



ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *Commiphora africana*

*¹Idris, M. M and ²Usman, S.J.

Department of Microbiology, Bayero University, P.M.B. 3011 Kano, Nigeria.

Correspondence Author: mimusa.mcb@buk.edu.ng, GSM: 07067654224

ABSTRACT

Commiphora africana has been used traditionally for generations in many parts of the world to treat a wide range of ailments. It has been reported to have bioactive compounds and has been recognized for its medicinal importance. The antimicrobial activity as well as the phytochemical screening of the aqueous, ethanol and petroleum ether crude extracts of *Commiphora africana* against clinical isolates of *Candida albicans*, *Epidermophyton floccosum*, *Staphylococcus aureus* and *Escherichia coli* were evaluated using agar well diffusion method. The results obtained from the phytochemical screening showed the presence of alkaloids, flavonoids, saponins and tannins. The crude leaf extracts at 400µg/ml, had high antifungal activity against *Candida albicans* (24mm), *Epidermophyton floccosum* (23mm) and *Staphylococcus aureus* (18mm). *Escherichia coli* resisted to all the extracts except for petroleum ether where it showed 10mm zone of inhibition. Clotrimazole and Ciprofloxacin were used as positive control and were active against ame isolates at 25µg/ml with zones 30mm and 19mm respectively. In conclusion, the study revealed that the crude leaf extracts of *Commiphora africana* had antimicrobial activity and can serve as a potential source of natural bioactive compounds for the treatment of fungal infections.

Keywords: *Commiphora africana*, extracts, antimicrobial activity, phytochemicals, clinical isolates, bacteria, fungi.

INTRODUCTION

Commiphora africana commonly referred to as African myrrh, is a shrub of the family Burseraceae. It has short lateral branches and forms small clusters of leaves at the end of the apex of the branches. It has wide geographic distribution in Africa, Asia and Middle East. In Nigeria it is found in the northern region, most commonly used to treat wide range of ailments. Different parts of *C. africana* like the bark, roots, leaves and fruit are used for in the treatment depending on the ailment (Kokwaro, 2009; Akor and Anjorin, 2009)

In Nigeria and many other developing countries, 80% of the population depend on indigenous medicinal plants as herbal remedies to treat infectious diseases, especially in the rural communities where availability and easy access to good synthetic drugs is still a problem for their primary health needs (Lifongo *et al.*, 2014). Moreover, the global health threat posed by drug resistance/failure and the slow pace of drug discovery and manufacture has made study of medicinal plants key in discovery of novel bioactive compounds. Nigerian scientists are now, with increased public awareness and improvements in methods, required to validate

and gather information on the antimicrobial activities of indigenous medicinal plants as well as their bioactive compounds and safety (Nasir *et al.*, 2015).

MATERIAL AND METHODS

Plant materials and Preparation

Fresh leaves of *Commiphora africana* were collected at Gwale local government area of Kano state and authenticated at the Herbarium of the Department of Plant Biology Bayero University, Kano with voucher number BUKHAN 0114. The leaves were cut into pieces and air dried at room temperature for 10 days, after which it was grinded using sterile electric blender. The powder was stored in air-tight bags for further use in extraction.

Extraction of Plant Material

The powdered leaves (30g) of *C. africana* were soaked in 300ml distilled water for aqueous extraction. Ethanol and petroleum ether extracts were obtained by soaking 30g of the powder in 300ml of each of the solvents. Crude extracts were then filtered using sterile Whatman No. 1 filter paper and concentrated with Rotary evaporator (Akinyemi *et al.*, 2005).

Preliminary phytochemical screening of crude extracts

The methods described previously by Trease and Evans (2002), and Sofowora (1993) were adopted. Standard procedure was used during procedure. The crude leaf extracts of *C. africana* were subjected to preliminary qualitative phytochemical screening for secondary metabolites such as alkaloids (Wagner's reagent), tannins, flavonoids, reducing sugars (alkaline reagent test), saponins (Frothing test) and starch (Molisch's).

Test Organisms

Staphylococcus aureus, *Escherichia coli* and *Candida albicans* were obtained from the Microbiology laboratory of Aminu Kano Teaching Hospital Kano, while *Epidermophyton floccusum* was obtained from the Kano State Dermatology Hospital, Bela. The isolates were identified by colony morphology, Gram's reaction and biochemical characterization (Cheesbrough, 2006).

Standardization of Inoculum

Loopful of the the test isolate was picked using a sterile wire loop and emulsified in 3ml of sterile physiological saline. The turbidity was then adjusted to 0.5 McFarland standards (Cheesbrough, 2006).

