Direct RESEARCH JOURNALS
Direct Research Journal of Biology and Biotechnology (DRJBB)
ISSN 4372-2608
Vol.5(2), pp.17-23, June 2019
DOI: https://doi.org/10.5281/zenodo.3246793
Article Number: DRJBB214335658

http://directresearchpublisher.org/journal/drjbb/

Full Length Research Paper

Phytochemical Screening, Chromatographic Evaluation and Antibacterial Activity of the Leaf Extracts of *Mitragyna Inermis* (Willd)

***[1]Onuh, O. A., [2]Machunga-Mambula, S. S. and [2]Akin-Osanaiye, B. C.**

[1]National Biotechnology Development Agency (NABDA), Along Umaru Musa Yar'adua Express Way, Airport Road, Lugbe, Abuja.
[2]Department of Microbiology, Faculty of Science, University of Abuja, Gwagwalada, Nigeria.
*Corresponding Author E-mail: kemmieonuh@yahoo.com

Received 18 April 2019; Accepted 9 June, 2019

Mitragyna inermis is a plant used in traditional medicine in Nigeria against bacterial infections, infectious diseases to mention a few. Dried crude methanolic extract of *M. inermis* leaf was fractionated successively using hexane, ethyl acetate and methanol as solvent systems. The column fractionation yielded eleven fractions (MC1 – MC11) while the thin-layer chromatography yielded six visible bands (MT-1 – MT-6). Antimicrobial activity of the fractions showed that the plant had some inhibitory effect against the test organism. The photochemistry of the eluted fractions showed varied contents of alkaloids, tannins, saponins, flavonoids, alkaloids and glycosides.

Keywords: Chromatography, fractionation, *Mitragyna inermis*

INTRODUCTION

Over five thousand (5,000) medicinal plants species have been identified in Africa (Kew, 1985; Gill, 1992). The properties of some of these indigenous plants are numerous and diverse and have been long exploited by the people (Arbonnier, 2004). In such African societies, the tradition of collecting, processing and application of the plant based medications are being handed down from generation to generation (Zongo, 2009). The recognition of medicinal plants for their clinical, pharmaceutical and economic values is still growing and varies widely between countries (Murraina, 2008). Medicinal plants are very important in pharmaceutical research and drug development not only when the bioactive phyto compounds are used directly as therapeutic agents, but also as starting materials in the synthesis of drugs.

Being naturally gifted by a suitable diverse range of climate from tropical to Savannah and fertile soil, Nigeria in West Africa possesses a rich flora of indigenous plants which are used in herbal medicine preparations to cure diseases and heal injuries (Ali, 2008; Sofowora 2008; Soladoye *et al*., 2010). *Mitragyna inermis* (Willd) is a medicinal plant with great potential. Its ethnomedical importance has been reported in various indigenous systems of folk medicine and scientific documents (Quedraogo *et al*., 2007; Ali, 2008; Sofowora 2008). These plants have been used widely in the treatments of diseases such as syphilis, typhoid fever, jaundice, Acquired Immune Deficiency Syndrome (AIDS), skin infections (Chaudhary *et al*., 2010). The powder/paste forms of the root, stem and leaf extracts of the two plants have also been reported to be used in treatment of asthma, pains, blood, skin and lung diseases, cataract and malaria and epilepsy (Mishana *et al*., 2000; Muazu and Keita, 2008; Simplice *et al.,* 2011).The following phytochemicals have been associated with *M. inermis*: saponin, tannin, phenol, terpenoids, carbohydrate, alkaloids, glycosides, flavonoids, sterols (Elmanama, 2002; Tor-Anyin and Orokpo, 2012). Thus in order to provide a scientific justification for the utilization of this plant, verification of the efficacy and safety through ethno pharmaceutical studies are being carried out to further prove these ethno medical claims. Therefore, it has now become evident that there is need for a holistic approach to health care and the untapped potential of traditional

medicines should be utilized. This will require proper guidelines for the validation of the scientific methods employed in assessing the antimicrobial potentials of the phytochemicals in the plants (Edeoga *et al*., 2005; Ode *et al*., 2011a). The purpose of this study is to evaluate the antibacterial properties of the fractions of *Mitragyna inermis* (Willd) and to screen the plants for some phytocompounds that are claimed to be responsible for the curative activities of the plant in ethnomedicine.

