Original Article

PHYTOCHEMICAL SCREENING AND ANT[redacted] *Commiphora Kerstingii* LEAVES EXTRACTS

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ABSTRACT

Commiphora kerstingii has medicinal properties for the effective management of several ailments including typhoid fever. To establish the ethnobotanical rationale for its traditional use, the powdered leaves were extracted with n-hexane, methanol and water. All extracts were subjected to phytochemical analysis and antibacterial activity against some selected gram-positive and gram-negative bacteria using the Kirby-Bauer disk diffusion method. Phytochemical screening of the plants revealed the presence of flavonoids, saponins, alkaloids, steroids and tannins. The methanol and aqueous extracts had activities against four test pathogens while n-hexane extract had inhibition on *Escherichia coli* only. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts ranged from 16-30 µg/ml to 20-40 µg/ml respectively.

Keywords: *Commiphora kerstingii*, phytochemical screening, antibacterial activity.

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INTRODUCTION

Medicinal plants have been used in Africa for many centuries and today almost every part of the world uses herbal plants for the treatment of different diseases (Adewunmi and Ojewole, 2004; Ogukwe *et al.*, 2004; Njoku and Ezeibe, 2007).These plants are of great importance to the health of individuals and communities (Edeoga *et al.*, 2005).

The research based on ethno-pharmacological information is generally considered as an effective approach not only for the discovery of new anti-infective agents from higher plants, but also because such information may be of value in disclosing new and economic materials like tannins, oils, gums, which are precursors for the synthesis of complex chemical substances (Mojab *et al.*, 2003; Duraipandiyan *et al.*, 2006). Therefore,

knowledge of the chemical constituents of plants is desirable.

The increasing interest for the use of plants as sources of new anti-infective agents has lead to the study of *Commiphora kerstingii* leaves. *Commiphora Kerstingii* is a tree of about 10m high and distributed along the arid region of Africa and it is often planted as a live-fence in towns for its aesthetic value (Kimura *et al.*, 2001; Mann *et al.*, 2003).The tree belongs to the family Burseraceae. Its bark is smooth, soft, green and peeling off in papery strips that roll off eventually. Its stem bark and leaves, which exudes resins is used traditionally in Northern Nigeria to treat typhoid fever, cancer, measles, asthma, rheumatism and venereal diseases (Mann *et al.*, 2003). Traditionally, a macerate of crushed leaves in oil is given in Cote D'ivorie and in Burkina Faso as a sedative and sopariffic (Adebayo *et al.*, 2006). Additionally, the resins of *Commiphora kerstingii* are used to treat gingivitis and inflammations (Kimura *et al.*, 2001) as well as fascioliasis (Massoud *et al.*, 2001). In Nigeria, a seed decoction is held to be a very effective purgative and verminfuge (Akor and Anjorin, 2009).

Kubmarawa *et al.* (2007) reported that a *Commiphora kerstingii* root contains secondary metabolites like saponins, tannins and volatile oils. These constituents were later established to be responsible for the inhibitory activity of the roots extract against *Bacillus subtilis*, *Candida albicans* and *Escherichia coli* (Kubmarawa *et al.*, 2007). Very recently, Musa, (2008) reported the antioxidant and antibacterial activity of *Commiphora kerstingii* stem bark extracts, which was also found to contain alkaloids, saponins, tannins, anthraquinones, flavonoids and cardiac glycosides.The methanolic extract of stem-bark were active against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyrogens*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa* (Musa, 2008). Equally, the antibacterial activity of a number of *Commiphora* species have been extensively studied (Hanus *et al.*, 2005). Rahman *et al.* (2008) examined the antibacterial terpenes obtained from the oleo-resin of *Commiphora molmol* and were found to be effective against several strains of *Staphylococcus aureus*. Adebayo *et al.* (2006) also studied the effect of ethanolic leaf extract of *Commiphora african* on some serum lipid profiles. The extract was found to possess antilipidaemic properties that may be used for the management of cardiovascular disorders. Aliyu *et al.* (2002) also reported the antimicrobial activity of *Commiphora african* ethanolic leaf extract which was found to contain tannins, alkaloids, triterpenes, sterols and phenolic compound.

Therefore, the objectives of this study is to screen the leaves of the plant for its phytochemical components, to determine the antibacterial activity of the extracts of the plant on selected bacterial species, and to determine the activity of the fully or partial purified fractions of extract obtained from thin layer chromatography.