Preparation of Stock

Stock solutions were prepared by dissolving 0.5 g of the crude extracts in 5ml of dimethyl sulphoxide (DMSO) under aseptic conditions to make 10% w/v working solutions. A serial dilution method was used to prepare four working solution of different concentrations (400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml)

Bioassay

The agar well diffusion method was used to test the antimicrobial activity of the plant extracts against different bacterial and fungal clinical isolates. The agar plates was prepared by pouring 15 ml of warm molten media into sterile Petri dishes and allowed to cool. The bacterial and fungal isolates were then inoculated on Mueller Hinton and Saboraud's Dextrose Agar respectively, then incubated at 37°C for about 6 hours after which the nutrient agar plates were seeded with the test microorganisms by the spread plate technique, and were left for about 30 minutes to dry (Ayo *et al.*, 2007). After allowing the plates to dry, wells with 6 mm diameter was punched on each agar plate. The wells were numbered accordingly to match with the code number of test extract concentrations; this was done in duplicate. Then, the

extracts were poured into wells, while matching the well number with the corresponding code number of the extract concentration. After allowing for about 15 minutes, the plates were incubated at 37°C for 24hrs. The assessment of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the wells. Ciprofloxacin (0.1 of 30µg/ml) and Clotrimazole (0.1 of 30µg/ml) were included to serve as control for bacteria and fungi respectively.

Minimum Inhibitory Concentration (MIC)

MIC is the lowest concentration of an antimicrobial compound that inhibits visible growth of microorganisms after 24hours of incubation for agar well diffusion method or no visible turbidity is observed in test tubes for broth method. The MIC was determined by preparing various concentrations of the extracts by serial doubling dilution using dimethyl sulfoxide and incorporated into 2ml nutrient both test tubes. Each test was inoculated with 0.1ml of the standardized inocula and incubated at 37 °C and 25 °C, for bacteria and fungi respectively. The lowest concentration of extracts without the inocula served as positive control while tubes containing broth and inocula without extracts served as negative control (Wiegand, *et al.*, 2008)

RESULTS

Table 1 shows the Phytochemical screening results of the ethanol, aqueous and petroleum ether leaf extracts of *Commiphora africana*. The crude extracts were found to contain saponins, alkaloids, flavonoids, tannins, reducing sugars and starch.

The antimicrobial activity of the ethanolic, aqueous and petroleum ether leaf extracts of *Commiphora africana* are presented in Table 2. The extracts were tested against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Epidermophyton floccusum*. All the isolates were susceptible to the extracts except *E. coli* that was only slightly susceptible to the petroleum ether extracts. Higher antifungal activity was observed.

The minimum inhibitory concentrations of all extracts were high for *S. aureus*. Ethanolic and petroleum ether extracts had lower MIC (200µg/ml and 250µg/ml) compared to the aqueous extract (300µg/ml). *Escherichia coli* only showed inhibition for petroleum ether extract, and at high concentration of 500µg/ml.

Table 1. Phytochemical constituents of *Commiphora africana*.

| Solvent | Saponin | Tannin | Flavonoids | Alkanoids | Reducing sugars | Starch |
|---------|---------|--------|------------|-----------|-----------------|--------|
| ELE | + | + | + | + | + | + |
| PLE | + | + | + | + | + | + |
| ALE | + | + | + | + | + | + |

Key: ELE: ethanolic leaf extracts; PLE: petroleum ether leaf extracts; ALE: aqueous leaf extracts; +: positive

Table 2. Antimicrobial activity of leaf extracts of *Commiphora africana* against some clinical isolate.

| Concentrations (µg/ml) | Extracts | <i>S. aureus</i> | <i>E. coli</i> | <i>C. albicans</i> | <i>E. floccusum</i> |
|------------------------|-----------------|------------------|----------------|--------------------|---------------------|
| | Aqueous | | | | |
| 400 | | 10mm | 6mm | 10mm | 12mm |
| 200 | | 8mm | 6mm | 8mm | 10mm |
| 100 | | 6mm | 6mm | 5mm | 6mm |
| 50 | | 6mm | 6mm | 2mm | 3mm |
| | Ethanol | | | | |
| 400 | | 18mm | 6mm | 23mm | 24mm |
| 200 | | 14mm | 6mm | 16mm | 18mm |
| 100 | | 8mm | 6mm | 10mm | 12mm |
| 50 | | 6mm | 6mm | 6mm | 6mm |
| | Petroleum Ether | | | | |
| 400 | | 8mm | 10mm | 12mm | 10mm |
| 200 | | 6mm | 8mm | 10mm | 8mm |
| 100 | | 6mm | 6mm | 6mm | 6mm |
| 50 | | 6mm | 6mm | 6mm | 6mm |
| 40 | CLO | NA | NA | 30 | 26 |
| 40 | CPX | 24 | 27 | NA | NA |

*6mm is the agar well diameter, CPX=Ciprofloxacin, CLO= Clotrimazole NA= Not Applicable

Table 3. Minimum Inhibitory Concentration (MIC) of leaf extracts of *Commiphora africana*.

| Test isolates | MIC(µg/ml)g/ml | | |
|------------------------------|----------------|---------|-----------------|
| | Aqueous | Ethanol | Petroleum Ether |
| <i>Staphylococcus aureus</i> | 300 | 200 | 250 |
| <i>Escherichia coli</i> | NA | NA | 500 |

Key: NA= No Activity

DISCUSSION

Table 1 showed that the leaf extracts of *Commiphora africana* contains alkaloids, flavonoids and tannins, saponin, reducing sugars and starch, which agrees with the work of Akor and Anjorin (2009), that studied the phytochemicals of *Commiphora africana* root extracts. Phytochemical are non-nutrient bioactive natural compounds that are produce by plants as secondary metaboht lites and are also very good antimicrobial agents. (Anyawun and Okoye, 2016).

