

PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF THE METHANOL LEAF EXTRACT OF *MITRAGYNA INERMIS* (WILD O. KTZE RUBIACEAE).

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ABSTRACT

Mitragyna inermis is a useful medicinal plant used in traditional medicine in Africa. It is used to treat several infectious diseases such as fever, headache, wounds, diarrhea, dysentery, cholera, malaria and other diseases. Fresh leaves of the plant were collected, authenticated, garbled, dried, grinded and extracted using 95% methanol. The extract was used for the phytochemical screening and bacterial susceptibility using well diffusion method.

The result obtained from the study showed the presence of tannins, flavonoids, alkaloids, cardiac glycosides, carbohydrate and reducing sugars in the methanol leaf extract of *Mitragyna inermis* while saponin was absent. The extract showed antibacterial activity on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* with minimum inhibitory concentration of 50mg/ml, 50mg/ml, and 25 mg/ml respectively. The activity was concentration dependent having no effect on tested concentration of 35.5mg/ml and 75mg/ml on *Staphylococcus aureus* and *E. coli* while at 18.75mg/ml on *K. pneumoniae*. *K. pneumoniae* was more susceptible to the extract. The extract was bactericidal at concentration of 100mg/ml on *S. aureus* and *K. pneumoniae* while 200mg/ml on *E. coli*. Thus the study demonstrated the antibacterial activity of methanol leaf extract of *Mitragyna inermis* and some possible phytochemical constituents that may be responsible for its activity.

Key words: Methanol leaf extract, *Mitragyna inermis*, phytochemical screening, antibacterial activity

INTRODUCTION

Health is the most precious of all things and it is the foundation of all happiness (Adesina, 2000). Traditional medicine has developed in various communities in Nigeria in response to the health needs of the people. African traditional medicine provides a holistic treatment (Ali, 2008). The type of treatment varies and include the use of vegetable organs (leaves, barks, roots etc) or product (latex, resins), whole or parts of animals (snail, born), and mineral substances (alum, kaolin). Others are fasting and dieting, hydrotherapy, treatment of burns, massage, psychotherapy, faith healing and therapeutic occultism (Sofowora, 2008). Attempt have been made by scientist to justify or rationalize on a scientific basis many aspect of the practices of the African traditional medical practitioner, some of these medical practices are inexplicable whereas others like the use of many of the herbs, can be rationalized (Aremu and Adekoya, 2009).

In recent times, the rapid development of multi resistance bacterial strains of chemically important pathogens attracts the interest of scientist to develop newer broad spectrum antimicrobials. The

development of synthetic antibiotics such as the third and fourth generation cephalosporin appears to be potent but are scarce, costly and not affordable particularly in developing countries and make compliance difficult (Ibukun, 2008). These necessitate looking for substance from alternative medicine with claimed antimicrobial activity. Ethno pharmacologist, botanist, microbiologists, and natural-products chemists are combing the earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases (Cowan, 1999). Effort in this regard have focused on plants because of their uses historically, and the fact that a good portion of the world's population (80%) particularly in developing countries rely on plants for the treatment of infectious diseases and non infectious diseases (Martinez, *et al*, 1996).

Mytragyna inermis is widely known and used in traditional medicine in the West Africa to treat several diseases (Konkon *et al*, 2008). The back 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 roots, barks and leaves are used for the treatment of anorexia, constipation and leprosy while the root, barks and stem are used for medical illness. The leaves as shown in figure 1 are used for the treatment of

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rheumatism, cramp, syphilis, jaundice, weakness and fatigue, child birth (make placental expulsion easier), serve as stimulant and diaphoretic agent (Konkon *et al*, 2008).

Mytragyna inermis has multiple properties that justify its multiple indications for which it's used in traditional medicine. Several compounds including alkaloids and non alkaloids have been purified from the plant parts (Fiot *et al*, 2005). Some studies showed that the pharmacological and biological properties were mostly due to the alkaloids present in the plant (Traore-Keita *et al*, 2000).

The aim of this study is to carry out the phytochemical screening of the methanol leaf extract of *Mytragyna inermis* and to determine the in-vitro activity of the plant extract on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiela pneumoniae* and also to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the leaf extract on the tested organism.

