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Antibacterial Activity of *Commiphora Africana* Leaf Extracts on Some Bacteria Associated with Persistent Diarrhea

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Abstract:

The study was aimed to investigate the phytochemical constituents and antibacterial activity of *Commiphora africana* leaf extracts against some bacteria associated with persistent diarrhea. Aqueous and methanol extracts from *Commiphora africana* leaves were prepared, screened for phytochemicals analysis and tested for antibacterial activity against *Salmonella typhi, Klebsiella* sp, *Shigella* sp and *Escherichia coli*. Phytochemical screening of the extracts showed that *Commiphora africana* leaf extracts contain Alkaloid, saponin, tannin, phenol, steroid, flavonoid, terpenoid and glycoside. Statistical analysis of the result showed that methanol extract demonstrated highest antibacterial activity with average zone of inhibition of 14.5 mm among the isolates than aqueous extracts (13.2 mm). Based on the susceptibility of the organisms to the extracts, *E. coli* was found to be the highest susceptible organisms with average zone of inhibition of 14.9 mm, followed by *Klebsiella* sp (14.2 mm), *Shigella* (13.9 mm), while least average zone of inhibition is shown by *Salmonella typhi* (12.4 mm). The MIC and MBC of the extracts ranges from 3.125 to 50 mg/ml There is no significant different on the susceptibility of the organisms against the extracts at $p<0.05$. The results of the present study have provided the justification for therapeutic potential of *Commiphora africana* leaf.

Keywords: Antibacterial activity, *Commiphora africana*, Bacteria, Phytochemicals

Introduction

Natural products, particularly from plants, have played a vital role in the discovery of drugs. Some of these drugs are completely derived from natural products, while some natural products serve as leads for novel drugs development [1]. One of the most important roles herbal medicines play in modern drug development is the identification of plants with useful therapeutic compounds [2]. The antibacterial activity of plant is due to present of phytochemical constituents in them. Phytochemicals are secondary metabolites present in medicinal plants which include terpenoid, flavonoids, steroid, Alkaloids and phenolic compounds. The phytochemicals have impressive pharmaceutical properties such as analgesics, aesthetic, antibiotics, antiparasitic, anti inflammatory, oral contraceptive, hormones and ulcer therapeutic laxative [3].

Commiphora africana commonly referred to as African myrrh, is a shrub of the family Burseraceae. The plant has short lateral branches, sharply pointed at the apex, bearing leaves in small clusters below the tip [4]. It has wide geographic distribution in Africa, Asia and Middle East [5]. 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 [6]. Parts of the plant are medicinally consumed in several West African countries possibly, because of the presence of phytochemicals [7]. Extracts of *C. Africana* is used to treat bronchitis, whooping cough and diseases of the genito-urinary organ [8] as well as exert antimicrobial effect [9]. The pharmacological activity and antimicrobial activity of *Commiphora africana* is due to the presence of phytochemicals such as methylisopropenyl furnace, sesquiterpenes, commiphoric acid, tannins, alkaloids, flavonoids and saponins [10]. The preliminary phytochemical screening of *C. africana* extract revealed the presence of flavonoids, tannin, anthraquinone, triterpenoides, saponins and alkaloids [11].

Bacterial resistance to antibiotics represents a serious problem for clinicians and the pharmaceutical industry and great efforts are being made to reverse this trend, and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infectious diseases [12]. With regard that, the study was aimed to determine the phytochemical constituents and antibacterial activity of *C. africana* leaf extracts on some bacteria associated with persistent diarrhea.

Materials and Methods

Sample Collection and Identification

The leaves of *C. africana* were collected at Rangaza town, Ungogo Local Government Area in Kano State, Nigeria. The identification and authentication of the plant leaves was done at the Herbarium in the Department of Plant Science, Bayero University Kano with the following voucher number BUKHAN 0276, and voucher specimens were deposited there for future reference. The leaves specimens were washed thoroughly with distilled water and air-dried in a shade for two weeks, then cut into pieces and milled into powder using a sterile pestle and mortar under laboratory condition. The powder was then kept in air tight container for future use as described by Ali *et al*. [13].

