International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 11 Issue 4, April 2024

ISSN: 2394-4404

Antimicrobial And Phytochemical Properties Of *Mitracarpus Villosus*

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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 villosus as syrup, the antimicrobial activity of the formulation was assessed using well diffusion method while determination of Minimum Inhibitory Concentration was assesses using paper disk method. The result revealed that the Mitracarpus villosus inhibited the growth of Pseudomonas aeruginosa and E. Coli, at highest concentration while Staphyloccous aureus, and Bacillus Subtilis displayed no sensitivity. Phytochemical screening was carried out on the aqueous and ethanolic extract and the qualitative determination of phytochemical constituent. The phytochemical analysis of the plants indicated the presence of tannins, flavonoids and saponins which may be responsible for the antimicrobial activity of the plants. This makes it a potential plant based pharmaceutical..***

Keywords: Antimicrobial activity, Extract, syrup, Bacteria and Mitracarpus villosus

I. INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Avoseh *et al.*, 2019). According to the World Health Organization (WHO) in 2018, more than 80% of the World's population relies on traditional medicine for their primary health care needs. Traditional medicine is an important part of African culture and local medicinal systems vary among cultural groups and regions (Adesina *et al.*, 2019). Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficiency and quality assurance of ethno- medicine (Adekunle 2020). In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as source of medicinal agents. Thus it is anticipated that phytochemicals with good anti-fungal activity will be used for the treatment of fungal infections. According to Abubakar, *et al.*, (2016), 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. These substances or compounds have potentially significant therapeutic applications against human pathogens including bacteria, fungi and viruses (Ouadja *et al.*, 2018).

Mitracarpus scaber (*Rubiaceae*) which is also known as *Mitracarpus villosus,* is a very common plant which can be found on cultivated or fallowed land. Recent studies have shown that alcoholic extracts of the aerial parts of *M.villosus* had in-vitro antimicrobial activities against *Dermatophilus congolensis*. Experiments have proved that ointments containing alcoholic extracts of *M. villosus* used topically had a high efficiency against *Bovine dermatophilosis* and cured tested animals without reoccurrence (Adesina *et al.*, 2019).

Different *Mitracarpus* species have demonstrated antibacterial and antifungal activities. Extracts obtained from the members of the family Rubiaceae have been used to treat liver diseases, acute hypertension, haemorrhagic shock,

ISSN: 2394-4404

diabetes mellitus, cancer, ischemia, and perfusion injury, although, only a few members of the genus *Mitracarpus* have been studied and documented in this regard (Aboh *et al.*, 2019). However, the vast medicinal uses of these species reported so far cannot be overemphasized. For instance, in Nigeria, the extract of *M. scabrum* has been used to treat ringworm, itching, wound, lice, and ulcer. The plant has been used to treat leprosy, skin diseases, toothache, headache, and dyspepsia, as well as tumour, wounds, burns, cuts, and boils. Moreover, literature has confirmed the use of *M. scabrum* to treat toothache, headaches, venereal and hepatic diseases, dyspepsia, amenorrhea, eczema, mycosis, and scabies. The leaves extract of *M. scaber* has been used to treat wounds, hepatitis, jaundice, inflammation, bacterial and fungal infections, toothache, headache, dyspepsia, amenorrhea, venereal diseases, liver diseases, leprosy, sore throat, respiratory diseases, skin diseases, dermatomes, amenorrhea, skin infections and as an antidote for poisons (Aboh *et al.*, 2019).

II. MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

The plant *Mitracarpus villosus* used for this research work was collected at the school botanical garden of the Department of Science Laboratory Technology, Federal Polytechnic Offa Kwara State. These were then collected in a sterile polythene bag, rinsed, air dried and made into powdery form before use.