MATERIALS AND METHODS

Plant materials

The leaves of *Mitragyna inermis* were collected around Idu, near National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. The plant was identified and authenticated in the herbarium of the department of Medicinal Plant Research and Traditional Medicine of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja Nigeria. The samples of the plants were deposited in the herbarium for reference purposes. The voucher numbers is: voucher specimen No NIPRD/H/6818 for *Mitragyna inermis*.

Preparation of plant materials

The leaves of the plant, *Mitragyna inermis* were separated, dried in a shade at room temperature. The completely dried parts were pulverized by grinding with electric blender.

Extracts preparation

The crude plant extracts were prepared by the methods of Olukoya *et al.* (1993), Aboh *et al.* (2014) with slight modifications. The samples were extracted using soxhlet apparatus on a rotary shaker with n-hexane, ethyl acetate, methanol and sterile distilled water respectively. Five hundred grammes of each pulverized parts was extracted using n-hexane for 48 h. The residue was dried in oven, while the collected extract was concentrated through evaporation under reduced pressure, packed and kept in refrigerator for further biological investigations. The dried residue was further extracted with ethyl acetate for another 48 h.

The extract was also concentrated as above while the residue was dried and extracted with methanol, concentrated as above and finally extracted with sterile distilled water. All the crude extracts were filtered and concentrated through evaporation under reduced pressure then transferred into sterile sample bottles, labeled appropriately and kept in refrigerator for further use.

Sterilization of the plant extracts

The extracted parts above were filtered using the membrane filtration system as described by (Sultana, 2007), with slight modifications. The membranes are held in holders, supported on a frame. Fluids are made to transverse membranes by negative pressure. The filter membrane disc used is made of cellulose ester having a nominal average pore diameter of 30 micron (0.30mm). The membrane was held firmly in a filtration unit which consists of supporting base for the membrane, a receptacle for the fluids to be filtered, and a collecting reservoir for the filtered fluid. This was carried out under aseptic condition.

Preliminary phytochemical screening

The phytochemical screening for the presence or absence of phytochemicals of the crude extracts was carried out using standard protocols as described by Sofowora, (1993), Trease and Evans (2002) and Aboh *et al.* (2014) with slight modifications. The following phytochemicals were screened for alkaloids, cardiac glycosides, tannins, flavonoids, phenols, saponins, anthraquinones, sterols and terpenes.

Standardization of plant extract

Five test tubes were labeled 1 to 5. A stock concentration of 50 mg/ml of the extract was prepared in the first test tube. 5 ml of distilled water was then introduced into the remaining four test tubes. The content of the first test tube was thoroughly mixed. 5 ml of this was withdrawn and added to the second test tube which was thoroughly mixed to obtain a concentration of 25 mg/ml. Another 5 ml was withdrawn from the second test tube and then transferred to the third test tube which was also thoroughly mixed to give a concentration of 12.5 mg/ml. In a like manner, a fourth concentration of 6.25 mg/ml was prepared from the third test tube, then, 3.125 mg/ml was prepared from the fourth test tube. 5 ml was removed from the last bottle and discarded.

Fractionation of the crude methanolic extracts of *Mitragyna inermis*

Fractionation of the crude methanolic leaf extracts of *Mitragyna inermis* was carried out in two parts: Column and Thin Layer Chromatography. The protocols of Nwodo *et al.,* (2010), Ode *et al.* (2011b) and Adefuye and Ndip, (2013) with slight modifications were employed.

Column chromatography

The crude leaf extracts of both plants were subjected to column chromatography to separate them into component