MATERIALS AND METHODS

Collection and Drying of Plant Materials

The leaves of *Commiphora kerstingii* were collected in Bosso town, adjacent to Federal University of Technology, Minna, Bosso Campus. The leaves were air dried at room temperature for two weeks and then crushed with mortar and pestle into powdery form using sieve.

Extraction

One hundred and fifty gram (150g) of the powered leaves was weighed and marcerated with 200ml of n-hexane for a period of 24hours. The extract was collected and filtered into a beaker, which was evaporated to dryness in a water bath, and then allowed to cool. It was then labeled n-H_x (n-hexane extract). The residue was air dried and the procedure repeated for methanol and aqueous which was then labeled M_x and Aq_x extracts respectively.

Phytochemical Screening

The extracts n-hexane, methanol and aqueous extracts were subjected to Phytochemical screening using standard methods (Sofowora, 1994, Oyewale *et al.*, 2001; Oyeleke *et al.*, 2009).

Test Microorganisms

The extracts of the leaves were assayed for antibacterial activity against six pure bacteria isolates which were obtained from the Department of Microbiology, Federal University of Technology, Minna, Nigeria.The bacteria includes *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis* and *Shigella spp.*

Antibacterial Bioassay

The standard Kirby-Bauer disk diffusion method described by National Committee for Clinical Laboratory Standards NCCLS (2002) was adopted for the antibacterial activity of the prepared extracts while sensitivity test was carried out by agar plates which were seeded with 1 ml culture of each bacteria isolated. The seeded plates were allowed to set and a standard cork borer of 4mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 0.1ml of each extract at a concentration of 100µg/ml. The antibiotic (Ampiclox) at 100µg/ml was used as a positive control. The plates were incubated at 37^0C for 24 hours after which the bioactivity was determined by measuring diameter of inhibition zones in mm using a transparent ruler.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

For MIC, two-fold serial dilutions of the extracts were performed. Each inoculum was prepared in its respective medium and density was adjusted to 0.5 Mcfarland standards (10^8 CFU/ml) and diluted to 1:100 for the broth micro dilution procedure. The test organisms were inoculated into each test tube which was then incubated at 37°C and the tube with the lowest dilution which had no detectable growth was considered as the MIC after 24 hours. The MIC is the lowest concentration of the

compound at which the microorganism tested does not demonstrate visible growth. MBC was determined by sub-culturing the test dilutions on to a fresh solid medium and incubated further for 24 hours. The highest dilution that yielded no bacterial growth on solid medium was taken a MBC (Suffredini *et al.*, 2004; Doughari *et al.*, 2007).

Thin Layer Chromatography

Mini thin layer chromatography and preparatory thin layer chromatography was carried out using standard method (Oyeleke *et al.*, 2008).

Determination of antibacterial activity of fractions separated from the preparatory Thin Layer Chromatography

The activity of reconstituted extract was determined against the test organisms using standard culture. 0.01 g of each extract was weighed and dissolved in 100ml of sterile water to give a concentration of 100µg/ml. Sterile nutrient agar was prepared and the standard culture inoculated wells were made and filed with the extracts. The plates were incubated for 24 hours at 37^0C after which zones of inhibition were measured.

RESULTS

Phytochemical Screening

The result of the phytochemical screening reveals the presence and absence of some constituents as shown in Table 1 below.

Table 1.Phytochemical constituents of *Commiphora kerstingii* leaf extracts

Constituent	n-hexane	methanol	aqueous
Saponins	-	++	++
Tannins	-	+++	+++
Flavonoids	++	-	++
Steroids	+	-	-
Cardiac glycosides	-	-	-
Alkaloids	-	+++	+++
Phlobatannins	-	-	-
Anthraquinones	-	-	-
Carbohydrates	-	++	+

Key: -= absent + = low ++ = moderate +++ = high.

Antibacterial activity of the Crude Extracts of *Commiphora kerstingii* leaves

The result of the antibacterial activity of the crude extracts of *Commiphora kerstingii* leaves on test organisms are shown in Table 2.