PREPARATION OF PLANT MATERIALS

The *Mitracarpus villosus* leaf was separately extracted using ethanol and distilled water. These were prepared using the method as describe by (Moody *et al*, 2016). These were carried out by suspending 40g of the finely ground leaves in 400 milimeter of distilled water and 95% ethanol. The ethanolic extraction was done for 24hours. The extract were then decanted and then filtered through a filtered paper. The filtered was then sterilized through a membrane filter and evaporated to dryness at 45 degree Celsius. The extract solution were then stored in the refrigerator at 4 ^{0}C until used

TEST ORGANISM

The test organism used for the antimicrobial assay of this medicinal plant include: *Pseudomonas aureginosa* (clinical strain), *Escherichia coli* (clinical strain), *Staphylococcus aureus* (clinical strain) and *Bacillus subtilis*. The test organism mention above was collected from the university of Ilorin teaching hospital and aseptically transported to laboratory for further characterization.

SCREENING OF EXTRACT FOR ANTIMICROBIAL ACTIVITY (USING WELL DIFFUSION METHOD)

To test for antimicrobial activity of plant agar well diffusion method was employed. 1g of aqueous was reconstituted in 5ml of sterile distilled water to 200 mg/ml concentration and was vortex for homogeneity. The broth culture of the test organism was compared to the turbidity of 0.5% MacFarland standard. 3 drops of standardize culture was transferred into a sterile petri dish. Freshly prepared cooled tempered sterile molten Muller Hilton agar was added to petri dish that contained standard organism, it was rocked gently and allow to set at room temperature a sterile cork borer was used to make 2 well of 6mm diameter on a solidified agar, a drop of (0.1ml) each extract of both aqueous and ethanolic was introduced into the well and labelled respectively, it was incubated at 37 ^{0}C for 24 hours control agar plate were made in parallel and included(OVC) Organism viability control, (MSC)Medium sterility control, Ethanotic extract was equally treated.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (USING PAPER DISK METHOD)

To determine the minimum inhibitory concentration (MIC) of the extract paper disk method was employed 0.9g, 0.8g, 0.7g, 0.6g, 0.5g, 0.4g, 0.3g, 0.2g and 0.1g of plant leave of both aqueous and ethanolic extract were accurately weighed on a weighing balance and dissolved in 5ml of sterile distilled water to make the concentration of 180 mg/ml, 160 mg/ml, 140 mg/ml, 120 mg/ml, 100 mg/ml and 80 mg/ml respectively. The sterile paper disk was soaked in each extract and allowed to solidify at room temperature after which they are labelled with the appropriate test organism, the organism were standardize as describe for antimicrobial assay. Each plate was then streaked with a loopful of standardized sensitive test organism (organism that were sensitive to plant extract during determination of antimicrobial activity) each paper disk of the extract of both aqueous and ethanolic extract as different concentration was placed on a Muller Hilton agar that contained the standardize organism. Control agar plate were made in parallel include organism viability control (OVC), medium sterility control (MSC), and extract sterility control (ESC). The plates were then incubated at 37 ^{0}C for 24hours. Ethanolic extract was equally treated.

III. RESULTS

TEST ORGANISM	ACTIVITIES	ZONE OF INHIBITION (mm)
Staphylococcus aureus	-	-
Bacillus subtilis	-	-
E. Coli	+	8
Pseudomonas aeruginosa	+	4

Key
(+) Activities
(-) No activities

Table 1: Antimicrobial activities of aqueous extract of Mitracarpus villosus leaves at 200 mg/ml concentration

IJIRAS

ISSN: 2394-4404

TEST ORGANISM	ACTIVITIES	ZONE OF INHIBITION (mm)
Staphylococcus aureus	-	-
Bacillus subtilis	-	-
E. Coli	+	15
Pseudomonas aeruginosa	+	18

Key
(+) Activities
(-) No activities

Table 2: Antimicrobial activities of ethanolic extract of Mitracarpus villosus leaves 200 mg/ml concentration

ORGANISM / CONC.	180	160	140	120	100	80	60	40	20
Pseudomonas aeruginosa	12mm	9mm	* 4mm	-	-	-	-	-	-
E.Coli	5mm	* 2mm	-	-	-	-	-	-	-

Key
(-) No activities
(+) Activities
(*) Mic

Table 3: Determination of minimum inhibitory concentration of aqueous extract prepared at various concentration in mg/ml

