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Antimicrobial activity of gridle pod leaves extract against some tested organism

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Abstract

The research is poised to examine the antimicrobial effects the leaves extract of Gridle pod leaves (*Mitracapus Scabber*). The antimicrobial assay was carried out using nutrients agar method. A well prepared nutrient agar were prepared in a sterile condition and allowed to solidified in 24 different petrish dish, they were inoculated with the tested organism in a sterile condition with a load of 1.0×10^6 cfu.. The leaves were obtained, air-dried and pounded to powder. Ethanol and water was used in the extraction in the Laboratory. The leaves extract was concentrated on electric water bath.The following concentration where prepared by serial dilution; 100%, 50%, 25% and 12.5% respectively and were used to test for antibacterial property in each petrish dish. The process was duplicated using a standard Ciprofloxaxin 500mg as the positive control. The results of the antimicrobial determination of gridle pod leaves show that *E.Coli* has a zone of inhibition of 40.11mm, 36.32mm, 21.12.mm 8.21 mmfor 100%, 50%, 25% and 12.5 % concentration while the extract when tested against *Staph. aureus* recorded the zone of inhibition of 32.51mm, 24.13mm 21.12,mm and 6.08mm for 100%, 50%, 25% and 2.5% concentrations. From the results *E.Coli* was found to be more susceptible to the test samples than *Staph. Areus*. The more the concentration of the extracts the wider the measured zone of inhibition hence in proportional to the antimicrobial activity. Phytochemical screening of the gridle pod extract was also carried out, the results revealed that both water and ethanol extract contain tannins, saponin, and phenol.The Terpeniod and Alkaloid where not detected. The phytochemical content revealed the medical potential of the extract from the leaves. Genarally the results indicate that Gridle pod leaves have a potent antimicrobial activity

Keywords: Zone inhibition; Extracts; Potents; Standard drug; Susceptible; Phytochemicals

1 Introduction

Mitracarpus scabe which is also known as *Mitracarpus villosus* (SW) DC, is a very common weed of cultivated or fallowed land it is called 'irawo ile' in Yoruba language. Recent studies have shown that alcohol extracts of the aerial parts of *M.villosus* had *in-vitro* antimicrobial activities against *Dermatophilus congolensis*. Experiments have proved that ointment containing alcohol extracts of *M. villosus* used topically had a high efficiency against bovine *Dermatophilosis* and cured tested animals without recurrence [1].

Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficiency and quality assurance of ethno- medicine [2]. In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents. Thus it is anticipated that phytochemicals with good anti-fungal activity will be used for the treatment of fungal infections. This is because according to Arora and Keur (2019), the success story of chemotherapy lies in the continuous search for new drugs to counter the challenges posed by resistant strains of micro-organisms. Studies indicate that in some plants, there are many substances such as peptides, tannins, alkaloids, essential oils, phenols and flavonoids among others which could serve as sources of antimicrobial production.

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These substances or compounds have potentially significant therapeutic applications against human pathogens including bacteria, fungi and viruses [3][4],. The development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants [5][6] Antibiotic resistance has become a global concernas the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens[7] [8]. Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for the development of novel drugs because of the great diversity in their chemical structure. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structure and novel mechanisms of action for new and re-emerging infectious diseases [9]. Therefore, researchers are increasingly turning their attention to ethno-medicine, looking for new leads to develop more effective drugs against microbial infections [10] and this has led to the screening of several medicinal plants for potential antimicrobial activity [11] [12]. Phytochemical composition, bioactivity and wound healing potential of *Euphorbia heterophylla* leaf extract by [13] showed that the phytochemical analysis revealed the presence of medicinally active constituents of the plant which is rich in Alkaloids, Flavonoids, Saponins and Tannins. The order of decreasing concentration of phytochemical from aqueous and ethanol extracts of fresh and dried sample are Alkaloids >Cyanide> Tannins> Flavonoids >Saponins.

The research into biologically active compounds from natural sources has always been of great interest for scientists looking for new sources of useful drugs against infectious diseases. *Mitracarpus scaber*is widely employed in traditional medicine in West Africa for headaches, toothache, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases and leprosy [14]. Among the folkloric uses, the juice of the plants is applied topically for the treatment of skin diseases (infectious dermatitis, eczema and scabies) [15] [16] observed that a lotion and a skin ointment made with the aerial part of *M.scaber* are used for skin infections or skin diseases and other infectious.

