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Original Research Article

Phytochemical and antibacterial studies on root extracts of *Entada africana* Linn.

Bashir M. Miko [1], Kabir Y Musa [1], Rashydah Ahmed [*,2], Atinga Vallada [3]

[1] Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[2] Department of Pharmacognosy and Drug Development, Kaduna State University, Kaduna State, Nigeria.
[3] Department of Pharmacognosy and Drug Development, Gombe State University, Gombe State, Nigeria

Corresponding Author:
Rashydah Ahmed
Email: rashmill@yahoo.com
Phone: +2348036427117

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Abstract

The root of *Entada africana* L was extracted with methanol, ethyl acetate and hexane. The extracts obtained were then screened for antibacterial activity against six wound isolates; *Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Pseudomonas florescence, Klebsiella pneumonia and Escherichia coli*. The results of this study showed that the methanol root extract of *Entada africana* produced the highest antibacterial activity, active on four out of the six organisms tested while that of hexane and ethyl acetate were only active on three organisms. Also, ethyl acetate extract produced a mild inhibition on *P. florescence* at 100mg/ml. The best inhibitory potential produced by the methanol extract was observed on *S. aureus* with minimum inhibitory concentration (MIC) value of 1.5mg/ml, followed by *Streptococcus pyogenes and Klebsiella pneumonia* both with values of 6.25mg/ml. *Proteus mirabilis, P. florescence* and *E. coli were not inhibited by the methanol extract. The root of E. africana showed strong antibacterial property on some common wound infectious bacteria, thus justifies the traditional use of E. africana in the treatment of wounds and skin infections.*

Keywords: Antibacterial; hexane extract; ethyl acetate extract; methanol extract.

1.0 INTRODUCTION

Medicinal plants are important sources of bioactive compounds, they play a vital role in traditional medicine for the treatment and management of diseases, medicinal plants cater for the health needs of about 80% of the world population [1]. These plants are natural substances that provide an alternative means to modern medicine [2]. Preparations from medicinal plants used in traditional practice from different parts of the world are useful leads for the development of new antibiotics [3]. Antibiotic resistance is a global concern [4] and in recent times more bacterial pathogens have emerged due to new modes of bacterial mutation. Scientists are continuously in search of new and better bioactive phytoconstituents that could be developed as useful anti-microbial for treatment of infectious diseases. Currently, out of 80% of pharmaceuticals derived from plants, only a little fraction of it is being used as anti-microbial [5].

Entada africana is a small tree, 4-10 m in height and 90 cm in girth with low branching that grows in high rainfall savannah areas. It

is known as Ogurobe in Yoruba and Tawatsa in Hausa Language [6]. The bark is brown-grey to black in colour, very rough, scaly and peels into long fibrous strips. The leaves are bipinnate, alternate with a rounded apex and a glabrous common stalk [7]. Traditionally, the bark is known to be abortive while decoction from the roots has a stimulating and tonic effects. The plant is reported to be used as an emetic, an antidote against toxic substances and the leaves are used in wound dressings while a decoction of the leaves, bark, roots and shoots is used in healing and reducing fever [7]. The plant has been reported to have hepatoprotective activity [8] and also analgesic and anti-inflammatory activity [9 -10].

2.0 METHODS

2.1 Collection, identification and preparation of *Entada africana*

Fresh *E. africana* root was collected in Giwa town, Giwa Local Government Area of Kaduna State, in April, 2016. The plant was taxonomically authenticated by Namadi Sunusi with Voucher number 0900376 deposited at the Herbarium section of the Department of Botany, Ahmadu Bello University, Zaria. Nigeria. The roots of the plant collected were washed, sliced into pieces, air dried for two weeks, size reduced into coarse powder using mortar and pestle.

2.2 Extraction

The powdered root was subjected to an extraction procedure using the method described by Kokate [11]. About 1000 g of the powdered root was successfully extracted with 2.5 litres of the solvents (methanol, n-hexane and ethyl acetate) with aid of a Soxhlet apparatus.

2.3 Phytochemical analysis

The phytochemical analysis of *E. africana* root was conducted to identify the presence / absence of phytochemicals, this was performed according to the methods previously described by Sofowora [12] and Evans [13].

