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Department of Chemistry, Federal University of Technology, Minna, Nigeria

Antimicrobial Efficacy of *Entada abyssinica* Rootbark Extracts

A. L. FADIPE*[1], F. EHINMIDU[2], A. K. HARUNA[1] AND M. ILYAS[3]

[1]Department of Chemistry, School of Science and Science Education, Federal University of Technology, Minna, Nigeria; [2]Department of Pharmaceutics and Pharmaceutical Microbiology; and [3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria

With 3 tables and 13 references

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ABSTRACT

Phytochemical screening of the rootbark extracts of *Entada abyssinica* using standard methods revealed the presence of alkaloids, saponins, flavoniods, steroidal nucleus, tannins and digitalis glycosides. The *in vitro* antibacterial and antifungal efficacy of these extracts in comparison with commercial antimicrobial agents were studied using the agar-well diffusion method against some Gram-positive and Gram-negative bacteria and some fungal strains. The results showed that the methanolic extract of the rootbark was quite active against all tested bacterial and fungal strains. The extract significantly inhibited the growth of Gram-negative *Salmonella typhi* and *Escherichia coli* where chloramphenicol, ciprofloxacin and ampicillin failed. The methanolic extract and the n-butanol soluble portion of the methanolic extract also significantly inhibited the growth of *Candida albicans*, an activity similar to that of terbinafine. In conclusion, the plant extract could be explored for possible antimicrobial agents after further work has been done to isolate and characterize the active constituents responsible for the observed effects and their minimum inhibitory concentrations determined..

Key words: *Entada abyssinica*, rootbark, methanol extract, antibacterial, antifungal, antimicrobial
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Introduction

Entada abyssinica Steud. ex. A. Rich. (Fabaceae) is a small low branching tree widely distributed in tropical Africa [Keay, 1989]. Locally, the plant is called`Tawatsa` in Hausa, `Angaramiri` in Igbo, and `Gbengbe` in Yoruba. A decoction of the rootbark of the plant is used by the Tangayikans as a remedy for rheumatism [Watt and Breyer-Brandwijk, 1962; Freiburghaus *et al.*, 1996]. Another report shows that a decoction of the root is used against malaria, cough and catarrh [Hardi, 1964]. In Tanzania, an infusion of the dried root is taken for epilepsy [Mathias, 1982], while in Uganda, the boiled root extract of the plant is used for the treatment of sleeping sickness [Freiburghaus *et al.*, 1996]. In continuation of the search in our laboratory to evaluate the antimicrobial potentials of *E. abyssinica*, the present study was undertaken to assess *in vitro* antibacterial and antifungal activities of various extracts of the rootbark of the plant against a broad spectrum of Gram-positive and Gram-negative bacteria and some fungal organisms using commercial reference antibiotics for comparative studies. A review of the literature

*Author for correspondence

reveals no report on the antimicrobial activity of extracts of the rootbark of *E. abyssinica*.

Materials and Methods

Plant materia and extraction

The rootbark of *Entada abyssinica* were collected from a farm along the Zaria - Jos road, Nigeria, in the month of November, 2004, and authenticated by a botanist at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A herbarium sample was made and a voucher (No. 900379) deposited in the same department. Two hundred gram of the air-dried and powdered rookbark was Soxhlet-extracted with petroleum ether at 60 - 80°C for 24 h. The resulting solution was concentrated *in vacuo* to yield an oily brown mass of 1.7 g, coded P. The defatted marc was air-dried and then Soxhlet-extracted with methanol for 48 h. The resulting solution was again concentrated *in vacuo* to yield a dark-brown mass of 9.2 g, coded M. The M dark-brown mass was suspended in water and filtered. The aqueous portion was successively partitioned with ethyl acetate and n-butanol to yield an 0.8 g golden brown ethyl acetate-soluble portion coded EM, and a 1.9 g dark-brown n-butanol-soluble portion coded BM. The residual aqueous dark-brown portion mass of 3.3 g was dried and coded AM.

Phytochemical screening

The P, M, EM, BM and AM portions were screened for the presence of alkaloids, flavonoids, saponins, steroidal nucleus, digitalis glycosides, phlobatannins, antraquinones and tannins using standard procedures [Trease and Evans, 1989; Harbone, 1991].

Antibacterial screening

Test bacterial strains

The bacterial strains used in this study were Gram-positive *Staphylococcus aureus* (ATCC 13709) and *Bacillus subtilis* (ATCC 607), Gram-negative *Pseudomonas aeruginosa* (ATCC 7853), *Proteus vulgaris, Salmonella typhi* and *Escherichia coli* (ATCC 9637) in nutrient broth at 37°C. These overnight cultures were standardized to 10^6 cfu/ml and used for the antibacterial susceptibility screening.