Table 2. Antibacterial activity of leaves of *Commiphora kerstingii* against test organisms zones of inhibition (mm)

Organisms	n-hexane extract	methanol extract	Aqueous extract	Ampiclox (Control)
Escherichia coli	21	-	24	34
Salmonella typhi	-	24	-	26
Pseudomonas aeruginosa	-	31	14	34
Streptococcus pyrogens	-	21	14	26
Staphylococcus aureus	-	-	24	29
Bacillus subtilis	-	18	-	32
Shigella spp	-	-	-	6

Minimum Inhibitory Concentration of the Crude Extracts.

The results of the MIC screening of crude n-hexane, methanolic and aqueous extracts of the leaves of *Commiphora kerstingii* is shown in Table 3.The results revealed that the minimum inhibitory concentration was between 16 to 30 µg/ml.

Minimum Bactericidal Concentration of the Crude Extracts

The results of the MBC analysis of crude n-hexane, methanolic and aqueous extracts of the leaves of *Commiphora kerstingii* is shown in Table 4.The results indicated that the minimum bactericidal concentration was between 20 to 40 µg/ml.

Thin layer Chromatography on leaves of *Commiphora kerstingii*

In the thin layer chromatography, 10ml of petroleum ether was used to elute n-hexane extract.While Ethylacetate, methanol and acetone (13:3:2) were used to elute both methanol and aqueous extracts, two spots were obtained for each extract with R_f value (0.60,0.62) for n-hexane, (0.71,0.67) for methanol and (0.61,0.64) for aqueous extracts respectively. None of these fractions exhibited antibacterial activity against the tested organisms.

Table 3: Minimum inhibitory concentration (µg/ml) of the extracts of *Commiphora kerstingii*

Organisms	n-hexane extract	Methanol extract	Aqueous extract	Ampliclox Control
Escherichia coli	18	-	20	28
Salmonella typhi	-	28	-	28
Pseudomonas aeruginosa	-	24	23	28
Streptococcus pyrogens	-	24	27	24
Staphylococcus aureus	-	-	30	20
Bacillus subtilis	-	30	-	20
Shigella spp	-	-	-	30

Table 4: Minimum Bactericidal Concentration (µg/ml) of the extracts of *Commiphora kerstingii*

Organisms	n-hexane extract	Methanol Extract	Aqueous extract	Control Ampliclox
Escherichia coli	20	-	24	32
Salmonella typhi	-	32	-	32
Pseudomonas aeruginosa	-	28	28	24
Streptococcus pyrogens	-	36	32	20
Staphylococcus aureus	-	-	32	24
Bacillus subtilis	-	40	-	24
Shigella spp	-	-	-	36

DISCUSSION

Phytochemical screening shows that some of the secondary metabolites tested were not present in the plant material and these include cardiac glycosides, phlobatannins and anthraquinones, Saponins and flavonoids were moderately present in both aqueous and n-methanol extract and completely absent in n-hexane extract. This does not actually tally with the result obtained from the previous findings on *Commiphora kerstingii* leaves by Dauda *et al.* (2008). This might be due to the difference in the solvent used for extraction as well as environmental factors. Tannins which were not observed in *Commiphora molmol* but were found in appreciable amount in methanol and aqueous extract of *Commiphora kerstingii* leaves. This is due to the fact that tannins contains different types of acid which are quite soluble in both aqueous and alcoholic media (Massoud *et al.*, 2001; Hanus *et al.*, 2005). Steroids were only observed in n-hexane extract which agrees with the results established and reported by (Wang *et al.*, 2004). Alkaloids were completely absent in n-hexane extract but present in substantial amount in methanol and aqueous extract. The result is in agreement with the findings of Musa (2008) that proves the presence of

these constituents in the stem-bark of *Commiphora kerstingii*. However in contrast to the previous findings of Kubmarawa *et al.* (2007) and Rahman *et al.* (2008) in which alkaloids was completely absent in the roots extract, alkaloids are found to be present in the methanolic and aqueous leaves extract of *Commiphora kerstingii*. This may be due to differences in species and the part of the plant that was *extracted* (Abdallah *et al.*, 2009).