2.4 Antibacterial study

The antibacterial activity of the *E. africana* root extracts was determined using the following bacterial wound isolates; *Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pyogenes, Pseudomonas florescence and Escherichia coli*. The bacterial wound isolates were obtained from the Department of Pharmaceutical Microbiology and Pharmaceutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

2.5 Antibiotics sensitivity testing

The antibacterial activity of *E. africana* root extract was evaluated by disc diffusion method on Mueller hinton agar (MHA) plates as described by Kabir and co-workers [14]. The Mueller Hinton agar media was prepared according to manufacturer's instruction and sterilized at 121°C for 15 min. It was subsequently transferred into sterile petri-dish, allowed to cool and solidify. 1 g of each of the three extracts of *E. africana* were taken and dissolved in 10 ml of distilled water to get a concentration of 100 mg/ml. These formed the initial concentration used to test the antibacterial activities of the extracts.

A 0.1 ml of the standard inoculums of the test microbes was taken and transferred onto the sterilized medium and evenly spread over the

surface of the medium with the aid of a sterile swab. At the center of each inoculated medium, a well was cut with the aid of a standard cork borer (10mm in diameter). After labelling the wells on the inoculated medium, 0.1 ml of each of the extracts in concentrations of 100, 50, 25, and 12.5 mg/ml was then transferred into the labelled wells. The inoculated plates were incubated at 37°C for 24 hours, after which each plate was observed for zone of inhibition of growth. The diameter of the zones was measured using a transparent ruler calibrated in millimeters and the results documented [15].

2.6 Determination of minimum inhibitory concentration (MIC)

The MIC of the three extracts were determined by two-fold serial dilution method using Mueller Hinton agar. The MIC of the extracts were determined at varying concentrations of 100, 50, 25, 12.5 and 6.25 mg/ml each. The initial concentration was obtained by dissolving 1 g of the methanol, n- hexane and ethyl acetate extracts each in 10 ml of the sterile broth. The initial concentrations were subsequently used to obtain the different concentrations of the three extracts in the broth. Thereafter, 0.1 ml sample was taken from a sterile test tube containing test microbe in normal saline and inoculated into different concentrations of each of the extracts. This was followed by incubation at 37 ^{0}C for 24 hours after which the test tube was observed for turbidity (growth). The lowest concentration of the three extracts in the broth which showed no turbidity or growth was noted and designated as the MIC [16].

2.7 Determination of minimum bactericidal concentration (MBC)

The MBC was determined to find out whether the test microbes were killed or their growth inhibited by the extracts. The Mueller Hinton agar was prepared, sterilized and transferred into sterile petri- dishes. The plates were then allowed to cool and solidify. The content of the MIC in the serial dilutions with no growth was collected and sub-cultured onto a prepared medium and incubated at 37 °C for 24 hours. Thereafter, the plates were observed individually for colony growth. The concentration at which no visible growth was seen was noted as the minimum bactericidal concentration [16].

3.0 RESULTS

Phytochemical screening of *E. africana* root extracts revealed the presence of tannins, flavonoids, saponins, steroids/triterpenes and alkaloids (see Table 1).

The results of antibacterial activity of *E. africana* root extracts against *Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis, Pseudomonas flourescense, Klebsiella pneumonia and Escherichia coli* showed various degrees of activities on the microbes (Table 2). Methanol extract was observed to have the best activity against most of the tested organisms among the three extracts. The minimum inhibitory concentrations of ethyl acetate extract ranged between 3.125 mg/ml for *S. aureus* and 50 mg/ml for *P. fluorescence*, this showed a

better antibacterial activity on the test organisms (gram positive and gram negative bacteria) compared to the n-hexane extract while the methanol extract showed the highest antibacterial activity on the test organisms with MIC values of 1.5 mg/ml for *S. aureus* and 12.5 mg/ml for *E. coli* (Table 5). The Standard (Sulphadizine) had MIC ranging from 50 to 100 µg/ml which is far lower than the results obtained for *Entada africana* root extracts. The zones of inhibition, minimum inhibitory concentrations and minimum bactericidal concentrations produced by n- hexane, ethyl acetate and methanol root extracts of *Entada africana* compared to that of a standard antibacterial agent (sulphadiazine) are presented in the Tables 2 -5.

