



An Investigation of the Phytochemical Composition and Antimicrobial Activities of Commiphora Africana Stem Bark Extract

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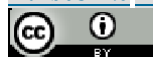
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ABSTRACT

Commiphora africana, a traditional African medicinal plant, has been used to treat various infections. This study investigated the phytochemical composition and antimicrobial activities of its stem bark extract. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, and terpenoids. The extract exhibited significant antimicrobial activity against a panel of microorganisms, including Staphylococcus aureus, Escherichia coli, and salmonella typhi, with MIC values ranging from 0.5-2.5 mg/mL. The antioxidant activity of the extract was also evaluated, showing a high scavenging capacity. The findings suggest that the stem bark extract of Commiphora africana is a potential source of natural antimicrobial and antioxidant agents, supporting its traditional use in medicine. Further studies are needed to isolate and characterize the bioactive compounds responsible for these activities

INTRODUCTION

Commiphora africana commonly referred to as African myrrh, is a shrub of the family Burseraceae and has short lateral branches and forms small clusters of leaves at the end of the apex of the branches (Idris, M.M. and Usman, 2018). It has wide geographic distribution in Africa, Asia and Middle East and in Nigeria it is found in the northern region, most commonly used to treat wide range of ailments (Idris, M.M. and Usman, 2018). Phytochemical studies have shown that the pharmacological activities of *Commiphora africana* are due to the presence of secondary metabolites such as flavonoids, coumarins, triterpenoids, saponins, and alkaloids (Mwangi et al., 2020).

Commiphora plants (myrrh plants) are well known in various cultures to have medicinal virtues including treating infectious diseases and the genus *Commiphora* has been exploited worldwide as a natural drug to treat pain, skin infections, inflammatory conditions, diarrhea, periodontal diseases, and wounds also in the Islamic inspired culture, it was utilized for the treatment of intestinal parasites, diarrhea, wound treatment, persistent chest ailments (Nimbeshaho et al., 2020). Different plant parts and components (roots, leaves, stem barks, flowers or their combinations, essential oils) have been employed in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin (Adekunle et al., 2009). Parts of the plant are medicinally consumed in several West African countries possibly, because of the presence of phytochemicals such as methylisopropenyl furane, sesquiterpenes and commiphoric acid and a macerate of crushed leaves in oil is drunk in Cote d'Ivoire and in Burkina Faso as a sedative and soporific (Akor J.S and Anjorin T.S., 2009). In addition to being widely used to prepare clean washes and showers for skin infections, bruises, and illness, bark extracts have been shown to have some insecticidal effect and to repel termites. Gum is frequently used to make antiseptic baths and washes for leprosy, skin infections, and ulcers. Bark extracts have also been demonstrated to have some insecticidal effect and to repel termites. The seed contains tannin, dye stuff, a fixed oil, dihydroflavonol glucoside and Z-guggulsterone (Akor J.S and Anjorin T.S., 2009). The study investigates the phytochemical composition and antimicrobial activities of *Commiphora africana* stem bark extract.

METHODOLOGY

Materials

The plant, *Commiphora Africana* was obtained from Malammadori local government Area Jigawa state, washed and air dried in the laboratory at room temperature for 25 days. Pulverization of the dried stem bark in to powder was carried out using mortar and pestle.

After drying, this was ground into a rough powder. The extraction solvents that were employed were silica gel (Kiesel gel S. 0.2 - 0.5 mm) for column chromatography (from Riedel-Dettaen Ag. Seelze Hannover), ethanol (from BDH Chemicals), ethyl acetate (Rectapur), and n-hexane (Merek). Using iodine vapor and a Camag Ultra Violet (U.V.) lamp 366-254 mm as detectors, R_f values were acquired on pre-coated Merek grade TLC plates.



Figure 1. A Picture of Commiphora Africana Plant
Microbiological Media: Test Organisms and Materials for Antibacterial Test

Below are list of the microbiological media used: Nutrient broth, Nutrient agar, McFarland turbidity standard scale. The following microorganism were tested for: Salmonella typhi (*S. typhi*), Escherichia coli (*E. COLI*), and Staphylococcus

Extraction

Each of the powdered sample was subjected to gradient extraction with cold ethanol. The sample was extract for 48 hours with each solvent. The extract was dried on a water bath (60 °C) and the residue cooled and stored in sterile container till required. The % yield of the crude extract was determined using remainder equation.

$$\% Yield = \frac{\text{Mass of extract}}{\text{Mass of the sample taken}} \times 100$$

This resultant extract was used for phytochemical screening test using standard procedure described by (Harbone 2012).

Phytochemical Screening

Extract will be subjected to phytochemical screening using standard technique as described by (Harborne, 2010), (Evans, 2009). The metabolites tested for includes alkaloids, carbohydrates, phenolic compounds, tannins, flavonoids, saponins, steroids, glycosides etc.

Tannins Test

Two milliliters of 5% ferric chloride were added to one milliliter of extract. The presence of tannins is indicated by the formation of dark blue or greenish black. (Harbrne, J.B. and Evence, W.C., 2012).