Bacteria Isolates

Isolates of *Salmonella*, *Shigella*, *Escherichia coli* and *Klebsiella* isolated from patients diagnosed with persistent diarrhea were obtained from pathology Department of Murtala Muhammad Specialist Hospital Kano. Identification of the isolates was conducted using conventional microbiological methods namely; Gram staining, cultural characterization using selective media and biochemical characterization as described by Cheesbrough [14]. The pure isolates of each of the test organisms were stored in peptone water and refrigerated at 4^0C before use.

Preparation of Plant Extract

Methanol and water were used in the extraction process. Fifty grams (50 g) of the powdered leaves were weighed out and mixed with 500 ml of distilled water and methanol respectively in a separate sterile conical flask and allowed to stand for three days. The mixtures were filtered using Whatman filter paper and the extracts were evaporated to dryness using rotary evaporator and water bath for methanol and aqueous extracts respectively. The solid residues obtained were measured and reconstituted in 10% DMSO at a stock concentration of 200mg/L; various concentrations were made from the stock solution and stored at 4^0 C until used [13].

Phytochemical Screening

Preliminary phytochemical screening was conducted to determine some bioactive component of *C. africana* leaf extracts. Presence of alkaloids, saponin, glycoside, tannin, flavonoids, steroids, terpenoid, anthraquinone and phenol were determined using procedures described by Sofowora [15] and Trease and Evans [16].

Test for Alkaloids

Wagner's test: To 0. l ml of the extract in a test tube, 3 drops of Wagner's reagent (Iodine in Potassium iodide) was added. Formation of brown/ reddish precipitate indicates the presence of Alkaloids.

Test for Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. The formation of yellow colored precipitate indicates the presence of flavonoids.

Test for Glycosides

Ten (10) ml of 50% Tetraoxosulphate (VI) acid was added to 1 ml of the extract in a separate test tube and the mixture was heated gently for 15 minutes followed by addition of 10 ml of Fehling solution and boiling. A brick red precipitate indicated the presence of glycosides.

Test for Saponins

Foam test: Half gram (0.5 g) of the powdered sample was dispensed in a test tube and 5 ml of distilled water was added and shaken vigorously. Persistent froth (foam) that lasted for about 10 minutes indicated the presence of saponin.

Test for Steroids

To 2 ml of the sample, 2 ml of acetic acid was added and the solution was kept under ice for cooling for few minutes. Then 2 ml of concentrated Tetraoxosulphate (VI) acid was added carefully. Color changes, from violet to blue/bluish green indicated the presence of steroids.

Test for Tannin

Gelatin test: To 2 ml of the extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicated the presence of tannins.

Test for Phenol

Ferric chloride test: Extracts were treated with 5 drops of ferric chloride solution. The formation of bluish black color indicated the presence of phenols.

Test for Terpenoid

Salkowski test: About 5 ml of extract was added with 2 ml of chloroform and 3 ml of concentrated Tetraoxosulphate (VI) acid. Reddish brown colour at the interface, indicates the presence of terpenoid.

Test for Anthraquinone

About 2 ml of extract was added into a test tube, 5 ml of benzene was added and shaken, then 5 ml of 10% Ammonia solution was also added followed by shaking. The formation of pink/red/violet color in the lower phase is positive for Anthraquinone.

