



Evaluation of Antibacterial Activities and Cytotoxicity of *Sclerocarya birrea* Stem Bark

Abigail John Mai*, Michael Emmanuel, Patrick Ayim, Mohammed Bello Magaji

Gombe State University, Tudun-Wada, Gombe, Gombe State, Nigeria

Email: *abinadmai@gmail.com, e.michael86@outlook.com

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Abstract

The research was aimed at the antibacterial studies and cytotoxicity of aqueous trona extract of *Sclerocarya birrea* (marula) stem bark. Extraction of the stem bark was carried out using decoction method from the dried stem bark of marula. The extract was subjected to phytochemical screening, antibacterial and cytotoxicity test. The extract indicated the presence of tannin, saponins and alkaloids with the absence of flavonoids, steroids and glycosides. An antimicrobial study of the extracts was carried out against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* using agar—well diffusion method. The extracts with trona have higher zone of inhibition at 100 mg/ml, *Escherichia coli*, (zone of inhibition = 16), *Staphylococcus aureus* (zone of inhibition = 14) and *Klebsiella pneumoniae* (zone of inhibition = 9). The cytotoxicity tests were carried out on the extracts using brine shrimp lethality assay, the extract showed a mortality with 9 nauplii dead upon exposure at 1000 ppm, and had an LC₅₀ value of 63.1 ppm. The FTIR characterization carried out on the extract indicates the presence of functional groups such as: O-H, C=O, C-N and C-C(O)-C indicating peak of alcohols, carbonyl group amine and ester.

Subject Areas

Electrochemistry, Organic Chemistry

Keywords

Trona, Marula, Cytotoxicity, Antibacterial Activity

1. Introduction

In recent years, herbal medicine has gained numerous attention due to its relatively low side effect [1]. It was reported that about 80% of the world population

especially in the developing countries, use herbs for the treatment of some disease [2]. Most pharmaceutical products depend on plants as the main source of material for their production [3]. Plant derived medicines can serve as a basis for the manufacture of different more active drugs [4] [5]. Most plants are made up of bioactive compounds such as lipids, phytochemicals, pharmaceuticals, flavonoids, fragrances and pigments. According to World Health Organization, even developed countries are beginning to turn to plants for their medicine source due to the increased resistance of most existing antimicrobial drugs [6].

Uses of *Sclerocarya Birrea* in Local Medicine

Sclerocarya birrea has been reported to have antiparasitic effect and can be used in the treatment of *trypanosomiasis* also known as African sleeping sickness [7] [8]. The methanolic extract of its leaves was reported to cause a complete mortality of the *trypanosoma brucei in vitro* [9]. *In vitro* antiplasmodial activity and *in vivo* antimalarial activity of *Sclerocarya birrea* was carried out against *P. falciparum* and *P. berghe* respectively. Results reported showed that *P. falciparum* was more sensitive to the extract compared to *P. berghe*, with the methanolic extract found to be more active than the water extract [10]. In south and east Africa, the stem-bark of *Sclerocarya birrea* is used as a potent remedy for dysentery and proctitis [11]. The Zulus of South Africa use a decoction of *Sclerocarya birrea* stem bark as a prophylactic remedy against gangrenous rectitis [12]. Decoctions or steam from boiled are prepared roots and used for the treatment of heavy menstrual flow in women, bilharzias, cough, weakness, sore eyes, and in heart pains. [13]. Traditionally the stem bark is used for the treatment of various gastrointestinal disorder especially dysentery/diarrhea, hemorrhoid, stomach ulcers and pain, sore throat/mouth and toothache [14]. Both the methanolic and aqueous extract of this plant was found to be anti-inflammatory in rats paw induced edema, and with some antioxidant activity [15].

In this research we evaluate the antimicrobial activity and cytotoxicity of the stem bark of *Sclerocarya birrea* trona extract

2. Materials and Method

Fresh stem bark of Marula (*Sclerocarya birrea*) was aseptically collected from Doho Ward Kwami Local Government of Gombe State, Nigeria using a cleaned cutlass, and was transported in cleaned polythene bag and sacks to the lab in the Chemistry Department of Gombe State University. A soft brush was used to remove any dirt/debris from the plant materials. The fresh stem bark of marula was air-dried under a shade for two weeks for proper drying and stored in an air tight container for used. The plant was identified at the Gombe state university herbarium of the department of biological science with a voucher number 460.

2.1. Water-Trona Extraction (Decoction Method)

500 g of dried sample of marula stem bark and 2 g of trona were extracted by

boiling in 2000 ml of distilled water for 60 minutes. The extracts were then filtered using Whatman Filter Paper No. 1, and the filtrate were concentrated using rotary evaporator, the crude extracts obtained were dried on a water bath and stored in an air tight container.