Previous studies by [17] reported the isolation of pentalogin from fresh, aerial parts of *Mitracapus scaber* which demonstrates a potent antifungal activity against *Candiba albican s*and *Trichophytonsoudanense.* Other investigations [18] showed that different extracts of *M*. *scaber* exhibited broad antibacterial and antifungal activity against standard strains and clinical isolates of *Staphylococcus aureus* and *C. albicans* responsible for common skin infections. More recently, [19] reported the hepato protective effects of *Mitracarpus scaber* decoction on tetrachloromethane (CCl4) induced hepatotoxicity *in vivo* as well as *in vitro* using isolated hepatocytes.

Figure 1 Image of *Mitracarpus scaber*

1.1 Statement of Research Problem

The use of *Mitracarpus scaber* has been on the basis of trial and error in different communities in Africa without any scientific basis. Plant parts have been used in different locations for the treatment of different ailments which sometimes bring about conflicting results.

Often traditional healers use plants according to their analogy and morphological similarities to the ailment being treated. For example, plants containing red juice are used to treat ailments connected to menstruation problems and bleedings [20][21]. There is therefore the need to ascertain the basis for the claims of the efficacy of the plants used locally in ethno-medicine.

1.2 Aims and Objectives of the Study

1.2.1 Aim of the Study

This project is aim at determining the phytochemical composition and anti-microbial potential of Gridlepod leaves (*Mitracarpusscaber)*.

1.3 Objective of the Study

The specific objectives of the study are to:

- Prepare ethanol extract of Gridlepod leaves (*Mitracarpusscaber)*.
- Use organic solvents in the extraction of bioactive components present in the plant.
- Assess the antimicrobial activities of the extracts of leaves of *M.scaber*on some selected microorganisms.
- To evaluate the phytochemical constituents Gridlepod leaves (*Mitracarpusscaber)*.

1.4 Justification of the Study

The increasing resistance to antibiotics has resulted in the research to form new organic molecules from plants with antimicrobial properties for treating diseases since some micro-organisms have developed resistance to many orthodox drugs [7]. There is the need to find an alternative approach in the treatment of infectious diseases. Using local plants will be a welcome development as the cost will be minimal.

The leaves and stem bark extracts of *Mitracarpus scaber* have been reported in the treatment of various ailments such as ulcer, cancer, skin diseases e.t.c. It is therefore important to scientifically investigate these plant parts to ascertain their therapeutic potentials. Determination of their chemical composition as well as antimicrobial efficacy against specific pathogens is important in the recognition of this plant as a potent commercial medicinal plant. Tests can determine its efficacy against a pathogen and thus, establish the minimal dosage required for the treatment of ailments.

2 Material and methodology

2.1 Collection and Preparation of Samples

The whole leaves of *Mitracarpus scaber* was collected from Felele in Lokoja Local Government of Kogi State. The plant samples were thoroughly washed with clean water to remove earthly materials and air-dried at room temperature for about three weeks. Dried samples were pulverized using pestle and mortar and a domestic electric grinder.

2.2 Preparation of Extracts

The powdered samples were soaked in distilled water and ethanol for 48 hours after which they were filtered using muslin cloth. The filtrate was then left to air dry. The crude extract gotten from the sample were stored in clean bottles and tightly covered to avoid contamination. The concentrated crude extracts were freeze-dried and kept under refrigeration until further use.

2.3 Bacterial Strain Collection

Standard strains of four pathogenic microorganisms were collected from the Microbiology Laboratory of specialist hospital Lokoja.

2.4 Acute Toxicity Study

The oral median lethal dose (LD50) of the extracts was determined using experimental rats accoding to the method of[22]

2.5 Preparation of test organisms

Standard strains of *S. aureus* and *E. coli*, were propagated on nutrient agar plates and maintained on the plate at 2-4^{o}C. Isolates were sub-cultured in Mueller-Hinton broth at 37^{o}C for 16 hours prior to antibacterial testing. 20 µL of these cultures were sub-cultured into 3 mL of sterile Mueller-Hinton broth and incubated at 37^{o}C for another 2 hours. The turbidity of the actively growing broth cultures was adjusted with sterile Mueller-Hinton broth to obtain turbidity optically comparable to that of 0.5 McFarland Standard (approximately 1-2 x 10^{8} CFU mL^{-1} for *E. coli*).

2.6 Test Microorganisms

The extracts were tested against four intestinal bacterial isolates *Escherichia coli,* and *Staphylococcus aureus.* The test microorganisms were maintained on nutrient agar slopes; and kept in refrigerator at 4^{o}C.