The antibacterial activity of the leaf extracts of *Commiphora kerstingii* were tested against some pathogens. The methanol and aqueous extracts had activities against four test pathogens. Although, the methanolic extract manifested the highest antibacterial activity while n- hexane extract had no activity against the organisms except *Escherichia coli.* According to Karaman *et al.* (2003) methanol is known to be a better solvent for more consistent extraction of antibacterial compounds from medicinal plants compared to other solvents such as hexane, chloroform and water. The strong activity of the methanolic leaves extracts suggest that this plant could be used for the treatment of infections caused by these organisms. The findings of this study is therefore in agreement with El-Ashry *et al.* (2003) who showed that *Commiphora* species have a considerable antibacterial activity against some gram negative bacteria especially *Salmonella typhi.* Recently, Rahman *et al.* (2008) found that *Commiphora molmol* has antibacterial activities against some strains of *Staphylococcus aureus*,*Salmonella enteric* and *Klebsiella pneumonia* Additionally, the result from this study are in line with Musa (2008) who stated that stem bark of *Commiphora kerstingii* demonstrated presence of antibacterial activities. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. These finding give credence to the traditional medicinal application of the plants as remedies for measles, gonorrhea and typhoid fever

The minimum inhibitory concentrations of the extracts were shown. The result obtained reveals the effectiveness of the plant extract as chemotherapeutic agents. The organisms were inhibited at concentrations ranging from 16 – 30 mg/ml. The minimum bactericidal concentration ranged from 20 – 40 mg/ml. The MIC values for most of the extracts were lower than their MBC values, suggesting that these extracts inhibited growth of the test microorganisms while being bactericidal at higher concentrations. The fractions obtained from the thin layer chromatography (TLC) showed less antibacterial activities against the test organisms. The lower antibacterial activities of the pure fractions of the extracts than the crude ones might have been as a result of the synergic action of the active components in the plant thereby agreeing with the views of Harborne (1984) and Oyeleke *et al.* (2008) who reported that the activities of plant extracts could sometimes change after fractionation with the obtained pure component eventually lacking in the activity of the original crude extract.

CONCLUSION

The antibacterial activity of aqueous and methanol extract which compared favourably with Ampliclox (commercial antibiotic) may help to discover new chemical classes of antibiotics that may be used as topical treatment of disorders resulting from the tested pathogens. It is equally suggested that more research be conducted that will further isolate, elucidate and characterized the active components and their mechanism of action.

REFERENCES

Abdallah, E. M., Khalid, A. S. and Ibrahim, N. (2009). Antibacterial activity of oleo-gum resins of *Commiphora molmol* and *Boswellia papyrifera* against methicillin resistant *Staphylococcus aureus* (MRSA). *Scientific Research and Essay* 4 (4): 351-356.

Adebayo, A. H., Aliyu, R. and Gatsing, K. (2006). The effects of *Commiphora Africana* (*Burseraceea*) on serum lipid profile in rats. *Int. J. Pharmacol.* 2(6): 618-622.

Adewunmi, C. O. and Ojewole, J. A. (2004). Safety of traditional medicines, complementary and alternatives medicines in Africa. *Afri. J. Tradi*, 1:1-3.

Akor, J. S and Anjorin, T. S. (2009). Phytochemical and antimicrobial studies of *Commiphora africana* (A. Rich) Engl. root extracts. *Journal of Medicinal Plants Research.* 3(5): 334-337.

Aliyu, R., Gatsing, D. and Umar, H. S. (2002).Phytochemical screening and antimicrobial properties of ethanolic leave extract of *Commiphora africana.West Afri. J. Biol. Sci.* 13: 75-80.

Dauda, B. E. N., Oyeleke, S. B. and Jimoh, T. (2008). The Phytochemical and Antimicrobial screening of extracts of *Commiphora kerstingii, Journal of National Teachers Technology,* 6(4): 112-120.

Doughari, J. H., Pukuma, M. S. and De, N. (2007). Antibacterial effects of *Balanites aegyptiaca* and *Moringa oleifera* Lam. on *Salmonella typhi. African Journal of Biotechnology* 6(19). 2212-2215.

Duraipandiyan, V., Ayyanar, M. and Ignacimuthu, S. (2006). *Antimicrobial Activity of Some Ethnomedical Plants Used by Paliyar Tribe from Tamil Nadu, India.* BMC complementary and alternative medicine. pp 635.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants, *Afr. J. Biotechnol.* 4(7): 685-688.

El-Ashry, E. S. H., Rashed, N., Alama, O. M. and Saleh, A. (2003). Components,therapeutic value and uses of Myrrh. *Pharmazie.* 3:163-168.