Antifungal screening

Test fungal strains

Sporulated cultures of *Aspergillus niger, Trichophyton rubrum, Trichophyton tonsurans* in Sabouraud dextrose agar slants, and an overnight culture of *Candida albicans* (ATCC 1023) in Sabouraud dextrose medium at 30°C were standardized to 10^6 cfu/ml and used for the antifungal susceptibility screening.

Commercial antimicrobial agents

Ciprofloxacin (Ranbaxy Lab., New-Delhi, India), chloramphenicol (Clarion Medical, Ltd., London, England), ampicillin (Laborate Pharm., New-Delhi, India), gentamicin (Jinlin Pharm. Group Corp., Shanghai, China), terbinafine (Sandox Pharm., Surrey, England), tioconazole (Neimeth Int. Pharm., Lagos, Nigeria), and ketoconazole (Hovid Pharm., BDH, Kuala Lumpur, Malaysia) were used as reference drugs for comparative purposes with the extracts.

Experimental protocol for antibacterial agents

Screening for antibacterial activity was by the agar-well diffusion method [Perez *et al.*, 1990; Dall' Agnol *et al.*, 2003]. Briefly, 20 ml of the molten and cooled nutrient agar (45°C) was poured into sterile Petri-dishes aseptically. On solidification, plates were separately inoculated with the standardized overnight nutrient broth cultures of the test organisms. Holes, 9 mm in diameter, were aseptically bored into the solid nutrient agar using sterile cork borer at equidistance. One millilitre of the test solutions (200 μg/hole) was introduced into the holes with the aid of a Pasteur pipette ensuring that no spillage occurred. The plates were then left at room temperature for 1 hour to allow for diffusion of extracts into the media and thereafter, the plates were incubated overnight at 37°C. At the end of the incubation period, the diameters of zones of inhibition of growth around the holes were measured to the nearest millimeter. Diameters of inhibition, expressed as the mean $\geq$ 10 mm, were considered active [Onocha *et al.*, 2003].

Solvents used for dissolution of extracts and solutions of reference antimicrobial agents (100 μg/hole) were set up as controls. Each test was carried out in triplicate and the results analyzed for statistical significance.

Experimental protocol for antifungal agents

The method employed was similar to that of antibacterial screening except that Sabouraud dextrose agar was used as the medium. Incubation was for 24 h at 30°C.

Results and Discussion

Table 1 summarizes the phytochemicals in the P, M, EM, BM and AM portions of the rootbark of *Entada abyssinica*. The M and BM extracts contained very high concentrations of alkaloids, flavonoids, saponins and digitalis glycosides, while tannins and steroidal nucleus were found in the M and P extracts in similar very high concentrations, respectively. Most of the phytochemicals were absent in the P and AM extracts.

Table 1. Phytochemicals present in the extracts of the rootbark of *Entada abyssinica*

Phytochemical	Observations				
	P	M	EM	BM	AM
Alkaloid	–	+++	++	+++	+
Flavonoid	–	+++	+++	+++	–
Saponins	–	+++	+	+++	+
steroidal nucleus	+++	++	++	–	–
Tannin	–	+++	+	++	–
Digitalis glycoside	–	+++	+	+++	++
Phlobatannin	–	–	–	–	–
Anthraquinone	–	–	–	–	–

P = petroleum ether extract; M = methanol extract; EM = ethylacetate-soluble portion of partitioned methanol extract; BM = n-butanol soluble portion of partitioned methanol extract; AM = residual aqueous portion of partitioned methanol extract; – = absent; + = low concentration; ++ = high concentration; +++ = very high concentration

The *in vitro* antibacterial screening of the various extracts of the rootbark of *E. abyssinica* (Table 2), revealed that the methanolic extract and its n-butanol soluble portion showed appreciable activities against both Gram-positive and Gram-negative bacteria with the methanolic extract giving significant inhibitions against Gram-positive *B. subtilis*, Gram-negative *S. typhi* and *E. coli* better than chloramphenicol, ciprofloxacin and ampicillin, which are commercial antibiotics. The petroleum ether, remaining water extracts and controls expressed no activity against all the tested bacterial strains.

