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PHYTOCHEMICAL STUDIES OF THE STEM BARK OF *Mitragyna inermis*

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ABSTRACT

Mitragynainermis is a tree species in the ***Rubiaceae*** family that is commonly used in traditional medicine to treat malaria. The plant's extract was obtained by macerating the powdered leaves in methanol. The extracts of the stem of *Mitragynainermis* were investigated for phytochemical constituents, antimicrobial activity, antioxidant activity, and acute toxicity. The results show the presence of alkaloids, flavonoids, steroids, cardiac glycoside, anthraquinone, tannins, and saponins, and the extract was also found to be effective against the clinical isolates: *Staphylococcus aureus*, *Escherichia coli, Aspergillus niger,* and *Candida albicans*. However, the extracts showed strong radical scavenging activity against DPPH for all three extracts. The LD_{50} of 158 mg/kg was calculated for the extracts, and the values were found to be within the practically slightly toxic range, so care should be taken when using the plants in traditional medicine healing.

Key Words: antioxidant, antimicrobial and acute toxicity

INTRODUCTION

For centuries, people have employed plants to treat widespread infectious ailments. Plants have been utilized for a long time because of their low side effects and numerous health advantages, which are primarily used to cure severe and chronic ailments [1] .People have traditionally looked for drug-based treatments for a variety of illnesses and discomforts. At every stage of human progress and in developed civilizations, certain medicinal plants' therapeutic properties have been identified, acknowledged, and passed down to following generations[2]. Many African plants have had their chemical composition studied, and some of the treated by massaging the back. Oedema is treated using ashes extracted from the wood. Leprosy, constipation, and anorexia are treated with the roots, barks, and leaves, while illnesses are treated with the roots, barks, and stem. The leaves are used as a stimulant and diaphoretic, as well as a treatment for rheumatism, cramps, syphilis, jaundice, weakness, and weariness during childbirth (making placental ejection simpler)[4]

This research is a preliminary attempt that tries to better understand the properties of *Mitragynainermis*, a plant that has historically been used as medicine in northern Nigeria, through phytochemical screening, antibacterial activity, antioxidant activity, and an acute toxicity investigation.

MATERIALS AND METHODS

Collection and preparation of plant materials

The plant stem bark has been collected in the Hadejia area and is air dried before being finely crushed in a laboratory environment. The plant was authenticated by the taxomonint at department of biological science Ahmadu Bello

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university Zaria. Our sample for analysis will now be the powder we collected. For a toxicity test, one portion of the leaf powder was freeze-dried, while the other portion was used for biological and phytochemical screening.

Extraction

Cold (Maceration) extractions were carried out using 85% methanol and water, each extract was then concentrate using rotary evaporator (Model RE52A – Shanghai Ya Rong Biochemical Instrument Factory), dried in moist free environment and kept for further uses.

Phytochemical screening

The extract was subjected to phytochemical analysis using standard procedures.

Antimicrobial Activity

A 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 been poured and allowed to set into a sterile Petri dish in order to evaluate the antimicrobial activities of the extract, according to[5]. A sterile cork borer was used to create wells in the set agar, which were then filled with 0.1 ml of extract solution (at various concentrations for each extract) and pre-diffused for 30 minutes. Streptomycin and ketomazole were utilized as antibacterial and antifungal agents, and dimethyl sulphate oxide was used as a negative control. The fungal plates were incubated for 72 hours at 35 degrees Celsius, while the bacterial plates were incubated at 37oC for 24hrs. The degree of inhibition were determine by the size of the zone of inhibition measured in mm and were taken as an evidence of the antimicrobial activity of the extract [6].

Antioxidant Assay

The free radical scavenging activity of plants extracts were measured using the methods describe by [7] with some modifications. 0.1g of each extract were dissolved in 95% methanol, 2ml of each extract solution were added to 2ml of DPPH methanol solution (0.004%), the mixture was shaken and allowed to stand in dark for 30 mins at room temperature similar concentration of ascorbic acid was used as positive control standard. The absorbance of the sample was measured at 517nmn. The absorbance of blank ($2cm^3$ 95% methanol added to $2cm^3$ DPPH solution) was also measured and converted into percentage antioxidant activity using the following formula.

$$AA\% = \frac{A_b - A_s}{A_b} \times 100$$

Where AA% = Antioxidant activity (%)

A_b = Absorbance of the blank

A_s = Absorbance of the sample

Acute Toxicity

The method [8] with slight modification was used for the determination of LD_{50} as a means of evaluating the safety of the extract in the pharmacological trials. Nine albino mice were ramdomaly divided in to 3 group of thrice. The animal in group 1, 2 and 3 recieved 10mg, 100mg/kg and 1000mg/kg respectively all

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treatment were given through intraperitoneal (IP)[8] recommend using single for the second phase of LD50 determination. The three animal were divided into three group of one. Mice in group 1,2,3 were given the extract 1600mg/kg,2900mg/kg and 5000mg/kg and observed the toxic sing and motarlity. Then the LD_{50} is calculated by the formula [9].