Hanus L.O., Rezanka, T., Dembitsky, V. M. and Moussaieff, A. (2005). *Commiphora myrrh* chemistry. *Biomed. Papers*. 149(1): 1-28.

Harbourne, J. B. (1984). *Phytochemical methods: A guide to modern techniques of plant analysis*. 2nd Edition. Chapman and Hall, London. Pp.1-19, 37-168.

Karaman, I., Sahin, F., Gulluce, M., Ogutcu, H., Sengul, M. and Adiguzel, A. (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus L. J. Ethnopharmacol.* 85:231- 235.

Kimura, I., Yoshikawa, M., Kobayashi, S., Sugihara, Y., Suzuki, M., Oominami, H., Murakami, T., Matsuda, H. and Doiphode, V.V. (2001). New triterpenes, myrrhanol A and Myrrhanone A, from guggul-gum resin and their potent anti-inflamatory effects on adjuvant-induced air-pouch granuloma of mice. *Bioorganic Med. Chem. Lett.* 11: 985-989.

Kubmarawa, D., Wase, G. A. and Ayinla, O. G. (2007). Preliminary studies on phytochemical analysis and antimicrobial evaluation of extracts of *Commiphora kerstingii J. Chem. Soc. Nigeria*, 32(1): 38-40.

Mann, A., Gbate, M. and Umar, A. N. (2003). *Medicinal and Economic Plants of Nupe land.* 1st Edn; Jube-Evans Books and publications, Bida, Nigeria, pp.42

Massoud, A., El Sisi, S., Salama, O. and Massoud, A. (2001). Preliminary study of therapeutic efficacy of a new fasciolocidal drug derived from *Commiphora molmol. American. J. Trop. Med. Hyg.* 65: 96-99.

Mojab, F., Kamalinejad, M., Ghaderi, N. and Vahidipour, H. (2003). Phytochemical Screening of Some Iranian Plants. *Iranian Journal of Pharmaceutical Research.* pp 77-82.

Musa, A. A. (2008). Antioxidant and antibacterial activity of *Commiphora kerstingii* Engl.Stem Bark extract. *Research Journal of Phytochemistry*, 2(2): 106-111.

National Committee for Clinical Laboratory Standards. (2002). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, NCCLS document M100-S12, Wayne, PA, USA.

Njoku, P. C. and Ezeibe, A. U. (2007). Phytochemical and elemental analyses of *Helianthus annus* and its use as blood clotting agent. *J. Chem. Soc. Niger.* 32(2): 128-132.

Ogukwe, C. E., Oguzie, E. E., Unaegbu, C. and Okolue, B. N. (2004). Phytochemical screening on the leaves of *Sansevieria trifasciata J. Chem. Soc. Nigeria*, 29(1): 8-10.

Oyeleke, S. B., Dauda, B. E. N. and Boye, O. A. (2008). Antibacterial activity of *Ficus capensis. African Journal of Biotechnology* , 7 (10), pp. 1414-1417.

Oyeleke, S. B., Dauda, B. E. N. and Damasus, I. (2009). Comparative analysis of the Antibacterial activity of *Citrus aurantifolia* on some selected Pathogens. *Journal of Science, Education and Technology*, 2(1). 343-345.

Oyewale, A., Musa, A. A. and Amupitan, J. O. (2001). Chemical composition and Biological activity of *Cochlospermum tinctorium.* (A.RICH). *J. Sci. Eng. Technol.* 8: 3300-3311.

Rahman, M. M., Garvey, M., Piddock, L. J. and Gibbons, S. (2008). Antibacterial terpenes from the oleo-resin of *Commiphora molmol*. *Phytother Res.* 10: 1356-60.

Sofowora, A. (1994). *Medicinal plants and traditional medicine in Africa*, University of Ife press Nigeria; pp.23.

Suffredini, I. B., Sader, H. S., Goncalves, A. G., Reis, A. O., Gales, A. C., Varella, A. D. and Younes. R. N. (2004). Screening of Antibacterial Activity Extracts Obtained from Plants Native to Brazilian Amazon Rain Forest. *Braz. J. Med. Ethnopharmacol*. 62:183-193.

Wang, X., Greilberger, J., Ledinski, G., Kager, K., Paigen, B. and Jurgens, G. (2004). The hypolipidemic natural product, *Commiphora mukul* and its component guggulsterone inhibit oxidation modification of LDL. *Atherosclerosis* 172:239-246.