



**PHYTOCHEMICAL SCREENING AND ANTI-BACTERIAL ACTIVITY OF
ETHANOLIC EXTRACT OF STEM BARK OF *COMMIPHORA AFRICANA* GROWN IN
SUDAN**

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ABSTRACT

The present study was designed to evaluate the phytochemical screening and antibacterial activity of the ethanolic extract of *Commiphora Africana* stem bark. The phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, sterols and glycosides. The in vitro antibacterial activity of the ethanolic extract of the stem bark of *Commiphora Africana* was investigated. The extract exhibited antimicrobial activities with zones of inhibition activity against *S. aureus*, *S. typhi*, *E. coli* and *B. subtilis*. The MIC against *S. aureus*, *S. typhi*, *E. coli* were 25, 6.5 and 12.5 µl respectively.

KEYWORDS: Phytochemical screening, antibacterial activity, antioxidant and *Commiphora Africana*.

INTRODUCTION

Plants which have one or more of its organs containing substances that can be used for therapeutic purpose are called medicinal plants (Sofowora, 1999). Acknowledge of the chemical constituents of plant desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in discovering new sources of such economic materials, such as tannins, oils, gums, precursors. In addition the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric medicine (Fanthwort, 1966). The development of microbial resistance towards antibiotics has heightened the importance of the search of new potential effective of plants and plants constituents against pathogenic microorganism (Owais, 2006). The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective or disease preventive properties (phytochemicals). These phytochemicals often secondary metabolites present in smaller quantities in higher plants includes the alkaloids, steroids, flavonoids tannins and many others (Nonitaet al., 2010).

It has been recorded in history that medicinal herbs has been used as form of therapy for the relief of pain The exploration of the chemical constituents from plant,

pharmacological and phytochemical screening would provide the basis for developing the new lead molecules in strategic favor for natural products drug discovery. The biologically active agent from natural sources have always been of great interest to working In various diseases. (Rajivelet al., 2012).

Tribal communities are using their traditional knowledge system to cure different diseases. They use plant as source of drug through trial and error method and the process is experienced over hundreds of years, which says that the medicinal plants have been in the focus as life saving drugs right from the beginning of the human civilization. The medicinal plants have been the object of the research in both systematic and advanced area of plant sciences. (Patil, 2012).

Commiphora Africana belongs to the family of burseraceae (Irvine, 1961). It is found on dry sites and savannah forest of Africa (Nair et al., 2005). It is traditionally used for the treatment of number of ailments including the treatment of typhoid and wound healing (Dalzeil, et al., 1956). *Commiphora Africana* is a small tree sometimes reaching 10 meters but usually not more than 5m high it can be recognized unmistakably from a distance by its outline a spherical top and a short trunk with low branches, crown is rounded, with the branches

ascending and then curving downwards. Many of the branchlets end in spine. The bark is grey-green sometimes shiny, peeling membranous scale; slash red, pleasantly scented, exuding a clear gum. Has a creeping root system that spreads several meters around the tree. Leaves trifoliate, leaflet cuneate at the base and with irregular and bluntly toothed margins, waxy grey-green above with a sparse covering of hairs, lighter in color and more densely hairy below, up to 4-2.5 cm, the middle leaflet larger than laterals. Flowers in axillary clusters of 4-10 petals. Fruits reddish, 6-8mm across but sometimes larger, almost stalkless, made up of tough outer layer, which splits when ripe to reveal a hard, furrowed stone embedded in a red resinous flesh, the generic name (*Commiphora*) is based on the Greek words (kommi) which means gum and (phero) which means to bear.

Sudan is rich in rare and useful medicinal plants from which medicine can be prepared. There is a need that the local herbs be evaluated for phytochemistry so as to determine the potential of this indigenous source of medicine. The aim of this work is to carry out a phytochemical screening, antibacterial activity and antioxidant activity of the ethanolic extract of the stem bark of *Commiphora Africana* in order to know the composition and the secondary metabolites.

MATERIALS AND METHODS

The bark of *Commiphora Africana* was purchased from a local herbarium market at Omdurman city and it was authenticated by a herbalist at Kordofan University.

Extraction of the plant materials

The bark of *Commiphora Africana* was first crushed by mortar and then was ground mechanically using grinder to a coarse powder to maintain a good extract. The ethanolic extract was prepared firstly by soaking 200g of the powdered bark in 600 ml of ethanol at room temperature for 72 hours. Secondly the ethanolic extract was filtered separately after 72 hours using Whatman filter paper NO. 1. Finally the volume of the extract was reduced by evaporation.

Phytochemical screening

The ethanolic stem bark extract of *Commiphora Africana* was screened for alkaloids, flavonoids, lignans, tannins, carbohydrate, saponins and sterols.

Test for alkaloids To 5ml of the prepared ethanolic extract 1ml of 2N hydrochloric acid was added and few drops of Dragendorff's reagent were added. Brown red precipitate was formed indicating the presence of alkaloids.

Test for flavonoids To 3ml of the ethanolic extract few drops of ethanolic aluminum chloride were added. Formation of a dark yellow color was taken as a positive test for flavonoids.

To 3ml of the ethanolic extract few drops of potassium hydroxide solution were added. A dark yellow color indicated the presence of flavonoids.

To 3ml of ethanolic extract few drops of ferric chloride solution were added. Development of a dark blue coloration was taken as a positive test for flavonoids.

