PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF THE STEM EXTRACT OF *MITRAGYNA INERMIS*

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Abstract

The stem bark of *Mitragyna inermis* was investigated for phytochemical constituents and antimicrobial activities. Air dried pulverized stem bark parts of *Mitragyna inermis* was sequentially extracted with n-hexane, ethyl acetate, acetone, methanol and water in order of increasing polarity using cold extraction (maceration). The acetone, methanol and water extracts were investigated and found to be effective against the clinically isolates*: Staphylococcus aureus, Escherichia coli, Aspergillus niger, and Candida albican.* Phytochemical analysis of these extracts revealed the presence of alkaloids, tannins, terpene, glycoside, protein, carbohydrate, flavonoids, saponins, steroids, and anthraquinone in all the three extracts. These results justified the traditional uses of the plant.

Key words: antimicrobial activity, extraction, *mitragyna inermis*, phytochemical constituents

Introduction

Mitragyana inermis is used in many countries by traditional medicine practitioners for the treatment of various ailments [1]. It is grown in sub-Sahara Africa [2, 3-5]. It is called Giyayya in Hausa. *Mitragyana inermis* is a bushy tree and grows up to 16 m high. It belongs to the Rubiac eae family. Its trunk is 60 cm diameter with scaly grayish bark. *Mitragyana inermis* is grown on dump perennially flooded site, swampy savannah or in inland site of coastal mangrove [6]. The plant is common across the region from Mauritania to west Cameroun; into the Congo basin and in Sudan [7].

Mitrragyna inermis is widely known and used in traditional medicine in West Africa to treat several diseases [5]. The bark is used for the treatment of fever, high blood pressure, dysentery, syphilis, wound and epilepsy. Ashes obtained from the wood are used for the treatment of oedema. The root, bark and leaves are used for the treatment of anorexia, constipation and

leprosy. The leaves are used for the treatment rheumatism, cramp, syphilis jaundice, weakness, fatigue, and in child birth (make placental expulsion easier). It also serves as a stimulant and a diaphoretic agent [5]. Other uses of *Mitragyana innermis* include as pain killer, also in treatment of arthritis, epilepsy, nasopharyngeal, affliction, stomach trouble, and venereal disposes [8]. The indiscriminate use of herbal medicines including *Mitragyna inermis* plant in our communities despite its claimed efficacy still poses a lot of medical challenges on the users and therefore its phytochemical constituents and antimicrobial activities need to be evaluated for scientific proof.

Experimental

Plants identification

The Plants were identified by a taxonomist at the Herbarium of Ahmadu Bello University, Zaria, and voucher number 259 was assigned.

Samples collection and treatment

Fresh stem bark of *Mitragyna inermis* was collected from Hadejia-Nguru wet land area of Jigawa State. They were washed in water and re-washed in distilled water, air dried and ground to fine powder.

Extraction

About 100 g of finely ground stem bark were soaked in *n*-hexane with occasional stirring for 48 h. The soaked material was filtered and the extract was concentrated using rotary evaporator, before drying and weighing in moist free environment, and then kept for further uses. The residue after extraction with hexane was soaked in ethyl acetate for 48 h with occasional stirring. The mixture was then filtered and the filtrate was concentrated using rotary evaporator, before drying and weighing in moist free environment, and then kept for further uses. The residue was soaked in acetone for 48 h followed by filtration, concentration, drying and weighing. The residue was also extracted with methanol which was filtered, concentrated, dried, and weighed. The residue was extracted with water followed by filtration, concentration, drying and weighing.

Preliminary phytochemical screening

The crude extracts were subjected to phytochemical screening to test for secondary metabolites as described by Chindo [9], Sofowora [10], and Trease & Evans [11].