fractions. The stationary phase (absorbent) used is column graded silica gel 60G (MERCK) while combinations of hexane, ethyl acetate and methanol were used as mobile phase (solvent system). In the setting up of the column chromatography, glass column of internal diameter 80 mm and length 100 cm (Quickfit, England) was used. The lower part of the glass column was plugged with glass wool with the aid of glass rod. Sand bed was placed over the glass wool. The sand bed served to give a flat base to the column of the absorbent. The wet packing method was used in preparing the silica gel column. 25 g of silica gel (200-425 mesh particles, size Å pore) was wet packed with 250ml hexane solvent system. The slurry of the silica gel and hexane was poured down into the column carefully. The tap of the column was left open to allow free flow of solvent into a conical flask below. The set up was seen to be in order when the solvent drained freely without carrying the silica gel, sand, glass or wool into the tap. At the end of the packing process, the tap was locked. The column was allowed to stabilize for about 24 h. Slurry of the crude extract was prepared in a ceramic mortar by adsorbing 5 g of the extract to 10 g of silica gel in 10 ml of methanol. The slurry was gently loaded onto the packed column. The column was then eluted with solvent systems (mobile phase) gradually in order of increasing polarity using hexane, ethyl acetate and methanol, at a ratio of 2:1 v/v. The following ratios of the solvent combinations were sequentially used in the elution process:
Hexane: ethyl acetate 100:0; 80:20; 60:40; 40:60; 20:80; ethyl acetate: methanol: 100:00; 80:20; 60:40; 40:60; 20:80; 0:100.

The above solvent systems (mobile phase) was continuously poured from the edge of the column with the aid of a dropper. The bottom outlet of the column was opened allowing eluent to flow through the column. As the eluent passed down the column, the compound fraction moved down the column. The separated fractions flowed out of the column where the different eluents were collected in properly labeled bottles. TLC analysis was carried out on fractions before they were evaporated over water bath. The weights of the dry fraction were recorded.

Thin layer chromatography

Analytical TLC plates were prepared by pouring homogenous silica gel (60, F_{254}, MERCK) slurry into aluminum plates by spreading technique. The silica gel layer was adjusted to 0.25 mm thickness. The coated plates were allowed to dry in air and activated by heating in hot air oven at 100-150ºC for 1 h.

Preparation of the development tank (mobile phase)

Solvent system used was hexane and ethyl acetate at the ratio of 4:1. v/v, then ethyl acetate (less polar) and methanol (more polar). The fractions obtained from the column chromatography were spotted with the help of capillary tube washed in acetone. Each fraction was applied as a single spot in a row along one side of the chromo plate, 2 cm from the edge and 1.5 cm from edge (known as the origin). The spotted chromo plate was placed at an angle of 45º in the development tank containing the solvent system, covering the bottom of the plate by the solvent up to nearly 1 cm. The solvent front was marked on the plate immediately after removing it from the chamber and allowed to dry. The mobile phase was not allowed to reach the end of the stationary phase. The plate was visualized and dried by hot air dryer. The plate was then viewed under daylight and UV light at 302 nm and 365 nm respectively. The plate was further exposed to iodine fumes in a chamber and finally sprayed with freshly prepared Vanillin reagent (0.16 g Vanillin powder + 14 ml of methanol + 0.5 ml concentrated sulphuric acid. The plate was carefully heated at 105ºC for optimal colour development. Characterization of the different compounds identified was done by calculating Rf values (Adefuye *et al*., 2013).

$$\text{Rf} = \frac{\text{Distance moved by the component from the origin to spot centre}}{\text{Distance moved from origin to solvent front}}$$

The fractions showing similar TLC mobility and band (that is, same RF) were pooled together. The fractions were kept at 4ºC in the refrigerator for further bioassay tests to confirm their biological activity.

Determination of minimum inhibitory concentration (MIC) of eluted fractions

The MIC was determined by using the micro-dilution method as described by Adefuye and Ndip, (2013) and Aboh *et al.,* (2014) with slight modifications. This assay was performed using round bottom 96-well microtitre plate. Two-fold serial dilutions of the eluted fractions were prepared in the test wells starting with 50 mg/ml stock. The dilutions were 25 mg/ml, 12.5 mg/ml, 3.125 mg/ml, 1.565 mg/ml. The bacterial strains were purified by standard bacteriological methods (Cheesebrough, 1984, CCLS, 2006). The bacterial strains were standardized to 0.5 MacFarland standard using Nephelometer (NCCLS, 1993) to give approximately 1.0 x 10^6 cfu/ml. The standardized cultures were maintained in sterile Muller Hinton agar. Eighteenth-hour broth culture of the *Salmonella typhi* was suspended into sterile Sabouraud dextrose liquid medium. It was standardized according to Clinical Laboratory Standards Institutes (CLSI, 2002) by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0 x 10^6 cfu/ml using Nephlometer.