Commiphora africana extracts have a dose dependent antimicrobial activity in which all extracts showed high activity, especially the ethanolic extract) against the fungi (*Candica albicans* and *Epidermophyton floccusom*) and Gram positive bacteria (*S. aureus*) tested; with no activity against the gram negative (*E. coli*).

This is in agreement with the finding of Toma *et al.* (2016) where the gram negative bacteria had least activity. This is concurrent with previous studies by which showed that *Commiphora* species have considerable antimicrobial activity. Akor and Anjorin (2009) reported the effect of root ethanolic extract from *Commiphora africana* to be active against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Musa (2008) showed that *C. kerstingii* stem bark contains antimicrobial activities against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Bacillus subtilis*. It was observed that *S. aureus* had minimum value for the ethanolic extracts, while *E. coli* only had minimum for Petroleum ether extract, 500µg/ml, which was higher than all concentrations used.

CONCLUSION

This study has shown that leaf extracts of *Commiphora africana* have antimicrobial activity against all tested isolates with the exception of *E. coli* which was resistant to the aqueous and ethanolic extract.

REFERENCES

- Adebayo, A.H., Aliyu, R., Gatsing, D. and Garba, I.H. (2006). The Effects of Ethanolic Leaf Extract of *Commiphora africana* (Burseraceae) on Lipid Profile in Rats. *International Journal of Pharmacology* 2: 618-622.
- Anyanwu, M.U, and Okoye R. C. (2017) Antimicrobial activity of Nigerian medicinal plants. *Journal of Intercultural Ethnopharmacology*. 6(2): 240-259
- Akinyemi K O, Oladapo O and Okwara C E, (2005) Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complement Altern Med.*;5:6.
- Akor, J.S. and Anjorin, T.S. (2009). Phytochemical and antimicrobial studies of *Commiphora africana* root extracts. *International Journal Agriculture and Biology* 11: 795-797.
- All, A.S. and Ali M.A.Z. (2007). Antibacterial Activity of *Commiphora quadricincta* from Saudi Arabia. *Journal of King Saud University* 1 (12):1420-2000.
- Ayo, R.G., Amupitan, J.O. and Zhao, Y. (2007). Cytotoxicity and antimicrobial studies of 1, 6, 8- trihydroxy-3-methyl-anthraquinone (emodin) isolated from the leaves of *Cassia nigricans* Vahl. *African Journal of Biotechnology* 6 (11): 1276-1279.
- Cheesebrough, M. (2006) District Laboratory Practices in Tropical Countries, 2nd ed. Part 2. Low price edition. Cambridge University press.

RECOMMENDATION

Further studies should be done to determine the active components of *C. africana* leaves and the safety the extracts. Also higher concentrations should be used in subsequent studies for the plants activity against *Escherichia coli*.

- Kokwaro, JO. (2009) Medicinal plants of East Africa: Third edition. Nairobi: University of Nairobi press., 478.
- Lifongo, LL, Simoben, CV, Ntie-Kang, F, Babiaka, SB and Judson PN. (2014) A bioactivity versus ethnobotanical survey of medicinal plants from Nigeria, West Africa. *Nat Prod Bioprospect* ;4:1-19.
- Musa, A.A. (2008). Antioxidant and antibacterial activity of *Commiphora kerstingii* (Engl.) stem bark extract, *Research Journal of Phytochemistry* 2: 106-111.
- Nasir, B, Fatima, H, Ahmed M, and Haq, IU. (2015) Recent trends and methods in antimicrobial drug discovery from plant sources. *Austin J Microbiol* ;1:1002.
- Sofowora, A.(1993) Screening Plants for Bioactive agents. *Medicinal Plants and Traditional Medicine in Africa*, 2nd ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. 134-156.
- Toma I, Gidado, Khan M and Abel A. (2016) Phytochemical Screening, Antioxidant and Antibacterial Activities of *Commiphora kerstingii*. *Int. Biol. Biomed. J. Summer*; 2- 3
- Trease GE and Evans WC (2008). *A Textbook of Pharmacognosy*, 16th ed. Bailliere Tinnall Ltd. London
- Wiegand, I, Hilpert, K and Hancock, RE. (2008) Agar and Broth Dilution Methods to Determine the Minimal Inhibitory Concentration (MIC) of Antimicrobial Substances. *Nat Protoc.* 3(2): 163-75