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality

RESULT AND DISCUSSION

Table 1: Phytochemical screening

Phytochemicals	
Alkaloids	+
Flavonoids	+
Steroids	+
Cardiac Glycoside	+
Tannins	+
Anthraquinone	+
Saponins	+

Antimicrobial activity

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

TEST ORGANISMS	Conc. (µg/ml)	Methanol Extract	+Control	-Control
E.coli	**10^{-1}**	20.00	28.50	0.00
	10^{-2}	19.00	22.00	0.00
	10^{-3}	18.50	21.00	0.00
	10^{-4}	16.00	20.00	0.00
S. aureus	**10^{-1}**	20.00	30.00	0.00
	10^{-2}	18.00	25.00	0.00
	10^{-3}	15.00	24.00	0.00

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	10^{-4}	14.00	21.00	0.00
Candida albican	**10^{-1}**	19.00	25.00	0.00
	10^{-2}	17.00	22.00	0.00
	10^{-3}	16.50	21.00	0.00
	10^{-4}	16.50	19.10	0.00
Aspergillusniger	**10^{-1}**	12.00	21.00	0.00
	10^{-2}	12.00	18.00	0.00
	10^{-3}	10.00	18.00	0.00
	10^{-4}	10.00	16.00	0.00

Acute Toxicity

TABLE 3: Acute toxicity of the methanol extact of stem of*mitragynainermis*

DOSES (mg/kg)	***Mortality***
Phase 1	
10	-
100	-
1000	*2/3*
Phase 2	
1600	*1*
2900	*1*
5000	*1*

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Table 4: The mean values of the antioxidants free radical scavenging activity of the *Mitragynainermis* stem by DPPH

Concentration µg/ml	% scavenging activity
1000	82.33±0.3%
500	81.7±2.6%
250	80.86±1.6%
125	79.12±1.01%

Discussion

The phytochemical components of plants determine their therapeutic qualities[10]. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and other essential phytochemicals are found in diverse plant sections[11]. They are in charge of the plant's color and organoleptic characteristics [12]. The presence of saponins, steroids, tannins, glycosides, terpenoids, flavonoids, anthraquinones carbohydrates and proteins in *Mitragynainermis* stem but alkaloid and phlabotanins are absences.The result of antimicrobial activity shows that the extract was active at different concentrations against the test organisms. Increase in concentration of extracts increase the zone of inhibitions. This is expected based on the phytochemical compositions of the extracts as[13] reported that some of the secondary metabolite particularly, the flavonoids are responsible for antimicrobial activity associated with ethnomedicinal plants. Previous studies have shown that extracts of plants inhibits the growth of various microorganisms at different concentration[14], [15]. The presence of alkaloids, tannins, and flavonoids, all of which have been demonstrated to have antibacterial activities, was cited in other research as the reason for the plant extracts' antibacterial action[16].Additionally, numerous research have documented the antifungal properties of natural compounds such as phenols, flavonoids, coumarines, quinones, saponins, xatonealkalnids, polypeptides, terpenoids, and essential oils [17], [18].Strong antibacterial activity has been demonstrated by alkaloids, and their structural underpinnings have allowed for the synthesis of several antibiotics with a variety of different modes of action.[19].The capacity of methanol extracts to scavenge free radicals has demonstrated that the extracts exhibit significant antioxidant activity, with a range of 79% to 82% when compared to ascorbic acid. These radical scavenging abilities in different concentration of the extracts were confirmed by the phytochemical components discovered in the extracts.

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Flavonoids have a variety of health advantages, such as antiviral, anticancer, antioxidant, and anti-inflammatory characteristics.[20].The flavonoids have also been shown to possess antimicrobial, and antioxidants activities[21].The study on the antioxidant activity of alkaloids indicated that alkaloids exhibited significant antioxidant activities measured by three antioxidant test systems [22]. Steroids have been reported to possess potent antioxidant activity[23].The 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing/antioxidant power (FRAP) model systems were used to test the tannins for possible antioxidant properties. For all of the extracted tannins exhibited excellent DPPH radical scavenging and ferric reduction activities[24] The saponins have also shown antioxidant activity[25].The acute toxicity effect of mitragynainermis stem extract shows that no animal died within 24 hrs of treatment with 10, 100, 1000mg/kg of the extract per body weight but the the toxicity sing of of general weekness, erection of hairs and rediness of eyes were observed within 24 hrs of administrations. However, for the second test (where three animal are divided in to three) all the three animal died when treated with 1600mg/kg, 2900mg/kg and 5000mg/kg interpretoneally and the same toxicity sing were also observed before death.

CONCLUSION

A pharmacological study was conducted to determine the tolerance limits for M. Inermis stem. This investigation found that no death by predefined dose method with an estimated LD50 greater than 5000 mg/kg was caused by the extracts utilized. The abundance of active chemicals in plantstem extract, including sterols, polyphenols, flavonoids, catechin tannins, alkaloids, and saponosidesmaight be responsible the discovered biological activities. This might provide justification for the various therapeutic uses it has in conventional medicine. After phytochemical analysis and toxicological research on Mitragynainermis, it is important to assess this plant's pharmacological effects on antimalarial activity in order to comprehend the benefits attributed to this plants species.

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