4.0 DISCUSSION

Preliminary phytochemical screening provides an insight of the type and chemical nature of bioactive constituents present in a given plant. In this study, phytochemical screening of root extracts of *E. africana* revealed the presence of carbohydrate, tannins, saponins, flavonoids, cardiac glycosides, steroids/ triterpenes, anthraquinone and alkaloids. This result is in agreement with the findings of [17] who also reported the presence of flavonoids, tannins, saponins, anthraquinone, cardiac glycosides, triterpenoids, alkaloids and reducing sugars in the plant. The information provided by phytochemical screening of plant materials on the nature and type of constituent present is very important in identification and differentiation of plant species thus, important in delimitation of taxa [18].

The results of the antibacterial study showed that the methanol extract of *E. africana* had the best antibacterial effect on the organisms tested and also had the lowest MIC values. *S. aureus* was seen to be the most sensitive with MIC value of 1.5 mg/ml, other organisms tested were less sensitive; *K. pneumonia* had 6.25 mg/ml, *E. coli* had 12.5 mg/ml while *P. mirabilis. P. flourescense* were not inhibited by the extract. However, the organisms not inhibited by the methanol extract may produce an inhibition if tested against a higher concentration of the extract. The MIC values are used as a tool to determine effectiveness while MBC values predict the possible mode of action of antimicrobials [14] thus, from the low MIC results obtained, the extract of *E. africana* has a strong antibacterial effect on the organisms tested and can be said to be bactericidal in mode of action. The presence of phytochemicals such as flavonoids, terpenoids, tannins, saponins and alkaloids in the root extracts of *E. africana* may account for the observed antibacterial activity in this study; these phytochemicals have been reported to possess antimicrobial activity [19-20].

5.0 CONCLUSION

From the results obtained in this study, it can be concluded that the root extracts of *E. africana* possess antibacterial activity and the methanol extract had the best activity compared to hexane and ethyl acetate extracts. This show that the roots of *E. africana* contain bioactive compounds that may be beneficial in skin infections thus justifies its use in traditional medicine for the management of burns, wounds and skin infections.

Table 1: Phytochemical Constituents of *E. africana* root extract

Tests	Hexane Extract	Ethyl acetate Extract	Methanol Extract
Carbohydrates			
Molich	+	+	+
Fehling	+	+	+
Flavonoids			
Shinoda	-	+	+
Sodium Hydroxide	-	+	+
Anthraquinones			
Borntrager	-	-	+
Modified Borntrager	-	-	-
Cardiac glycosides			
Keller- kiliani	-	+	+
Kedde	-	+	+
Saponins			
Frothing	-	+	+
Haemolysis	-	-	+
Steroids/Triterpenes			
Salkowski	+	+	+
Lieberman-Burchard	+	+	+
Alkaloids	-	+	+
Dragendorff	-	+	+
Mayer	-	+	+
Wagner's			

Key: (-) Absent; (+) Positive

Table 2: Zone of inhibition produced by n-hexane extract of *E. africana* and Standard Antibacterial agent in MHA (mm)

Organism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Sulphadizine 100 µg/ml
S aureus	15	13	00	00	20
S pyogenes	16	14	12	00	24
P mirabilis	13	11	00	00	24
P fluorescence	00	00	00	00	22
K pneumonia	00	00	00	00	22
E coli	00	00	00	00	20

Table 3: Zone of inhibition produced by Ethyl acetate extract of *E. africana* and Standard Antibacterial agent in MHA (mm)

Organisms	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Sulphadizine 100 µg/ml
S aureus	21	17	15	14	20
S pyogenes	20	18	16	13	22
P mirabilis	19	16	14	00	24
P fluorescence	12	00	00	00	20
K pneumonia	00	00	00	00	22
E coli	00	00	00	00	22

Table 4: Zone of inhibition produced by Methanol extract of *E. africana* and Standard Antibacterial agent in MHA (mm)

Organisms	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Sulphadizine 100 µg/ml
S aureus	26	25	22	19	22
S pyogenes	22	16	14	00	22
P mirabilis	00	00	00	00	22
P fluorescence	00	00	00	00	20
K pneumonia	22	20	16	14	20
E coli	18	17	14	00	20

Table 5: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of extracts of *E. africana*

Organisms	hexane	Ethyl acetate (MIC mg/ml)	Methanol	Hexane	Ethyl acetate (MBC mg/ml)	Methanol
S aureus	6.25	3.12	1.56	25	12.5	6.25
S pyogenes	12.5	6.25	6.25	50	25	25
P mirabilis	25	12.5	0	100	50	0
P fluorescence	0	50	0	0	0	0
K pneumonia	0	0	6.25	0	0	25
E coli	0	0	12.5	0	0	50

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