ORGANISM / CONC.	180	160	140	120	100	80	60	40	20
Pseudomonas aeruginosa	16mm	11mm	6mm	* 2mm	-	-	-	-	-
E.Coli	13mm	10mm	* 4 mm	-	-	-	-	-	-

Key
(-) No activities
(+) Activities
(*) Mic

Table 4: Determination of minimum inhibitory concentration ethanolic extract prepared at various concentration in mg/ml

PHYTOCHEMICAL COMPOUND	AQUEOUS EXTRACT
Flavonoids	+
Tannis	+
Saponin	+
Alkanoids	+
Glycosides	+
Anthraquinone	-

Key
(+) indicates presence
(-) indicates absence

Table 5: Result of Phytochemical analyses of aqueous extract from leave of Mitracarpus villosus

PHYTOCHEMICAL COMPOUND	ETHANOLIC EXTRACT
Flavonoids	+
Tannins	+
Saponin	+
Alkanoids	-
Glycosides	-
Anthraquinone	-

(+) indicates presence
(-) indicates absence

Table 6: Result of Phytochemical Analyses of Ethanolic Extract from leave of Mitracarpus villosus

IV. DISCUSSION

The result of antimicrobial activity of aqueous extract of *Mitracarpus villosus* leaves were shown in Table 1 The result revealed that the aqueous extract of *Mitracarpus villosus* inhibited the growth of *Pseudomonas aeruginosa* and *E. coli*, at highest concentration of 200 mg with the zone diameter of 4 mm and 8 mm while *Staphyloccous aureus*, and *Bacillus subtilis* displayed no sensitivity and absence of zone of inhibition at 200 mg concentration.

The result of antimicrobial activity of ethanolic extract of *Mitracarpus villosus* leaves were shown in Table.2. The result revealed that the ethanolic extract of *Mitracarpus villosus* inhibited the growth of *pseudomonas aureginosa* and *E. coli* at the higest concentration of 200 mg with the zone diameter of 18 mm and 15 mm, while *Staphyloccous aureus* and *Bacillus Subtilis*, displayed no sensitivity and absence of zone inhibition at 200 mg concentration

The result of minimum inhibitory concentration of aqueous extract during determination of antimicrobial activity is shown Table .3. The result revealed that *Pseudomonas aureginosa* and *E. coli*, displayed sensitivity and clear zone at different concentration of the extract used. i.e 40 mg and 160 mg respectively.

The result of minimum inhibitory concentration of the ethanolic extract of the plant material respectively on the test organism that were sensitive to the plant extract during determination of antimicrobial activity is shown in Table .4. The result revealed that *Pseudomonas aureginosa* and *E. coli* displayed sensitivity and clear zone of inhibition at different concentration of extract used. i.e. 120 mg and 140 mg respectively.

The result of the phytochemical screening of aqueous extract of *Mitracarous villosus* leaves was shown in Table 5. The result shows that flavonoids, tannins, saponin, alkanoids, and glycosides were found present, only anthraquineon were absent.

The result of phytochemical screening of ethanolic extract of *Mitracarpus villosus* leaves was shown in Table .6. The result shows that flavonoids, tannis, saponins, were found present, only glycosides, alkanoids anthraquineon were absent. The result is in conformity with that of Crespan, (2019) which expressed antibacterial activities in its phenolic compounds of *Mitracarpus villosus* which may be because of iron deprivation or hydrogen bounding with vital proteins

V. CONCLUSION

The phytochemical analysis of the plants indicated the presence of tannins, flavonoids and saponins which may be responsible for the antimicrobial activity of the plants. The observed antibacterial activities on the susceptible organisms studied were due to the presence of the bioactive metabolites. The activities manifested by the plant extracts against the test microorganisms testify to the fact that, these plants could be used as antimicrobial agents. The Ethanolic extract of *Mitracarpus villosus* leaves possess better antimicrobial activity compared to other solvents. The plant may be useful in the development of drugs for the treatment of infections.

IJIRAS

ISSN: 2394-4404

The findings of the study suggest the great value of the species *Mitracarpus villosus* for use in pharmacy and phototherapy therefore it could serve as a natural source of antimicrobial substances of high importance.

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