Determination of antimicrobial activity of crude extracts

The organisms used in this study were *Staphylococcus aureus and Escherichia coli* (for antibacterial test); *Aspergillus niger* and *Candida albican* (for antifungal test). They were clinically isolated and obtained from ATBU Teaching Hospital. The strains were maintained and sub-cultured on nutrient ager (bacteria) and sabroud dextrose ager (fungi). Ager well diffusion method was used to evaluate the antimicrobial activities of the extract as described by [2]. Culture of 0.1 ml of various organisms in nutrient broth was seeded in molten nutrient ager and SDA (bacteria and fungi respectively) that had poured and allowed to set in to sterile Petri dish. Wells were made into the set ager using sterile cork borer and the wells were filled with the extracts solution of 0.1ml (at different concentration for each extracts) left to pre-diffuse for 30 min. Dimethyl sulphur oxide was used as negative control and streptomycin (antibacterial) and ketomazole (antifungal) as positive controls. The bacteria plates were incubated at 37^{o}C for 24h, while the fungi plate were incubated at 25^{o}C for 72 h.

The degree of inhibition were determined by the size of the zone of inhibition measured in mm and were taken as evidence of antimicrobial activity of each of the extracts.

Results and discussions

Table 1: Phytochemical constituents of crude stem extracts of *Mitragyna inermis*.

Extracts/Phytochemical	Acetone	Methanol	Water
Alkaloids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Terpenes	+	+	+
Phlobatanins	-	-	-
Glycosides	+	+	+
Anthraquinones	+	+	+
Steroids	-	-	-
Carbohydrates	+	+	+
Proteins	+	+	+

Table 2: Zone of inhibition (mm) of crude stem extracts of *Mitragyana inermis* at different concentration (μg/ml) against clinical isolates.

TEST ORGANISMS	**Extract Conc. (μg/ml)**	**ZONE OF INHIBITION OF EXTRACT (mm)**				
		Acetone Extract	**Methanol Extract**	**Water Extr.**	**+Control**	**- Control**
E.coli	**10^{-1}**	21.00	20.00	21.50	28.50	0.00
	10^{-2}	20.00	19.00	20.00	22.00	0.00
	10^{-3}	20.00	18.50	16.50	21.00	0.00
	10^{-4}	18.00	16.00	16.00	20.00	0.00
S. aureus	**10^{-1}**	19.50	20.00	21.50	30.00	0.00
	10^{-2}	16.00	18.00	20.60	25.00	0.00
	10^{-3}	15.00	15.00	17.00	24.00	0.00
	10^{-4}	13.00	14.00	14.00	21.00	0.00
Candida albican	**10^{-1}**	19.00	19.00	19.50	25.00	0.00
	10^{-2}	17.00	17.00	17.00	22.00	0.00
	10^{-3}	16.50	16.50	17.00	21.00	0.00
	10^{-4}	16.50	16.50	16.00	19.10	0.00
Aspergillus niger	**10^{-1}**	11.00	12.00	17.00	21.00	0.00
	10^{-2}	11.00	11.00	10.00	18.00	0.00
	10^{-3}	10.00	10.00	10.00	18.00	0.00
	10^{-4}	10.00	10.00	09.30	16.00	0.00

The results of the phytochemical screening of all the three extracts showed the presence of alkaloids, saponins, flavonoids, glycosides, terpene, carbohydrate and protein, and the absence of

phlobatannins, and steroids. Antibacterial and antifungal assay were performed on the acetone, methanol and water crude extracts against the clinical isolates, *Eschericia coli*, *Staphylocolus aureus, Aspergillus niger* and *candida albican*. The results showed that all the extracts were active at different concentrations against the test organisms. Increase in concentrations of the extracts increased the zone of inhibitions. This is expected based on the phytochemical compositions of the extracts. Singh and Bhat [12] reported that some of the secondary metabolites, particularly the flavonoids, were responsible for antimicrobial activity associated with ethanomedicinal plants. Other studies have shown that extracts of plants inhibit the growth of various microorganisms at different concentrations [13-15]. Some studies attributed the antibacterial activity of the plant extracts to the presence of alkaloids, tannins, and flavonoids that possess antibacterial properties [16]. The observed antimicrobial properties may be attributed to the phytochemical constituents of the extract which justified the use of the plant in traditional medicine.

Conclusion

In this work, it was observed that the solvents used, namely, acetone, methanol, and water, can extract certain compounds from plant parts. Acetone, methanol and water extracts were tested for antimicrobials activity and were found to be active against clinically isolates: *Staphylococcus aureus, E. coli, Aaspergilus niger and Candida albican.* The results of this study justified the traditional uses of *Mitragyna inermis* in the treatment of various diseases.

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