIALS AND METHODS

Nutrient agar base,Distilled water ,Flat bottom flask,Autoclave machine,Petric disc ,Wire loop,3 Gram positive, 3 Gram negative and 1 fungal isolates ,Test tubes ,Test rack,Coke borer,Incubator,Peptone water,Cotton wool,70 percent methanol,Tape water,1ml needle,Masking paper/Tape,Weighing machine Set square divider,Meter rule.

3.4 PREPARATION OF CULTURE MEDIA (NUTRIENT AGAR AND PEPTONE WATER)

24g of nutrient agar powder was weighed using a weighing balance. The powder was dissolve in 1000ml of distilled water, the solution was mixed up homogenously until the crystals are completely dissolved, using autoclave tape, the cover was tightly raped to avoid spillage when autoclaving. The solution was loaded inside a basin, and then put into the autoclave machine. The temperature and pressure gauge was set and the machine was tight in all the opposite direction, finally, it was plugged to a light source for sterilization. The machine was allowed to work at 121^0c for 15 minutes at 15lbs (pounds pressure). The machine was kept off and allowed to cooled for 1 hour, gently remove the cap and the media. The media was allowed to cool at 45 ^{0}C and dispensed 10-15ml in a sterile Petric dish, allowed to cooled and store in refrigerator for further uses.

3.5 INOCULATION

The sterile nutrient agar plat was loaded inside a drier to drain excess water of condensation using a sterilized wire loop, I obtained a descret colony of the young cultured isolate (bacteria) of respective, 3 gram positive, 3 gram negative and 1 fungal (streptococcus app., Staphylococcus aureas, Crynebacterium app., Pseudomonas aeruginosa, Shigella app., salmonella spp and candida albicans.)

The isolates are transferred to the nutrient agar plates by a pour plate method and swan to the entire surface, drain off the excess fluid and allow the plate right side up.

3.6 SUSCEPTIBILITY TEST USING THE EXTRACT AS ANTIBIOGRAM

3.6.1 DITCH PLATE METHOD

Using a coke borer of 4mm in diameter, hole is obtained by ditching into the center of the nutrient agar plate with bacterial inoculated. This is done into three places to obtained an average reading. 0.1ml of the extract of a known concentration 5g/5ml, 5g/10ml, 2.25g/10ml and 1.25g/10ml are dispensed into the respective hole against all the inoculate in the nutrient agar plates, gently take to incubator without shaking to avoid spillage and was incubated at 35°c for 18-24 hours.

3.6.2 READING SHOWING ZONES OF INHIBITION

The zone of inhibition is considered as the logarithmatic power or the capability of the extract to inhibit bacterial metabolic activities. These are seen by physical examination from the hole of the extract to the diameter where the growths are cleared.

Using a set square divider and a meter rule, the zone of the inhibition is taken and measured with a meter rule. Then, the average was noticed and was repeated in all the plates.

3.7 MINIMUM INHIBITORY CONCENTRATION VALUE (MIC)

These are the concentration set to determine the drugs or extract concentration in g/ml. However, for the purpose of this research 5g/10ml is taken as the highest concentration.

3.7.1 METHOD

Sterile test tubes are arranged in a test tubes rack, 2ml of small letter water was poured into all the tubes and 1 loopful of the isolate was added. Add 2ml of the extract into the first tubes and titrate across the test tubes and discard the 8 tubes, repeat all this to all the isolates and incubate at 37 ^{0}C for 18-24 hours.

3.7.2 READING

Reading was taken by the physical examination, notice where turbidity start to show bacterial growth. This is termed as T, where the tube is clear is termed as C. The minimum inhibitory concentration value (mic) is taken from the first clear tube. Read and records from all the tubes.