Table 1. Qualitative phytochemical contents of *Mitragyna inermis* fractions.

Constituents	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
Alkaloids	-	-	-	-	+	+	+	-	-	-	-
Tannins	-	-	-	-	-	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	+	+	-	-	-	-	-	-
Terpenes	-	-	-	-	+	+	+	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	-	-	-	-	-
Phenols	-	-	-	-	-	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-

Key: M1- M11 = eluted fractions of M. inermis, + = present, - = absent

Control wells were prepared using Ciprofloxacin 20 mg/ml as positive for bacteria. Dimethyl sulfoxide (DMSO) was used as negative control. The plates were then sealed with parafilm and incubated at 37°C for 24 h for bacteria and yeast. After incubation, 40 µL of 0.2 mg/ml of Tetrazonium dye (p-iodonitrotetrazoluim violet) was added to well and incubated for an hour, after which the uninhibited organisms would have converted the dye from blue to pink in the well. The MIC was defined as the minimum concentration at which growth was inhibited with no visible change. Sterility test was performed to verify whether the broth used in the assay was contaminated before test procedures. 50 µL of broth was dispensed into a well, without both extract and inoculums.

Preliminary *In-vivo* efficacy study

Twenty five healthy male and female rats weighing between 250 g and 300 g were used. They were divided into five groups (I, II, III, IV, V), of the five rats each. Group I- Group IV were infected with a single dose of 1ml suspension of *Salmonella typhi* having being certified pure by morphological cultural and biochemical identification using protocols described by Cheesbrough (2002) and CLSI, (2006). Daily monitoring of animals for clinical signs were taken, observed and recorded. Faecal droppings of each animal were collected for colony count. Infectivity was established eight days post- inoculation. Infected animals were treated with extracts and standard drugs for Group IV. Standard drug, Amoxycillin 20 mg/mL was used for Group IV. The extracts, MI was administered in doses of 500 mg/kg, 1000 mg/kg and 1500 mg/kg to Group I, II, III respectively. On the seventh day, the animals were sacrificed. Group V (control) was given only physiological saline.

RESULTS

Phytochemical analysis of the fractionated methanol extract of *Mitragyna inermis* leaf revealed the presence of the following; alkaloids, tannins, flavonoids, terpenes, phenols, saponins, sterols, carbohydrates. Eleven fractions were eluted (M1-M11). No single fraction contained all the phytochemicals screened, but all the phytochemicals in the crude were present in the fractions. Table 1 shows the details of the result obtained. Anthraquinone was absent in all the fractions. All the fractions were further subjected to thin layer chromatography. Table 3 shows the details of the Thin Layer Chromatography profiles of the leaf extracts of *Mitragyna inermis.* The weight and percentage yield of the fractions of the plant and the retardation factor (Rf) values are also shown in the above (Tables 1 and 2). Fractions with same Rf values for *Mitragyna inermis* were pooled together to give a total of 6 fractions (Table 4).

MT1- fraction LT1
MT2- combination of fractions 2 and 3
MT3- fraction 4
MT4- fraction 5
MT5- combination of fractions 6 and 7
MT6- combination of fractions 8, 9, 10, 11.

The minimum inhibitory concentration (MIC) of the fractions of *Mitragyna inermis* leaf (methanol extract) is presented on (Table 5). In the experimental infection carried out, the plant extract (MI) showed mild inhibitory effect against the infective organism *S. typhi* in wistar rats. Two extract concentrations, 500 mg/kg and 1000 mg/kg of fractions MT5 were relatively safe experimentally for treatment. However, extracts at 1500 mg/kg showed toxic effects on liver and kidney. Infectivity was established eight days post inoculation (Figure 1).

DISCUSSION

The screening of the fractionations of the extracts of *M. inermis* for phytochemicals revealed that alkaloids were present in fractions MC5, MC6, MC7. The absence of some of the phytochemical in some fractions could be

Table 2. Details of fractions from column chromatography of crude methanolic extract of *Mitragyna inermis* leaf.