Glycosides Test

Three milliliters of chloroform and a 10% ammonia solution were added to two milliliters of extract. Glycosides are present when a pink hue forms. (Harbrne, J.B. and Evence, W.C., 2012).

Phenol Test

One milliliter of the extract was mixed with two milliliters of distilled water and a few drops of 10% ferric chloride. Phenols are present when a blue or green hue forms. (Harbrne, J.B. and Evence, W.C., 2012).

Quinones Test

One milliliter of pure sulfuric acid was applied to one milliliter of extract. Quinones are present when a red color forms. (Harbrne, J.B. and Evence, W.C., 2012).

Terpenoide Test

Two milliliters of chloroform and concentrated sulfuric acid were added to 0.5 milliliters of the extract. Terpenoids are present when a reddish-brown tint forms at the contact. (Harbrne, J.B. and Evence, W.C., 2012).

Preparation of Culture Media

Nutrient agar was used as the medium. The media was prepared in accordance with the manufacturer's guidelines. In a screw-cap container, 35g of medium will be combined with 1 liter of distilled water, then autoclaved for 15 minutes at 1210c. After that, the medium will be poured into 90 mm sterile agar plates and let to set. To ensure sterility, the agar plates were incubated at 370°C for 24 hours.

Antibacterial Activity of the Plant Extract

Activity test

The agar well diffusion method (Irobi et al., 1994) will be used to conduct the plant extract activity test. In order to create homogenous inoculums, *E. Coli*, *S. typhi*, and *S. aureus* were clinically isolated and incubated independently on the surface of nutrient agar plates using a sterile cotton swab. Each bacterium was uniformly distributed throughout the whole agar plate surface. A sterile glass borer will be used to create five wells on the solid agar on each plate, each measuring 6 mm in diameter and 5 mm in depth. These wells will be numbered according to the various extract concentrations.

To get a 50 mg/ml concentration, 0.5g of the extract will be diluted in 10ml of distilled water. To get the remaining concentrations of 25, 12.5, and 6.25 mg/ml, serial dilution was used. After that, the various extract concentrations were poured into the corresponding wells on the inoculation plates. Since erythromycin is a wide spectrum antibiotic, 30 mg/ml of 10µg of it will also be utilized as a control. After that, the setup will be incubated at 370C for 24 hours. A clear ruler was used to measure the zones of inhibition in millimeters (mm) following incubation; they were classified as sensitive if they were greater than 10 mm or resistant if they were less than 10 mm.

Minimum Inhibition Concentration (MIC) Evaluation

Each extract's MIC was calculated using the procedure outlined by Cheruiyot et al. (2009). Plant extracts that demonstrated antibacterial activity in the agar well diffusion experiment were used to test the MBC. Then, using a doubling dilution of plant extracts in nutritional broth up to the fifth dilution, it was carried out for the concentrations of each extract (50, 25, 12.5, and 6. mg/ml). A test tube was filled with one milliliter (1 ml) of the nutritional broth. An equal quantity (1 ml) of the extract was then added to the first test tube. A serial dilution was carried out, with the last 1 ml being thrown away. Before adding 1 cc to each test tube and incubating the mixture for 18 hours at 370C, each organism will be suspended individually in 5 ml of nutritional broth and left overnight. MBC was defined as the lowest extract concentration that, during

an overnight incubation period, did not exhibit any discernible growth of the injected microbe. (Murugappan and colleagues, 2006)

Minimum Bactericidal Concentration (MBC) Evaluation

The technique outlined by Adegboye et al. (2008) was used to determine the MBCs of the extracts. Samples were extracted from tubes that showed no signs of growth in the MBC test, subcultured onto newly made nutrient agar medium, and then incubated for 48 hours at 37°C. The MBCs were determined to be the lowest extract concentration that prevented bacterial growth on the agar plate surface.

RESULTS AND DISCUSSION

Result of Extraction of the Plant *Commiphora Africana*

Mass of the powdered Steam bark used = 200g

Table 1. Characteristic of Plant extract

Solvent	Color of extract	Weight of Extract (g)	Percentage extracted (%)
Ethanol	Reddish Brown	14.96	7.48

Weight of Extract (g) = (Weight of bottle - Weight of extract) - Weight of bottle

$$\text{Weight of extract (g)} = 51.575 - 36.615 = 14.96\text{g}$$

$$\text{Percentage extracted (\%)} = \frac{W_1}{W_2} \times 100$$

$$= 7.48\%$$

W_1 is the powder's net weight in grams following extraction, and W_2 is the total weight of the powder in grams taken during extraction.

The percent recovery of the ethanol extract was calculated to be 7.48 %

Volume of the liquid extract after rinsing with the solvent = 43 ml.

The Phytochemical Screening Result

Phytochemical screening result showed that *commiphora Africana* contains the presence of Tannin, Terpenoids, Glycoside, phenol and Quinone's. The presence of this diverse secondary metabolite in the plant justified its use in folklore medicine, and have been associated with antibacterial activities.