RESULT AND DISCUSSION

Results of Analysis ,

90% ethanol and 10% distilled water extraction

Weight of sample = 250g

Weight of beaker = 105.98g

Weight of empty tray = 363.9g

Weight of beaker + extract = 130.5g

(Weight of beaker + extract) – Weight of beaker

130.5g – 105.98g = 24.52g

Therefore extract = 24.52g

4.1 DETERMINATION OF PERCENTAGE YIELD

$$\% \text{ yield} = \frac{\text{weight of extract (g)}}{\text{weight of sample (g)}} \times 100$$

$$= \frac{24.52\ (g)}{250\ (g)} \times 100$$

$$= 9.81\%$$

4.2 RESULT OF ANTIMICROBIAL ACTIVITY OF THE EXTRACT

TABLE 2: antimicrobial susceptibility test using plant extract as antibiogram (mitracarpus scaber)

S/NO	Isolates	Concentration g/ml (5/5)	Zone of inhibition(mm)			Average/error
1	Streptococcus spp	A	40	41	43	41.33 ± 1.53
2	Staphylococcus aureus	A	43	40	44	42.33 ± 2.08
3	Shigella spp.	A	34	34	31	33.00 ± 1.73
4	Corynebacterium spp	A	36	36	41	37.67 ± 2.89
5	Pseudomonas aeruginosa	A	29	29	28	28.67 ± 0.58
6	Salmonella spp	A	34	33	37	34.67 ± 2.08
7	Candida albicans	A	31	30	30	30.33 ± 0.58
1	Pseudomonas aeruginosa	B	30	30	29	29.67 ± 0.58
2	Candida albicans	B	30	32	30	30.67 ± 1.15
3	Corynebacterium spp	B	35	32	37	34.67 ± 2.52
4	Salmonella spp	B	33	33	35	33.67 ± 1.15
5	Staphylococcus aureus	B	41	40	41	40.67 ± 0.58
6	Streptococcus	B	45	45	45	45.00 ± 0.00

	spp					
7	Shigella spp.	B (2.5/10)	26	30	30	28.67 ± 2.31
1	Candida albicans	C	22	22	21	21.67 ± 0.58
2	Corynebacterium spp	C	30	28	27	28.33 ± 1.53
3	Streptococcus spp	C	28	27	28	27.67 ± 0.58
4	Shigella spp.	C	27	29	28	28.00 ± 1.00
5	Salmonella spp	C	38	35	35	36.00 ± 1.73
6	Staphylococcus aureus	C	39	38	38	38.33 ± 0.58
7	Pseudomonas aeruginosa	C (1.25/10)	30	29	28	29.00 ± 1.00
1	Candida albicans	D	No activities			
2	Pseudomonas aeruginosa	D	26	25	26	25.67 ± 0.58
3	Shigella spp	D	24	25	24	24.33 ± 0.58
4	Staphylococcus aureus	D	31	30	31	30.67 ± 0.58
5	Streptococcus spp	D	27	27	31	28.33 ± 2.31
6	Salmonella spp	D	27	26	27	26.67 ± 0.58
7	Corynebacterium spp	D	29	28	29	28.67 ± 0.58

The table above shows the result of the sensitivity and resistance of the extract of mitracarpus scaber, where only three bacterial, that is staphylococcus aureus,

Corynebacterium spp. And salmonella spp. were sensitive to the extract with the highest zone of inhibition as 2.98mm, 2.08mm and 2.08mm respectively at the concentration of 5g/5ml while the lowest was streptococcus spp, With the zone of inhibition of 0.00mm at the concentration of 5g/10ml. Other includes candida albicans, which is fungi is resistance at the concentration of 1.25g/10ml.