MATERIALS AND METHODS

Fresh leaves of *Mytragyna inermis* were collected in the month of December 2010 between 5.00 to 6.00 pm at Girmari village, Borno state. The plant was identified and authenticated by a plant Taxonomist in the Department of Biological Sciences, University of Maiduguri and deposited in the Department of Pharmacognosy Herbarium where it was assigned a voucher specimen number PCG0025. The leaves were garbled to remove extraneous material. They were dried under shade, powdered and kept in an airtight container.

Extraction of *Mytragyna inermis* leaves using Methanol

Maceration large cylindrical glass method was used for the extraction.

A 400g weight of the powdered leaves was weighed and put into a large cylinder glass and 1250ml of 95% methanol was poured on the powder, stirred and then covered. It was allowed to stand for 24 hours at room temperature with intermittent stirring. The mixture was filtered and the residue was washed severally with 150ml of methanol. The filtrate obtained was concentrated by evaporating it to dryness using a Rotary Evaporator set at 45°C. The concentrated extract was then collected, dried on a stainless steel tray and weighed.

The dried extract was then packed, labeled and stored in air tight container away from light.

The percentage yield of the extract was calculated using the expression:

$$\text{Percentage yield} = \frac{X}{Y} \times 100$$

Y

Where:

X = weight of the dried concentrated extract.

Y = weight of the powdered leaves.

Phytochemical Screening

Test for Carbohydrate;

Molisch Test

A 0.5mg weight of *Mitragyna inermis* leaf extract was dissolved in 3ml of water and heated. 3 drops of Molish's reagent was added and small amount of concentrated sulphuric acid was carefully added from the side of the test tube to form a lower layer. A reddish coloured ring at the interfacial ring was observed.

Test for Reducing Sugar

A 0.1g weight of the extract was dissolved in 5ml distilled water; 5ml of equal mixture of Fehling's solution A and B was added and boiled. A precipitate of brick red colour was formed.

Test for Saponins

Frothing test

A 0.5g weight of *Mitragyna inermis* leaf extract was shaken with water in a test tube. A honey comb froth which does not persist on standing was observed.

Haemolysis test

About 0.5g of *Mitragyna inermis* leaf extract was dissolved in 3ml of distilled water and filtered, 0.5ml of animal blood was added to the filtrate in the test tube. Another 0.5ml of animal blood was added to 0.5ml of normal sodium chloride solution (0.9%) for control and allowed to stand for 10 minutes. Solution in the test tube containing extract and blood turned dull red with no precipitation while the tube containing normal sodium chloride and blood maintained the initial red colour.

Test for Tannins

About 0.5g of *Mitragyna inermis* leaf extract was stirred with 1ml of distilled water, filtered and 2ml of ferric chloride reagent was added to the filtrate. A blue-black precipitate was observed.

Test for Cardiac Glycosides

i. Salkowski Test

About 0.5g of extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of steroidal ring (i.e. aglycone portion of cardiac glycoside) (Sofowora, 2008).

The methanol extract of *Mitragyna inermis* was tested using the above procedure. A reddish brown colour was observed.

ii. Keller-Kiliani Test

A 0.5g weight of *Mitragyna inermis* leaf extract was dissolved in ethanol; 2ml glacial acetic acid and 2ml concentrated sulphuric acid were added, followed by 3 drops of 10% ferric chloride solution. At the junction of the liquid, a reddish-brown colour was produced which gradually becomes blue.

Test for Alkaloids

About 0.5g of *Mitragyna inermis* leaf extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath. 1ml of the filtrate was treated with a few drops of Wagner's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. A reddish-brown precipitate was observed.

Test for Flavonoids

Ferric chloride test

About 0.1g of *Mitragyna inermis* leaf extract was dissolved in 1ml ethanol. 1ml of 10% ferric chloride was added to it. A brown solution with dirty green precipitate was observed.

Antibacterial Screening

Sterilization of equipments

All work surfaces were mopped with moist hand towel and was disinfected with Dettol® (chlorhexidine and cetrinimide) so as to reduce microbial load on working surface.

Dry heat sterilization

Hot air oven was used to sterilize the conical flasks, pipettes, forceps, glass Petri dishes, wire loop, and beakers at 160°C for 2hours.

Moist sterilization

Moisture insensitive equipments and materials used for microbiological processes were sterilized in an autoclave at 121°C, for 15 minutes.

Preparation of Growth Media

Nutrient agar and broth were prepared as per manufacturers specifications.