Table 2. Phytochemical Screening of Ethanol Crude Extract of Plant C. Africana.

S/N	Phytochemicals	Tests	E.E Results
1.	Tannin	Ferric Chloride Test	+ +
2.	Glycoside	Chloroform Test	+
3.	Terpenoids	Chloroform Test	+ + +
4.	Phenols	Ferric Chloride Test	+
5.	Quinones	Sulfuric Acid Test	+ +

Key

+ = Present - = Absent

E.E = Ethanol extract

The Minimum Bactericidal Concentration Result

The ethanolic extracts' concentrations demonstrate their efficacy against *S. aureus*, *S. typhi*, and *E. coli*. This suggests that the crude ethanolic stem bark extract of the plant *Commiphora Africana* contains a component with antibacterial action.

The outcome demonstrated a strong association with the documented anti-disease use of the herb in traditional medicine. *Staphylococcus aureus*, *salmonella typhi*, and *Escherichia coli*'s sensitivity to the ethanolic bark extract suggests that the extract's compound can be further developed to combat this microorganism. As a result, the plant's use in treating skin diseases, diarrhea, dysentery, and external pile is justified because this bacterium is the cause of these illnesses.

Table 3. Minimum Bactericidal Concentration of Plant Extract of *C. Africana*

Test Organism	MBC (mg/ml) Ethanolic Extract		
<i>E.coli</i>	+	-	-
<i>S.typhi</i>	-	-	-
<i>S.aureus</i>	-	-	-

Key

+ = Bacterial Growth

- = No Growth of Bacteria

DISCUSSION

According to the results of a phytochemical test, *Commiphora Africana* contains phenol, quinones, glycosides, tannin, and terpenoids. The plant's usage in traditional medicine was supported by the existence of this varied secondary metabolite, which has been linked to antibacterial properties. In addition to causing vomiting and stomach cramps, tannin has been demonstrated to form irreversible compounds with proline-rich proteins that hinder the production of new proteins. Terpenoids have been shown to possess physiochemical and pharmacological properties; they are in charge of maintaining the health of the skin and shielding the body against bacterial and fungal invasions (Evanco W.C. and Harbrne, J.B, 2012). Ethanolic extract concentrations demonstrate efficacy against *S. aureus*, *S. typhi*, and *E. coli* (Kingston, 2008). Thus, research suggests that the crude ethanol of the bark of the plant *Commiphora Africana* contains a chemical with antibacterial action. This finding was in good agreement with the plant's documented anti-disease use in traditional medicine. The fact that *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* are sensitive to the ethanolic bark extract suggests that the extract's compound can be further developed to combat these microorganisms. As a result, the plant's use in treating skin diseases, diarrhea, dysentery, and external pile is justified because these illnesses are caused by this bacterium (Genellian et al., 2006). The lowest concentration of extract that prevented any bacterial growth on the agar plate surface is shown by the minimum bactericidal concentration (MBC) of the

extract on the test organism (i.e. the ethanolic steam bark extract killed all the bacteria).

Summary

The screening of phytochemicals was conducted in accordance with the standard protocol outlined by Harbone (2015). A yield of 1.56 percent was obtained from a sample of 10g that was sucked in 100ml of ethanol. Phenol, tannin, quinone, terpenoide, and glycoside are the secondary metabolites that are plentiful, according to the results of phytochemical screening. The steam bark extract of the plant *Commiphora africana* contains this secondary metabolite, which makes it effective against bacteria like *E. coli*, *S. aureus*, and *S. typhi*.

CONCLUSION

The ethanolic bark extract of *Commiphora Africana* had secondary metabolites such as tannin, glycosides, phenol, quinones, and terpenoids that were also present in the steam bark extract. *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* were all sensitive to the steam bark extract. This study might provide a scientific foundation and support the traditional medicine practitioners' assertion that *Commiphora Africana* steam bark extract is a practical herbal therapy with broad-spectrum antibacterial activity against the tested microorganisms (Adekunle A. A. 2009). It supports the assertion that *Commiphora Africana* steam bark extract is effective in treating conditions such as skin disorders, diarrhea, dysentery, and external pile that are specifically linked to *E. coli*, *S. typhi*, and *S. aureus* (Atmani D. N. 2009). It also comes to the conclusion that the dosage given, which might change depending on the target organism or illnesses, determines how effective the herbal mixture is.

Recommendation

It is recommended that more studies be conducted on the plant *Commiphora africana* in order to fully investigate all of its therapeutic or medicinal potential.

1. To evaluate the ethanolic extracts' effectiveness against *E. coli*, *S. aureus*, and *S. typhi*, experiments were conducted at high doses.
2. To determine the extract's efficacy against additional disease-causing agents, it was tested on other bacteria.
3. To identify, separate, and describe the bioactive components of the plant extract, research will be conducted utilizing bioassay guided fractionation.
4. The Ministry of Health demonstrated that all herbal products must undergo scientific validations prior to being marketed as treatments.

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