Antimicrobial Activity of the Extracts

The agar well diffusion method was used to determine the antibacterial activity of the plant extracts as described by Ali *et al*. [13]. A 0.1 ml volume of the different standardized organisms (0.5 MacFarland) was introduced onto the surface of freshly prepared Mueller Hinton Agar in a sterile Petri dish and allowed to set and then labeled. A 6 mm sterile cork borer was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four of the wells were filled with different concentrations of the extract which were labeled accordingly; 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml, while the 5th well contained the control, 50 mg/L of Tetracycline (Pal Pharmaceutical), used as control in this research. These were then left on the bench for 1 hour to enable proper diffusion of the extracts and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each well was measured to the nearest millimeters along straight line.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts

The MIC of the extracts was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2ml of 200 mg/ml of the extract into a test tube containing 2 ml of Nutrient broth, thus producing solution containing 100 mg/ml of the extract. The process continue serially up to test tube No. 5, hence producing the following concentrations; 100, 50, 25, 12.5, 6.25 mg/ml. Test tube No. 6 do not contain extracts and serve as Control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37°C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity. The least concentration of the extract where there was no growth in tube was taken as the MIC. From each tube that did not show visible growth in the MIC, 0.01ml was aseptically transferred into extract free Mueller Hinton agar plates. The plates were incubated at 37°C for 24 hour.

The MBC was recorded as the lowest concentration (highest dilution) of extract that had less than 99% growth on nutrient agar plates [17].

Statistical Analysis

The data on the average zone of inhibition produced by the isolates against the extracts used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

Results

Phytochemical Screening

The phytochemical screening of aqueous and methanol *C. africana* leaf extracts is presented in Table 1. The result indicated the presence of Alkaloid, terpenoids, flavonoids, anthraquinones, phenol, saponin and tannin while are steroid is absent.

Table 1: Phytochemical screening of C. africana leaf extracts

S/N	Phytochemical	Aqueous extract	Methanol extract
1	Alkaloids	+	+
2	Flavonoid	+	+
3	Glycosides	+	+
4	Saponin	+	+
5	Steroids	-	-
6	Phenols	+	+
7	Terpenoid	+	+
8	Anthraquinones	+	+
9	Tannin	+	+

Antibacterial activity of Aqueous Leaf Extract

The antibacterial activity of aqueous *C. africana* leaf extract is presented in Table 2. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *E. coli* (17.5 mm) at 100 mg /ml. The zone of inhibition of the control (Tetracycline 50 mg/ml) ranges from to 20 - 22 mm

Table 2: Antibacterial activity of aqueous C. africana leaf extract

	Concentration (mg /ml)/zone of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Salmonella typhi	09.20±0.00^{a}	10.80±0.00^{a}	12.50±0.11^{b}	13.50±0.13^{b}	20
Klebsiella sp	11.30±0.15^{a}	12.40±0.13^{b}	15.40±0.22^{c}	16.50±0.26^{c}	21
Shigella sp	11.70±0.17^{a}	12.30±0.20^{a}	13.80±0.09^{b}	14.20±0.31^{b}	22
Escherichia coli	12.30±0.20^{a}	14.40±0.12^{b}	14.70±0.17^{b}	17.50±0.36^{c}	22

***Key**: Values having different superscript on the same row are considered significantly different at p<0.05*

Antibacterial Activity of Methanol Extract

The antibacterial activity of methanol *C. africana* leaf extract is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *Shigella sp* (18.7 mm) at 100 mg /ml. The zone of inhibition of the control (Tetracycline 50 mg/ml) ranges from to 20 - 22 mm

Table 3: Antibacterial activity of methanol C. africana leaf extract

	Concentration (mg /ml)/zone of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Salmonella typhi	11.30±0.20^{a}	12.40±0.12^{a}	14.20±0.17^{b}	15.50±0.17^{b}	20
Klebsiella sp	12.80±0.12^{a}	13.50±0.17^{b}	15.40±0.25^{c}	16.40±0.20^{c}	21
Shigella sp	11.70±0.32^{a}	12.80±0.25^{a}	16.10±0.32^{b}	18.70±0.37^{c}	22
Escherichia coli	13.3±00.32^{a}	14.20±0.20^{a}	15.80±0.12^{b}	17.30±0.32^{b}	22

***Key**: Values having different superscript on the same row are considered significantly different at p<0.05*

MIC and MBC of the Extracts

Minimum inhibitory concentration of aqueous and methanol extract of *C. africana* leaf is represented in Table 4. The result showed dilutions of various concentrations of aqueous and methanol extracts can inhibit and/or kill the isolates. Lower MIC (6.25 mg/ml) was shown by methanol extract than aqueous extract. MBC of the extract ranges between 12.5-50mg/ml.