2.2. Phytochemical Screening

The phytochemical composition of the extracts of the plant materials were analyzed for the presence of saponins, steroids, tannins, flavonoids, alkaloids and glycosides according to standard methods [16].

2.3. Antibacterial Activity

The antibacterial activity of the crude extract was determined in accordance with Agar-well diffusion method [17]. The test organisms (*Echerichia coli*, *Klepsiella pneumoniae* and *Staphylococcus aureus*) were collected from Microbiology Department, Gombe State University and were authenticated by biochemical tests. The test organisms were aseptically sub cultured on nutrient agar for twenty four hours inside of incubator at 37°C. Using inoculation platinum wire loop, enough material from an overnight culture of the test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard [18]. Sensitivity test was conducted following the agar-well diffusion method [17].

2.4. Cytotoxicity Test

Brine Shrimp Lethality Test

Artemia salina nauplii (<48 hrs old) was exposed to a sample solution for 24 hrs and frequencies of immobility of the 10 nauplii in 5 ml solution were scored.

2.5. FTIR Analysis

The FTIR analysis of the two extracts was carried out in Gombe University, Faculty of Pharmaceutical Sciences. The FTIR spectra of the extracts which extracted with trona and the extract without trona were recorded in the range 4000 - 450 cm^{-1} using Perkin Elman Spectrum version 10.03.06.

3. Results and Discussion

3.1. Phytochemicals Screening

The result shown on **Table 1** indicated that marula stem bark extract contains tannins, alkaloids, and saponins while flavonoids, steroids, and glycosides are absent. The results of the current research are in agreement with the one reported by Kutama *et al.*, (2013) [19], with an observed difference which might be due to the difference in method of extraction, trona, high temperature and sample sizes.

3.2. Antimicrobial Activity

The result of antibacterial study of the stem bark of *Sclerocarya birrea* (marula)

and the standard antibiotic used are presented in the **Table 2**. The aqueous extract with trona was found to be effective in inhibiting the growth of the three tested bacteria as demonstrated by an agar well diffusion method. The aqueous extract shows higher zone of inhibition at a concentration of 100 mg/ml, and the zone of inhibition increases with the increase in concentration of the extract.

3.3. Cytotoxicity Test

The result obtained from cytotoxicity of the marula stem bark extracts using brine shrimp lethality assay as shown in **Table 3** shows that the effects of the

Table 1. Results of phytochemicals screening of marula stem bark with trona.

Phytochemicals	Aqueous Extract
Flavonoids	-
Saponins	+
Steroids	-
Alkaloids	+
Glycosides	-
Tannins	+

Key: + = Present; - = Absent.

Table 2. Results of antimicrobials activity of marula stem bark extract with trona.

Test Organisms	Concentrations (mg/ml)	Zone of Inhibition Diameter (mm)	Ciprofloxin
<i>Escherichia coli</i>	100	16	24
	50	9	
	25	7	
	12.5	NA	
<i>Staphylococcus aureus</i>	100	14	23
	50	10	
	25	6	
	12.5	NA	
<i>Klebsiella pneumoniae</i>	100	9	23
	50	6	
	25	4	
	12.5	NA	

Key: NA = No activity.

Table 3. Result of Brine Shrimp lethality assay of extracts with trona.

Concentration (ppm)	No. of dead nauplii	% Mortality	LC50 Value (ppm)
1000	9	90%	63.1
100	6	60%	
10	3	30%	

crude extract of *Sclerocarya birrea* on the mortality rate of the brine shrimp (*Artemia salina*) depend on the types of crude extracts and its concentration. For aqueous extract with trona 9 nauplii died upon exposure at 1000 ppm whereas at 100 ppm and 10 ppm 6 and 3 nauplii died. This indicates that the number of mortalities increases with the increase in concentration of the extract and aqueous extract with trona has lethal concentration for 50% mortality (LC_{50}) value of 63.1 ppm. Values of the LC_{50} greater than 1000 ppm indicates poor activity, and values lower than 1000 ppm indicates higher activity of the plant extract [20].

4. Conclusion

An improved antibacterial activity was obtained from the trona extract of the neem stem bark. The extract was found to have potential for the treatment of diseases related to *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* with high efficiency. Since the activity of a plant extract is mostly related to the values of LC_{50} such that values below 1000 ppm are found to be active and values above 1000 ppm are found to be inactive, with the obtained value of 63.1 ppm, we can say that the plant extract is active and effective as an anti-bacteria for the treatment of diseases caused by *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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