Susceptibility Testing

Preparation of crude extracts stock concentration

Five test tubes were labeled 1 to 5. A stock concentration of 300mg/ml of the extract was prepared in the first test tube, subsequently; 5ml of distilled water was then introduced into the remaining four test tubes. 5ml of the stock was withdrawn and added to the second test tube which was mixed thoroughly to obtain a concentration 150mg/ml. Another 5ml was withdrawn from the second tube and then transferred to the third tube which was also thoroughly mixed to give a concentration of 75mg/ml. In a like manner, 37.5mg/ml and 18.75mg/ml concentrations were made in test tubes four and five of respectively.

Zone of inhibition- Well Diffusion Method

The prepared plates were inoculated with an overnight culture suspension of organisms by pouring 1ml of it on each media plate to flood while the excess was discarded to allow uniform growth of the organism. Each inoculated plate was labeled with the organism and the concentration of the test extract to be used. A cork borer of 6mm in diameter was used to create three wells on each inoculated plate. Each concentration of the extract was filled into their corresponding plate as labeled. This was allowed to stand for diffusion into the agar and then incubated at 37°C for 24 hours. The plates were observed for zones of inhibition, measured with a transparent ruler and recorded. Same was done for the control agents (Gentamicin 40mg/ml and distilled water).

Determination of activity index

The selectivity index of crude leaf extract was calculated as:

Activity index (AI) = mean zone of inhibition of extract

Zone of inhibition for standard antibiotic drug

Determination of minimum inhibitory concentration (MIC) using broth dilution method

This was carried out using procedure described by Ibukun (2008). A double strength stock concentration of 400mg/ml was prepared by dissolving 6g of crude extract in 15 ml of distilled water. Single strength concentrations were

prepared in the broth from this stock using aseptic technique throughout. The Bijou bottles were numbered from 1 through 7 and the bottle containing the double strength broth (5ml) was numbered 1. Another 5ml of the single strength broth was added to bottles 2 to 7. Five milliliter of the double strength stock was transferred to the first bottle. Using a separate pipette, the content was mixed and 5ml was transferred to the second bottle. This dilution was continued to bottle six while separate pipette was used for each to give concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. Five milliliter was removed from bottle 6 and discarded. The seventh bottle contains no extract and served as the control. 0.1ml of an over night culture suspension of test organism was inoculated into all the bottles. The bottles were then incubated at 35°C for 24 hour and examined for growth (turbidity). This was done for three organisms.

RESULTS

Table 1: Percentage yield of methanol extract of dried leaves of *Mitragyna inermis*.

Weight of powdered leaves for extraction (g)	400
Weight of dried extract (g)	38.97
Percentage yield (%)	9.7

Table 3: Antibacterial activity of methanol leaf extract of *Mitragyna inermis* using agar diffusion technique.

Leaf extract conc. (mg/ml)	Mean zone of inhibition (mm)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
300	16±0.5	11±0.3	18±0.1
150	12±0.6	9±0.2	15±0.1
75	9±0.2	7±0.1	11±0.2
37.5	0	0	8±0.1
18.75	0	0	0
Control			
Gentamicin 40mg/ml(+)	18±0.1	15±0.2	27±0.1
Distilled water	0	0	0

Result expressed as mean ± SD of triplicate measurement

The smallest concentration of extract that visibly inhibits growth (without turbidity) was recorded as the minimum inhibitory concentration (MIC).

Determination of minimum bactericidal concentration (MBC)

All the bottles content showing no turbidity in the MIC assay were sub cultured onto a nutrient agar plate. The contents of the MIC in the serial dilution bottles without turbidity were sub cultured onto the prepared nutrient agar plates and incubated at 35°C for 24 hours and observed for colony growth. The MBC was the plate with the lowest concentration of extract without colony growth.

Table 2: Results of phytochemical screening of methanol extract of *Mitragyna inermis*.