Table 4: Minimum inhibitory concentration (MIC) and MBC of the extracts

	Aqueous extract		Methanol extract	
Isolates	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
Salmonella typhi	12.5	50.0	6.25	25.0
Klebsiella sp	6.25	12.5	6.25	25.0
Shigella sp	12.5	25.0	6.25	12.5
Escherichia coli	6.25	25.0	3.125	12.5

Discussion

Medicinal plants represent a rich source from which antioxidant and antibacterial agents may be obtained. Plants are used medicinally and can be a potent source of many drugs [18]. The results of the present study suggested that several phytochemicals were present in *C. africana* leaf extracts. Several studies were conducted to ascertain the phytochemical constituent of *C. africana* [6,10]. This finding is in conformity with that of Idris and Usman [19] who found the presence of alkaloids, flavonoids and tannins, saponin and reducing sugars in *C. africana* leaf extracts. The presence of the phytochemicals can be correlated with the fact that solvent extracts showed antibacterial activity against the bacterial isolates. Phytochemicals give plants their colour, flavor, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites [20]. Alkaloids are known to play some metabolic roles and control development in living system [21]. It also interferes with cell division, hence the presence of alkaloids in clove could account for their use as antimicrobial agents. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory and immunomodulatory properties [22]. Flavonoids are also present in the extract as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [23]. Steroids are importance in pharmacy as they possess compounds like sex hormones and can be used for drug production [24]. Saponins protect against hypercholesterolemia and antibiotics properties [25]. In addition, it has been found that saponins have antitumor, antioxidant and anti-mutagenic activities and can lower the risk of human cancers by inhibiting the growth of cancer cells [26,27]. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins [28].

The results of antibacterial activity of *C. africana* leaf extracts (Table 2 and 3) showed that the methanol extract is more effective against the tested isolates than aqueous extracts. This is in agreement with the *in vitro* studies by Paraskeva *et al*. [29] using selected African *Commiphora* species. The methanolic extract ranked higher in inhibiting the growth of the tested bacteria, with largest inhibition. The better efficacy of the ethanol extract as against the aqueous extract may be because different solvents have different polarities, hence different degrees of solubility for the various phyto-constituents [30]. *E. coli* and *Klebsiella* sp were more susceptible to the extracts in comparison with the rest with average zone of inhibition of 14.90 mm and 14.20 mm respectively. The result of antimicrobial activity of *C. africana* leaf in this study was inconformity with the study conducted by many researchers [6,19,31]. Based on the result of the present study, the antibacterial activity of the extract against the extract was dose dependent. This is in

agreement with previous studies conducted by Bakari *et al*. [32] and Ibrahim *et al*. [33] which demonstrated that several *Commiphora* species had considerable antibacterial activity against some gram positive and gram negative bacteria. The antibacterial activity of the extracts was due to the presence of phytochemical constituents such as alkaloid, tannin, terpenoid and saponin which possessed antibacterial agents.

The result of MIC and MBC of the extracts showed that dilutions of various concentrations of aqueous and methanol extracts of *C. africana* leaf can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by methanol extract than aqueous extract. MBC of methanol extract ranges between 12.5 - 50mg/ml

Conclusion

In conclusion, this study revealed that *C. africana* leaf extracts possess antibacterial activity that inhibits bacterial growth. The results of the present study show that methanol extracts of *C. africana* leaf are more effective against all tested bacterial strains than aqueous extracts. *E. coli* and *Shigella* were also more susceptible to the extracts while *Klebsiella* was the least susceptible. The antibacterial activities of the extracts are due to the present of bioactive compounds like Alkaloid, Terpenoid, Saponin, Tannin, flavonoids and flavonoids which were dissolved in the solvents. It is recommended that the use of natural herbs should be encouraged. Further study on toxicity effect of the plant is also recommended.

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