2.7 Qualitative Phytochemical Analysis

The phytochemical analysis of the extract was carried out to determine the presence of tannins, saponins, alkaloids, phenols and glycoside as described result are as follows;

2.7.1 Test for Saponins

5ml of the extract with 10ml of water in a test tube was shaken and then a full mass of small bubbles formation was taken as an indication of the presence and absence of saponins.

2.7.2 Test for Tannins

Extract plus 4ml of water and drops of ferric chloride was mixed and then immediately green precipitate formation was taken as an indication for the presence of tannins.

2.7.3 Test for Alkaloids

2ml of the extract plus picric and drops were mixed; and orange coloration was taken as an indication for the presence of Alkaloids.

2.7.4 Test For Phenols

Equals volume of the extract was added to equal volume of ferric chloride, a deep bluish green solution was taken as a positive test for the presence of phenols.

2.7.5 Test for Terpenoids

In brief, 5ml of extract (0.5mg/ml) was mixed with 2ml chloroform and 3ml concentrated H_2SO4 was carefully added to form a layer. A reddish brown colouration of the interface was indication of terpenoids.

2.8 Antimicrobial Activity Assay

Antimicrobial activities of the plant extracts were tested using agar-well diffusion method [23]. Mueller- hinton agar was prepared and allowed to solidify. Swab stick was used to swab the overnight culture of the selected strains of bacteria (1.0× 10^{-6} cfu) on the molten Mueller hinton agar and 8mm sterile cork borer was used to bore wells on the agar surface. The extracts at concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml were poured into the wells using sterile syringe and allowed to diffuse and then incubated at 37^{o}C for 24 hours. The plates were observed for the zone of inhibition around the wells and the zones of inhibition were recorded in millimetres. The processabove were repeated with a Ciprofloxacin 500mg instead of the extracts aspositive control.

3 Result

3.1 Result of Acute Toxicity

No sign of toxicity or mortality observed even up to a dose of 5000 mg/kg of the aqueous extracts. The result of the acute toxicity studies. the oral LD50 of each of the extracts was then taken to be > 5000mg/kg.

3.2 Result of the Antibacteria effects and phytochemical screening of Gridlepod leaves extract

The Phytochemical screening of the extract test positive for Tannin, Saponin, and Phenol except Alkaloid and Terpenoids using methods variously described by [24] and [25]

Table 1 Phytochemical Composition of GridlepodLeaves

Phytochemical compound	Ethanol extracts	Water extract
Tannins	+	+
Saponins	+	+
Phenol	+	+
Alkaloid	-	-
Terpenoids	-	-

Key:+ =Detected; -=Not detected

Table 2 Antimicrobial test for ethanol extracts of Gridlepod leaves (mm)

	100%	50%	25%	12.5%
Escherichia coli (diameter in mm)	40.11	36.32	21.12	8.21
Staphylococcus genus (diameter in mm)	32.51	24.13	12.09	0

Table 3 Antimicrobial test for Ciprofloxacin (Standard drug)

	100%	50	25%	12.5%
Escherichia coli (diameter in mm)	73.20	60.30	30.00	25.40
Staphylococcus (diameter in mm)	70.10	57.20	31.50	24.10

The results of the ntimicrobial test for ethanol extracts of Gridlepod leaves (mm) is shown in table 1, while the result of Antimicrobial test for Ciprofloxacin as a standard drug is shown in table 2