PHYTOCHEMICAL CONSTITUENTS	STATUS
Saponins	-
Tannins	+
Cardiac glycosides	+
Alkaloid	+
Carbohydrate	+
Reducing sugar	+
Flavonoid	+

Key: + = present and - = absent

Table 4: Activity index of methanol leaf extract of *Mitragyna inermis* (75mg/ml) with respect to Gentamicin (40mg/ml)

Organism	Activity index
<i>Staphylococcus aureus</i>	0.5
<i>Escherichia coli</i>	0.5
<i>Klebsiellia pneumoniae</i>	0.4

Table 5: Results of minimum inhibitory concentration (MIC)

Concentration of leaf extract (mg/ml)	Organisms		
	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>E. coli</i>
200	+	+	+
100	+	+	+
50	+	+	+
25	+	-	-
12.5	-	-	-
6.25	-	-	-

Key: += Inhibition, -= No inhibition

Table 6: Results of minimum bactericidal concentration (MBC)

Concentration	Organisms		
	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>E. coli</i>
200	+	+	+
100	+	+	-
50	-	-	-
25	-	NT	NT

Key: += Inhibition, -= No inhibition, NT= Not tested

DISCUSSION

The phytochemical screening revealed the presence of tannins, cardiac glycosides, and alkaloids, reducing sugar, carbohydrate and alkaloid (Table 2). This agrees with the phytochemical findings of Konkon *et al* (2008) who found flavonoid, catechic tannins and alkaloids in methanol solution of the leaf extract. Saponins were found to be absent in the extract. This also disagrees with the report of Zongo *et al* (2008) in which the chloroform leaf extract of *Mitragyna inermis* was found to contain alkaloids, sterols, and triterpenes and were responsible for the antibacterial activity. The presence of some of these phytochemical constituents may be responsible for some of the observed antibacterial activity of the leaf extract. In addition, tannins have been reported to prevent the development of micro organisms by precipitating microbial protein and making nutritional protein unavailable for them (Fluck, 1973).

The result obtained from the study also showed that the methanol leaf extract of *Mitragyna inermis* has *in-vitro* antibacterial activity against the clinical isolates tested (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiellia pneumoniae*). It revealed a concentration dependent activity at the tested concentration (table 3). The result of this findings agrees with the report of Zongo *et al* (2009) in which the chloroform leaf extract showed *in-vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiellia pneumoniae* (Gram negative), having the largest zone of inhibition was more susceptible to the plant extract as compared to *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive). This does not agree with the literature report that Gram positive bacteria are more sensitive to plant extract and their components

than Gram negative bacteria (Kelmanson *et al.*, 2000; karou *et al.*, 2006; and Mosoodi *et al.*, 2008).

The effect produced by the positive control agent (Gentamicin, 40mg/ml) on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiellia pneumoniae* was higher than the tested concentration of the plant extract since the activity index of the extract at 75mg/ml with respect to gentamicin was below unitary value (<1) (Table 4). There was no observed anti bacterial activity with negative control agent (distilled water) while on the other hand, it was observed on the plate containing leaf extract. This implies that the tested organisms were susceptible to the plant extract.

The minimum inhibitory concentration (MIC) of the leaf extract on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiellia pneumoniae* were 50mg/ml, 50mg/ml and 25mg/ml respectively (Table 5). This implies that a concentration lower than the MIC for each organism will be ineffective at inhibiting their growth.

The extract was bactericidal at concentrations of 100mg/ml on *S. aureus* and *K. pneumoniae* while 200mg/ml on *E. coli* (Table 6).

Therefore, it is evident from the result of the study that the antibacterial activity of the methanol leaf extract may be due to the presence of some of the phytochemical constituents. Moreover, the presence of flavonoids and tannins may be responsible for the antibacterial properties as they do play a major role in bacteria growth inhibition (Wunwisa and Areeya, 2005).

CONCLUSION

From this study, it can be concluded that *Mitragyna inermis* methanol leaf extract contains tannins, cardiac glycosides, alkaloids, reducing sugar, carbohydrate and flavonoids while saponins were found to be absent. Some of these phytochemical constituents may be responsible for its activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiellia pneumoniae*. The MIC of the crude methanol extract of *M. inermis* against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiellia pneumoniae* were 50mg/ml, 50mg/ml and 25mg/ml respectively. The MBC against *Staphylococcus aureus* and *Klebsiellia pneumoniae* were 100mg/ml each while that of *Escherichia coli* was 200mg/ml. The result shows that crude extract of *M. inermis* was found to be more effective against *Klebsiellia pneumoniae* than against *Staphylococcus aureus*, *Escherichia coli*. Thus the study justifies the use of *Mitragyna inermis* plant in traditional medicine for the treatment of infectious diseases.



Figure 1: A photograph showing the leaf of *Mitragyna inermis*.

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