Vanillin/sulphuric acid test: 5ml of the prepared ethanolic extract was introduced to Whatman filter paper No.1. 5 ml of vanillin/sulphuric acid reagent was put in porcelain dish. The filter paper with the extract was introduced to the vanillin/sulphuric acid reagent. Red color was developed in the filter paper indicating the presence of flavonoids.

Test for tannins: To 3ml of the prepared extract few drops of ferric chloride were added, bluish black color was formed indicating the presence of tannins.

Test for saponins: 20 ml of the prepared extract was vigorously shaken in a test tube. The presence of a froth that could persist for one hour indicated the presence of glycosides.

Test for Sterols: Liberman – Buchard test: 1ml of acetic anhydride was mixed with 1ml of chloroform and cooled to 0°C. One drop of concentrated sulphuric acid was added; when the sample was added either in solid form or solution in chloroform, red color that changed with time was taken as a positive result.

Salkowski reaction: dissolve 1-2 mg of the sample was dissolved in 1ml of chloroform and 1ml of concentrated sulphuric acid was added; forming two phases with a red color was taken as a positive result indicating the presence of sterols.

Test for carbohydrates: Molisch's test: 2ml of the prepared extract were mixed with 0.2ml of 10% aphanaphthol 10% in addition to 2ml sulphuric acid, a bluish violet zone was formed indicating the presence of carbohydrates.

Fehling's test: to 1ml of the extract were treated with 5ml Fehling's solution (A,B) and heated; the appearance of a red precipitate was taken as a positive result for carbohydrates.

Antibacterial Activity Determination

Standard strains of bacteria were tested. The standard strains included, *S. aureus* (ATCC 259230), *S. typhi* (ATCC 259525), *E. coli* (ATCC 259523) and *B. subtilis*. The antibacterial test was performed using the agar diffusion method (Nair R. et al., 2005). The bacteria suspensions were prepared equal to the turbidity of Mac Farland 0.5 standard and were cultivated (100 µl). Wells of 6 mm diameter were made on the nutrient agar plates using a sterile cork borer. The cut agar disks were carefully removed using forceps that had been sterilized

by flaming. To each well were introduced different concentrations (25µl - 50µl and 100 µl)ethanolicextract of *commiphora Africana* stem bark. The plates were allowed to stand for 1 h at room temperature (25 ± 2 °C) for diffusion of the substances to proceed before the growth of organisms commenced. The plates were incubated at 37 °C for 24 h to observe inhibition zones.

Determination of Minimal Inhibitory Concentration (MIC)

The extracts of the test samples were tested in three dose levels of 25µl, 12.5µl, 6.25µl. The minimal inhibitory concentration was determined at different concentrations among the tested microorganism most were sensitive for ethanolic extract of *commiphora Africana* stem bark. It can be recommended as an easily available and renewal source of antimicrobial agent.

RESULT AND DISCUSSION

Thousands of diverse natural products are produced by plant and many of these are involved in plant defense. The phytochemical diversity of antimicrobial compounds includes terpenoids, saponins, phenolics and phenyl propanoids, stilbenes, alkaloids, glucosinolates, hydrogen cyanide and indole (Cooper, J., 2006). In this study, the phytochemical analysis of the ethanolic extract of the stem bark of *commiphora Africana* table (1) showed the presence of different groups of secondary metabolites which are alkaloids, flavonoids, tannins, saponins, sterols and carbohydrates which are of medicinal importance.

The antibacterial test of ethanolic extract of *C. Africana* stem bark presented in Table 2 indicated that the plant has activity against *S. aureus* (ATCC 259230), *S. typhi* (ATCC 259525), *E. coli* (ATCC 259523) and *B. subtilis*. with inhibition zones ranging from 8 to 33 mm.

The minimum inhibitory concentration values of the ethanolic extract of *C. Africana* stem bark against the test organisms in Table 3 showed that bacteria vary in the degree of their susceptibility to antibacterial agents. As expected, antibacterial agents with low activity against organisms have a high minimum inhibitory concentration, and vice versa (Crosnow, 1983). The MIC is the parameters used to evaluate the efficacy of agents such as antiseptics, disinfectants and chemotherapeutic agents (Mann A, et al., 2008). The results of this study show that the ethanolic extract of *C. Africana* stem bark has activity against several bacteria, and thus it could be useful as a source of antibacterial agents.

Table (1): The phytochemical screening on the ethanolic extract of the stem bark of *Commiphora African*.

Compound	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Sterols	+
Carbohydrates	+

+ = presence of the constituents

Table (2): Antibacterial effect of ethanolic extract of *commiphora Africana* stem bark.

Micro organism	Concentration		
	25µl	50µl	100µl
	Inhibition zone (mm)		
<i>E. coli</i>	13	17	22
<i>Staphylococcus aureu</i>	11	15	33
<i>Salmonella typhi</i>	14	18	27
<i>Bacillus subfills</i>	08	10	19

Table (3): Minimum inhibitory concentration of ethanolic extract of *commiphora Africana* stem bark.

Micro organism	Concentration			
	0 µl	25µl	12.5µl	6.25 µl
	Results			
<i>E. coli</i>	+	-	+	-
<i>Staphylococcus aureu</i>	+	-	+	+
<i>Salmonella typhi</i>	+	-	-	+
<i>Bacillus subfills</i>	+	+	+	+

(+) = presence of growth; (-) = absence of growth; MICs were presented as the lowest concentrations that did not permit any visible growth of test organisms in broth culture.

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