Fractions	Eluent (solvent system)	Ratio v/v	Colour of fraction
MC1	Hex	100	No
MC2	Hex : EA	80 : 20	Grayish
MC3	Hex : EA	60 : 40	Greenish
MC4	Hex : EA	40 : 60	Dark green
MC5	EA	20 : 80	Yellowish brown
MC6	Hex : EA	0 :100	Yellowish brown
MC7	EA : Met	80 : 20	Brownish
MC8	EA : Met	60 : 40	Reddish
MC9	EA : Met	40 : 60	Reddish
MC10	EA : Met	20 :80	Reddish
MC11	Met :	100	Reddish brown

Key: Hex = Hexane, EA = Ethyl Acetate, Met = Methanol, MC = *Mitragyna inermis* fraction from column chromatography.

Table 3. Details of thin layer chromatography of methanolic extract of *mitragyna inermis* leaf.

Eluted Fractions	Weight of Fraction (g)	Percentage (%) Yield	Rf Value
MT- 1	0.184	3.70	0.42
MT- 2	0.235	4.71	0.56
MT- 3	0.246	4.92	0.66
MT- 4	0.237	4.74	0.78
MT- 5	1.091	21.83	0.7
MT- 6	2.611	52.22	0.89

Note: Mobile System/ Solvent (Eluent) HEM = Hexane Ethyl Methanol Ratio of 4: 4: 1 (v/v)
MT = *Mitragyna inermis* fraction from TLC

Table 4. Zone of inhibition of the fractions of mitragyna inermis against salmonella typhi mean zone of inhibition (mm).

Fractions	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	Positive Control	Negative control
MT- 1	0.0± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
MT- 2	6.0 ± 1.0	4.0 ± 1.5	4.0 ± 0.5	0.0 ± 0.0	21.0 ± 0.0	0.0
MT- 3	7.0 ± 0.5	5.0 ± 0.5	4.0 ± 0.0	2.0 ± 0.0	20.0 ± 0.0	0.0
MT- 4	9.0 ± 0.0	7.0 ± 1.0	5.0 ± 1.0	3.0 ± 0.5	18.0 ± 0.0	0.0
MT-5	11.0 ± 0.0	9.0 ± 0.5	6.0 ± 1.5	4.0 ± 0.0	19 ± 0.0	0.0
MT- 6	10.0 ± 0.5	8.0 ± 0.0	7.0 ± 0.0	5.0 ± 0.0	20.0 ± 0.5	0.0

Values are mean inhibition zone (mm) ± SD of the three replicates, $p < 0.05$
Key: MT1 – MT6 = *Mitragyna inermis* fractions of TLC
Positive control = Chloramphenicol (20mg/ml)
Negative control = Distilled water

linked to loss during the process of fractionation. Also, degradation or transformation of the active plant constituent may occur during fractionation due to hydrolysis, esterification, oxygenation or ultraviolet radiation (Ode *et al*., 2011a). Again, decrease in activity after fractionation may be as a result of the phenomenon of synergy between the active ingredients. Thus, during the process of fractionation, the different constituents and secondary metabolites are separated according to their polarities. There is also possible synergy between the constituents (Nwodo *et al*., 2010; Ode *et al*., 2011a). Tannins were present in fractions M6, M7, M8, M9, M10 and M11 (Table 1).

The column chromatography profile of the methanol leaf extracts gave 11 fractions (MC1- MC 11) in *M. inermis* (Table 2). After spotting on Thin Layer chromatography, 11 bands produced 6 MT1- MT6 for *M. inermis* (Table 3). MT2 to MT6 also showed activity against the microorganisms tested. Previous study by Hefferon, (2012) reported a reduced biological action of the fractions of some medicinal plants as compared to the crude plants. This suggests that the biological activity

Table 5. Minimum Inhibitory Concentration (MIC) of Methanol Fractions of *Mitragyna inermis* against *Salmonella typhi*

Fractions	MIC (mg/ml)
MT-1	0
MT-2	25.00 ± 0.00
MT-3	12.50 ± 0.00
MT-4	12.50 ± 0.00
MT-5	12.50 ± 0.00
MT-6	25.00 ± 0.00

*Values are mean inhibitory concentration (µg mL -1) ± of three replicates. MT- Fraction of *Mitragyna inermis* , Chloramphenicol 20 mg/ml as positive control, DMSO- Negative control (0.00)