4.3 RESULT OF MINIMUM INHIBITORY CONCENTRATION (MIC)

TABLE 2: Minimum inhibitory concentration (MIC) of mitracarpus scaber extract

S/NO	Organisms	Concentration used (5g/10ml) value						
		5	2.5	1.25	0.63	0.31	0.15	0.12
1	Streptococcus spp	C	C	C	C	MIC	T	T
2	Corynebacterium spp	C	C	C	MIC	T	T	T
3	Pseudomonas aeruginosa	C	C	C		MIC	T	T
4	Shigella spp	C	C	MIC	T	T	T	T
5	Salmonella spp	C	C	C	IC	MIC	T	T
6	Candida albicans	C	C	C	MIC	T	T	T
7	Staphylococcus aureus	C	C	MIC	T	T	T	T

C= Clear, T= Turbidity, MIC = Minimum inhibitory concentration value

The above table shows the minimum inhibitory concentration of the extract. Shigella spp. And streptococcus spp. has the highest MIC at the concentration of 1.25ml while the lowest was staphylococcus aureus, Pseudomonas aeruginosa and salmonella spp. At the concentration of 0.31ml.

4.4 REULT OF PHYTOCHEMICAL SCREENING.

S/NO	METABOLITES TEST	RESULT
1	TEST FOR CARDIAC GLYCOSIDE	
	(i) salkowski test	+
	(ii) Liebemann-Burchard tes	_
2	TEST FOR TERPENNOID	
	Acetic anhydrite test	+
3	TEST FOR TANNINS	
	(i) Ferric chloride test	+
	(ii) Lead ethanoate test	
4	TEST FOR CARBOHYDRATE	
	Molisch`s test	+
5	TEST FOR MONOSACCHARIDE	
	Barfoed`s test	_
6	TEST FOR ANTHRAQUINONES	
	BORNTRAGER`S TEST	_
7	TEST FOR KETOSE	+
	Salivanoff`s test	+
8	TEST FOR PENTOSE	
	Hydrochloric acid test	+
9	TEST FOR PHLOBATANNINS	
	Hydrochloric acid test	+
10	TEST FOR SAPONIN	
	FROTHING TEST	+
11	TEST FOR PHENOLIC	

	HYDROXYL	
	Ferric chloride test	+
12	TEST FOR FLAVONOID	
	(i) Sodium hydroxide test	+
	(ii) Lead ethanoate test	+
13	TEST FOR ALKALOID	
	(i) Dragendorff`s reagent	+
	(ii) Mayer`s reagent	+
14	TEST FOR SOLUBLE STARCH	
	Hydrochloric acid test	+
15	TEST FOR RESIN	
	Copper acetate test	
16	TEST FOR CARDENOIDLIDES	+
	Keller – killiani test	

+ = present

- = absent

The above table shows the metabolites test result carried out during the phytochemical screening of mitracarpus scaber. Some of the metabolites were presence while others are absence such as Monosaccharide, Anthraquinone and soluble starch.

4.5 DISCUSSION

Report have shown that the extract of mitracarpus scaber "Zucc"has antibacterial and antifungal activities (Ahonkhai et al., 1999). This work equally has confirmed such findings. Although, the application of heat in the extraction method may have affected

the potency of the antiFungal organisms in the different formulations.

Mitracarpus scaber "Zucc" is currently being used in the treatment of various diseases conditions without standardization. The standardization of a crude drug is an integral part of establishing it's correct identity. Before any crude drug can be includes it a herbal pharmacopoeia, pharmacognostic parameters and standards must be established. (Abere et al., 2007). The results of these investigation could therefor, serve as a basis for proper identification, collection and investigation of the plant. Phytochemical evaluation revealed the presence of tannins and other metabolites which has been claimed to be responsible for it's antimicrobial activity.

CONCLUSION

Medical plants are plant which have a recognized medicinal uses. These parameters, which aree being reported for the first time , could be useful in the preparation of the herbal section of proposed Nigerian pharmacopoeia. Any crude drug which is claimed to be mitracarpus scaber but whose characters significantly deviate from the accepted standard above would then be rejected as either contaminated, adulterated or downright fake. It phytochemical evaluation revealed the presence of different metabolites such as alkaloids, carbohydrate, resin and many others. In conclution, these findings should be suitable for inclusion in the proposed pharmacopceia of Nigerian medicinal plants.

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