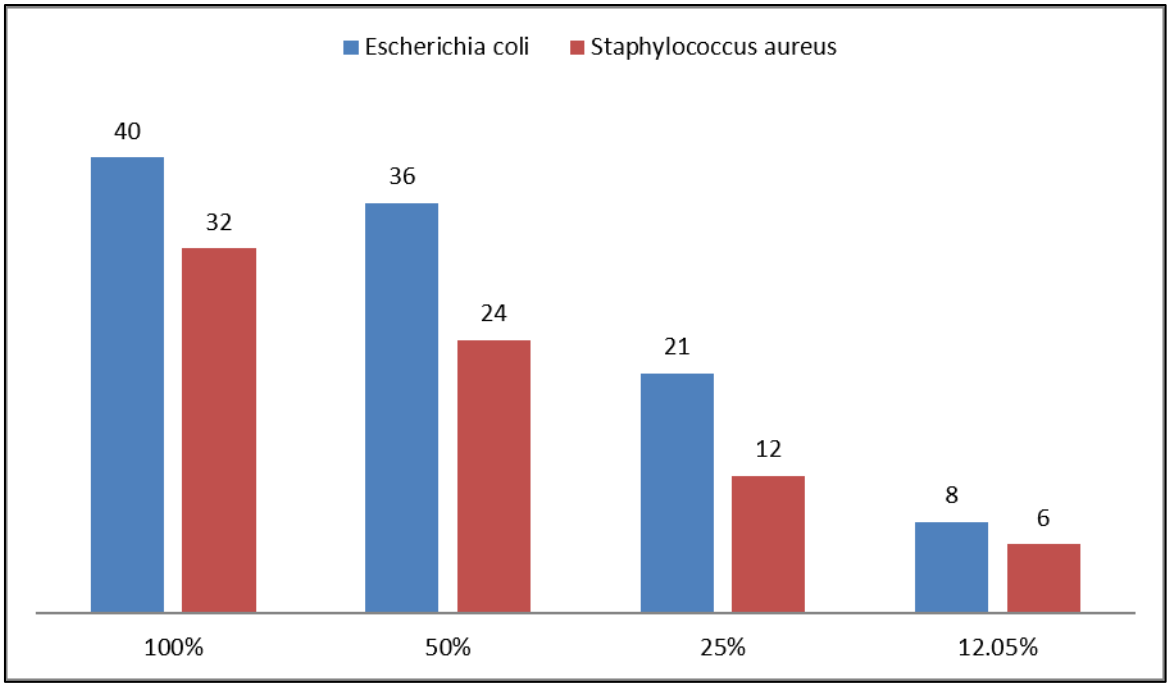

Figure 2 Zone f Inhibition Effect Of Ethanol Extracts Oon The Organism

4 Discussion

The results of the phytochemical screening of gridlepod leaves revealed the presence of Tannins, Saponins and phenol in both water and ethanol extracts. While Terpenoids and Alkaloid where absent. Their presence shows the medicinal properties of gridlepod leaves. Tannins for example are known to be made up of phenolic compounds, Tannins possess homeostatic properties and therefore is widely used as tropical application on superficial wounds and infections.

Tannins is used in treatment of urinary tract infection, diarrhea, healing of wound and dysentery. The present of tannins and saponins in medicinal plant explain why each sample can be used for diabetis treatment because both constituents ethno pharmaceutical uses are to treat diabetes and hyperglycemia disorder often associated with diabetes.

The presence of phenols in medicinal plant have been used for medicinal applications for example as laxatives and antimicrobial and anti-inflammatory agents.

Saponins are a class of organic compounds that increase the output force of the heat and increase its rate of contraction by acting on the cellular sodium-potassium at phase pump. Each of the phytochemicals present in the sample have its unique medicinal properties that isthe reason for it antimicrobial activity

The result of the antibacterial activity of gridle pod leave against E-coli has 40.11mm, 36.32mm, 21.12 mm and 8.21 mm for 100%, 50%, 25% and $12\frac{1}{2}$% respectively while the extract tested against *staph. aureus* recorded 32.511mm, 24.13mm, 12.09 mm and 6.08 mm for 100%, 50%,25% and $12\frac{1}{2}$% zone of inhibition respectively. From the results, *E. coli* is more susceptible to test sample than *staph. aureus*. For example, in figure 2 compare the two results of the two cultured organism at different concentration. Gridle pod leaves extract however show a significant antibacterial activity is dose dependent. The more the concentration of the extract the wider the zone of inhibition hence the more the antibacterial activity.

Interestingly,[26] reported antimicrobial effect of chloroform and water extracts of Gridlepod leaves on hospital strains of bacteria. It was concluded from the preliminary phytochemical analysis shows that phenols and tannins detected in the extracts may contribute to the antimicrobial effect.

This may be the reason why Gridlepod leaves also showed similar anti-microbial activities in this research, indeed, previous study on Gridlepod leaves have also shown that this plant has anti-bacterial property over bacteria [27].

5 Conclusion

From the figures and the tables above, all extracts of Gridlepod leaves showed varying degrees of invitro antibacterial activity on microorganism tested. The zones of inhibition for the isolates are different which could be due to the nature and concentration of bacterial vis-à-vis their mode of action . It is therefore concluded that Gridlepod leaf has a tremendous latent in terms of medicinal uses. Constantly, it contains different chemical constituents. This review comprises the pharmacological aspects of gridlepod leaves as a rich source of phytonutrients. Numerous studies have shown that gridlepod leaves exhibit numerous properties like Anti-flammatory, Antiseptic , Stimulant , anti-malarial. However more research is necessary to investigate further on the antmicrobial activity.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby declare no conflict of interest.

No conflict of interest to be disclosed.

Informed consent was obtained from each of the individual participants included in this study.

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