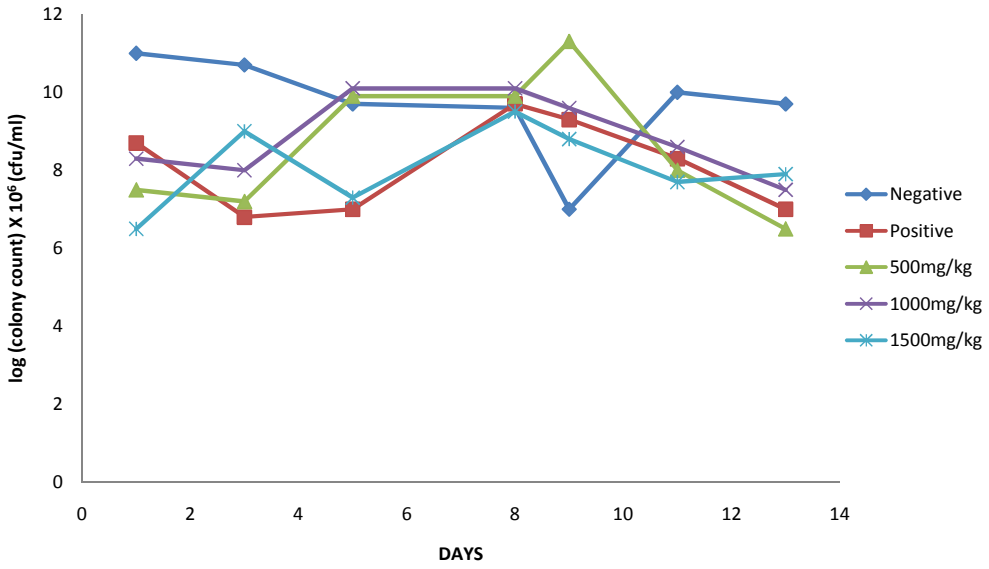

Figure 1. *In vivo* antibacterial activity of MI on *S. typhi.*

of some plants is due to a combination of phytochemicals which are separated into smaller entities with varying biological efficacy during the process of fractionation (Shafi *et al*., 2013).

Conclusion

From the various parameters studied on the medicinal plant, *Mitragyna inermis,* the plant was found to be very rich in phytochemicals such as flavonoids, sterols, polyphenol, alkaloids and saponins. The richness could justify the multiple therapeutic indications for which the various parts especially the leaf of the plant are used for traditionally. The plant exhibited antimicrobial activities against the micro organisms tested. Its activities were highest in the leaves and it increased its increasing concentration of the plant extract. This compared favorably with positive control (standard antibiotics used).

Authors' Declaration

We declared that this study is an original research by our research team and we agree to publish it in the journal.

REFERENCES

Aboh MI, Olayinka BO, Adeshina GO, Oladosu P (2014). Antifungal Activities of Phyto compounds from *Mitracarpus villosus* (Sw) DC from Abuja, Nigeria. *Journal of Microbiology Research,* 4(2): 86-91.

Adefuye AO, Ndip RN (2013). Phytochemical analysis and antibacterial evaluation of the ethyl acetate extract of the stem bark of *Bridelia micrantha. Pharmacognosy Magazine,* 9(33):45-50.

Ali M (2008). *Isolation of natural products. Pharmacognosy (Pharmacognosy and Plant Cultivation)* vol. 2 Eds Satish Kumar Jain for CBS Publishers and Distributors, New Delhi India, Pp. 469 – 475.

Arbonnier M (2004). *Trees, shrubs and lianas of West African dry zones*. CIRAD MARGRAF publishers, GMBH. AJ Wageningen, the Netherlands, p. 573.

Chaudhary G, Goyal S, Poonia P (2010). *Lawsonia inermis* Linnaeus. Phyto-Pharmacological Review. *International Journal of Pharmaceutical Sciences and Drug Research*, 2(2):91-98.

Cheesbrough M (2002). *Medical Laboratory Manual for Tropical Countries.* ELBS edition Tropical Health Technology Publications, U.K, p. 392.

CLSI (2002, 2006). Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts, Approved standard M27-A2. Wayne (PA),

Edeoga HO, Okunu EO, Mbaebie (2005). Phytochemical constituent of some Nigeria medicinal plants. *African Journal of Biotechnology*, 4(7):685 – 688.