Table 2. *In vitro* antibacterial activities of *Entada abyssinica* rootbark extracts and some commercial antibiotics

Test compound	Zone of inhibition of test organisms (mm)					
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudonomas aruginosa*	*Proteus vulgaris*	*Salmonella typhi*	*Escherichia coli*
P	–	–	–	–	–	–
M	14.0 ± 1.41	18.5 ± 0.71	13.5 ± 0.71	14.5 ± 0.71	19.0 ± 1.41	20.0 ± 0.71
EM	–	–	–	–	–	–
BM	12.5 ± 0.71	15.5 ± 0.71	15.5 ± 0.71	–	11.5 ± 0.71	14.5 ± 0.71
AM	–	–	11.5 ± 0.71	0.95 ± 0.71	–	10.5 ± 0.71
Chloroform	–	–	–	–	–	–
Methanol	–	–	–	–	–	–
Chloramphenicol	–	–	–	–	–	–
Ciprofloxacin	34.5 ± 0.71	24.0 ± 1.41	35.5 ± 0.71	31.0 ± 0.00	–	–
Gentamicin	36.5 ± 0.71	22.5 ± 0.71	30.5 ± 0.71	20.0 ± 0.00	22.5 ± 0.71	36.5 ± 1.73
Ampicillin	–	–	23.5 ± 0.71	–	–	–

P, was dissolved in $CHCl_3$ (2000 μg/ml); M, EM and BM were dissolved in MeOH (2000 μg/ml); standards were dissolved in distilled water (1000 μg/ml); $CHCl_3$ and MeOH served as negative controls; – = no inhibition; values are mean of triplicate readings; P = petroleum ether extract; M = methanol extract; EM = ethylacetate-soluble portion of partitioned methanol extract; BM = n-butanol soluble portion of partitioned methanol extract; AM = residual aqueous portion of partitioned methanol extract;

The *in vitro* antifungal activities of the extracts (Table 3), revealed that all the fungal strains were weakly susceptible to the methanol extract of the rootbark of *E. abyssinica*. The methanolic extract and the n-butanol soluble portion of the methanolic extract significantly reduced the growth of *C. albicans* with inhibitory activities similar to that of terbinafine and better than that of tioconazole and ketoconazole. Earlier, Jones [1984] has listed

Candida sp. as a resistant strain to ketoconazole *in vitro*, while Smit *et al.* [1986] suggested the emergence of resistant strains of *Candida* spp. during treatment with ketoconazole. All other extracts displayed weak antifungal activities against the fungal strains in comparison with the reference antifungal agents.

Table 3. *In vitro* antifungal activities of *Entada abyssinica* rootbark extracts and some commercial antifungal agents

Test compound	Zone of inhibition of test organisms (mm)			
	Candida albicans	*Aspergillus niger*	*Trichophyton rubrum*	*Trichophyton tonsurans*
P	–	20.5 ± 071	22.0 ± 1.41	–
M	20.0 ± 0.00	16.8 ± 2.86	16.8 ± 1.49	12.8 ± 0.95
EM	–	–	20.5 ± 0.71	20.0 ± 0.00
BM	20.0 ± 0.00	–	–	13.5 ± 0.71
AM	–	–	–	–
Chloroform	–	–	–	–
Methanol	–	–	61.3 ± 6.85	65.0 ± 4.24
Terbinafine	19.9 ± 1.26	62.0 ± 5.40	42.0 ± 7.35	42.3 ± 4.35
Tioconazole	14.3 ± 1.50	31.0 ± 4.56	17.3 ± 4.42	24.0 ± 1.41
Ketoconazole	–	13.0 ± 5.45		

P. was dissolved in $CHCl_3$ (2000 μg/ml); M, EM and BM were dissolved in MeOH (2000 μg/ml); standards were dissolved in distilled water (1000 μg/ml); $CHCl_3$ and MeOH served as negative controls; - = no inhibition; values are mean of triplicate readings; P = petroleum ether extract; M = methanol extract; EM = ethylacetate-soluble portion of partitioned methanol extract; BM = n-butanol soluble portion of partitioned methanol extract; AM = residual aqueous portion of partitioned methanol extract

The methanolic and methanolic-based extracts of the rootbark of *E. abyssinica* displayed moderate activities against the tested bacterial and fungal strains in this study. This could be due to the presence of the high concentrations of one or more of the active phytochemicals of medium or high polarity such as steroids, alkaloids, flavonoids, saponins and tannins in the plant as shown in Table 1. These metabolites even in relatively low concentrations, could be responsible for the antimicrobial activities detected [Dall' Agnol *et al.*, 2003]. However, minor components, as well as possible synergic effect(s) between these constituents could also contribute to the observed antimicrobial effects [Paulo *et al.*, 1997].

The present investigation, thus justifies the traditional uses of the rootbark of the plant in the treatment of infections. It also suggests that the plant could be explored for possible antimicrobial agents. However, further work is required to isolate and characterize the active constituents responsible for the observed effects and to determine their minimum inhibitory concentrations (MIC).

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