Elmanama AA, Alyazji AA, Nedaa A, Gheneima A (2011). Antibacterial, Antifungal and synergestic of Effect of *Lawsonia inermis*, *Punica granatum* and *Hibiscus sabdariffa*. *Annals of Alquds Medicine*, 7(1432): 33 – 41.

Gill LS (1992). In: *'The ethnomedical uses of plants in Nigeria.'* Published by University of Benin Press, University of Benin, Benin city, Edo State, pp 46-143.

Hefferon KL (2012). Plant-made vaccines. *Journal of Vaccines and Vaccination*, 3(4):108-110.

Keay RWJ (1989). *'Trees of Nigeria'.* Clarendon Press, Oxford, p. 874.

Muazu J, Kaita AH (2008). A review of traditional plants used in the treatment of epilepsy amongst the Hausa/Fulani tribe in Northern Nigeria *African Journal of Traditional, Complementary and Alternative medicines,* 5(4).

Murraina IA (2008). *In vitro* screening for Antimycoplasmal activities and toxicity of the acetone extracts of selected plants from Northern Nigeria. M. Sc. thesis. Ahmadu Bello University, Zaria, Nigeria, p. 185.

Nwodo UU, Ngene AA, Iroegbu CU, Obuyeke GC (2010). Effects of fractionation on antibacterial activity of crude extracts of *Tamarindus indica. African Journal of Biotechnology,* 9(42): 7108-7113.

Ode OJ, Asuzu IU, Ajayi IE (2011a). Bioassay-Guided Fractionation of the crude Methanol Extract of *Cassia singueana* Leaves. *Journal of Advanced Scientific Research,* 2(4): 81-86.

Ode OJ, Sanni S, Oladele GM (2011b).The Global Relevance of Traditional Medicine and Herbal Plants, The Nigerian Perspective. *International Journal of Applied Biology and Pharmaceutical Technology*, 2(4).

Olukoya DK, Idika N, Odugbemi T (1993). Antimicrobial activity of some medicinal plants from Nigeria. *Journal of Ethnopharmacology*, 39: 69-72.

Quedraogo S, Ranairo HR, Ndiaye M, Kabore ZI, Guisson IP, Bucher B Andrian T (2004). Cardiovascular properties of aqueous extract from *Mitragyna inermis* (Willd) Oktze Rubiaceae. *International Journal of Pharmacology,* 3: 80 – 85.

Simplice DK, Tchadjobo T, Liboudo DP (2011). Sub-saharan Rubiaceae: A Review of their traditional uses, phytochemistry and biological activites. Pak. J. of Bio. Sci. 14: 149 – 69.

Sofowora A (2008). Medicinal plants and traditional medicine in African, 3rd Edition, Spectrum Books Ltd. Ibadan, Nigeria.

Sofowora A, Ogunbodede E, Onayade A (2013).The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. *African Journal of Traditional Complimentary and Alternative Medicines,* 10(5):210-229.

Soladoye M, Amusa NA, Raji EO, Chukwuma EC, Taiwo AA (2010). Ethnobotanical survey of Anti – Cancer Plants in Ogun state, Nigeria. *Annals of Biological Research*, 1 (4): 261 – 273.

Sultana Y (2007). Pharmaceutical Microbiology and Biotechnology. Jamia Hamdard, Hamdard Nagar, New Delhi-110062, p. 30.

Tor-Anyiin TA, Orokpo AM (2012). Phytochemical Screening, Chromatographic Evaluation and Anti-bacterial Activity on stem, bark extracts of *Mitragyna inermis.*

Trease G, Evans WC (1999). "Pharmacognosy," 14th Edition, Bailiere Tindal W. B. Sauders company ltd; London, Pp. 224-275.

Wunwisa K, Areeya K (2005). Antimicrobial properties of Thai traditional flower vegetable extract. *Australian Journal of Technology,* 9(2):71-74.

Zongo C, Etienne-Francois AO, Savadogo OL, Koudou CJ, Traore AS (2009). *In vitro* Antibacterial Properties of Total Alkaloids Extracts from *Mitragyna inermis*(Willd) O. Kuntze, a West African Traditional Medicinal Plant. *Asian Journal of Plant Sciences,* 8 (2):172-177.