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Preliminary Phytochemical, Antibacterial and Antifungal Properties of *Alafia barteri* Stem Grown in Nigeria

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Research Article

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ABSTRACT

The preliminary phytochemical studies of *Alafia barteri* stem extracts revealed the presence of reducing sugar, steroids, glycosides, flavonoids and anthraquinones. Hexane, ethylacetate and methanol successive extracts of *A. barteri* stem showed inhibition on the six test bacteria. *Escherichia coli* and *Pseudomonas aeruginosa* were sensitive to methanol extract at concentrations ranging from 25 to 200mg/ml using agar disk diffusion procedure, while hexane and ethylacetate extracts of the plant inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa* at concentrations between 50 and 200mg/ml. Hexane and ethylacetate extracts showed lower inhibition on *Staphyloccocus aureus and Bacillus subtilis* (gram positive), and *Klebsiellae pneumonae* (gram negative). Meanwhile, methanol extract exhibited antibacterial properties on *Staphyloccocus aureus* at concentrations between 50 and 200mg/ml, and *Bacillus subtilis, Klebsiellae pneumonae* and *Salmonellae typhii* at concentrations between 100 and 200mg/ml. The three extracts exhibited higher antifungal properties on *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum* and *Epidermophyton floccosum* with activity comparable to that of the reference drug tioconazole trosyd.

Keywords: Alafia barteri, bioactivity, phytochemical screening, agar diffusion method, sensitivity;

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1. INTRODUCTION

Herbal remedies have played important roles since early times in the treatment of all kinds of diseases in Africa and other continents, owing to the challenges confronting the appropriate delivery of official health care to millions of people in urban, remote and rural communities. Herbal remedies constitute a strong component of traditional, complementary and alternative medicine. In realization of the inherent value of herbal remedies to primary health care and the fact that over three quarters of the world's population rely on plants for medicinal care, the world health organization (WHO) has called for the identification, sensible exploitation, scientific development and appropriate utilization of herbal medicines which provide safe, cheaper and effective remedies in medicare (Wambebe, 1998). Hence, pharmacological evaluations are critical in drug development. The results obtained from this study revealed the stem extracts of *Alafia barteri* as potential and reliable herbal remedy for the treatment of fungal infections.

Alafia barteri (Apocynaceae) is a high-climbing, scandent shrub with small, pure white or pink flowers (Irvine, 1961). It is used in ethnomedicine for the treatment of sickle cell anaemia, rheumatism, eye infections, febrifuges, as chewsticks and toothache. The twining stem of *A. barteri* is used for the treatment of fever, inflammation and as binding materials for roots (Leeuwenberg 1997; Irvine, 1961; Burkill, 1985; Daziel, 1937; Nadkarni, 1976).

Antifungal properties of ethanol and water extracts of *A. barteri* leaves were reported (Adekunle and Okoli, 2002). The phytochemistry of the plant has not been established.

In continuation of our studies on biological activities of medicinal plants grown in Nigeria, we report the preliminary phytochemistry, antibacterial and antifungal properties of the hexane, ethylacetate and methanol extracts of *Alafia barteri* stem.

2. EXPERIMENTAL DETAILS

2.1 COLLECTION AND AUTHENTICATION OF THE PLANT MATERIAL

The whole plant material of *Alafia barteri* was collected from Ibadan area of Oyo State, Nigeria, in the month of November 2009. Botanical identification and authentication was done by Mr. A.W. Ekundayo of the Forestry Research Institute of Nigeria (FRIN), Ibadan where a voucher specimen was deposited with the herbarium file number FHI108800.

2.1.1 Preparation of plant extracts

Fresh whole plant of *Alafia barteri* was air-dried, weighed and separated into leaves and stem (leaves 574g and stem 1000g). The dried stem was successively extracted in hexane, ethylacetate and methanol for 10 days respectively using cold extraction method. The resultant hexane (5g), ethylacetate (7g) and methanol (7.5g) extracts were obtained by evaporation and stored in the refrigerator for further use.

2.2 PHYTOCHEMICAL STUDIES

The Preliminary phytochemical screening of the hexane, ethylacetate and methanol extracts of *Alafia barteri* stem was done using standard procedures (Trease and Evans, 1983; Trease and Evans, 1989; Harborne, 1991; Harborne, 1998; Edeoga et al; 2005).

2.3 ANTIMICROBIAL ASSAY

2.3.1 Microorganisms

Cultures of six human pathogenic bacteria. Four gram negative bacteria, *Salmonella typhii* (UCH 4801)*, Escherichia coli* (UCH 00260)*, Pseudomonas aeruginosa* (UCH 1102) and *Klebsiellae pneumonae* (UCH 2894), and two gram positive bacteria, *Bacillus subtilis* (UCH 74230) and *Staphylococcus aureus* (UCH 2473) (gram positive) were used for the antibacterial assay. For the Antifungal assay, six fungi were also utilized. These were; *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum.* The organisms were clinical strains from the Department of Medical Microbiology, University College Hospital, Ibadan and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria.

2.3.2 Media

Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. Hexane, ethylacetate and methanol were also used in solubilizing the extracts and as negative controls in the assays.

2.3.3. Antimicrobial agents

Gentamycin (10µg/ml) and Tioconazole 0.7mg/ml were included as standard reference drugs in the study.

2.4 ANTIMICROBIAL ACTIVITY DETERMINATION

2.4.1 Agar diffusion-pour plate method (bacteria)

An overnight culture of each organism was prepared appropriately from its stock and inoculated each into the sterile nutrient broth of 5ml, each incubated for 18-24hrs at 37^{o}C. From overnight culture, 0.1ml of each organism was taken and put into the 9.9mls of sterile distilled water to get (1:100) 10^{-2} of the dilution of the organism.

From the diluted organism (10^{-2}), 0.2ml was taken into the prepared sterile nutrient agar cooled to about 40-45^{o}C, then poured into sterile Petri dishes and allowed to solidify for about 45-60mins. Using a sterile cork-borer of 8mm diameter, the wells were made according to the number of the test tubes for the experiment. For this work 8 wells were made. The graded concentrations of the extracts were put into the wells accordingly including the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 18-24hrs at 37^{o}C.

2.4.2. Agar diffusion-surface plate method (fungi)

A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in duplicates and solidified properly. 0.2ml of the (1:100) 10^{-2} of the organism was spread on the surface of the agar using a sterile Petridish cover to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8mm diameter. The graded concentrations of the extracts were put into the wells accordingly including the controls. All the plates were left on the bench for 2hrs to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25^{o}C for 72hrs (Kavanagh, 1972; Zwadyk, 1972; Van den Berge and Vlietinck, 1991).

3. RESULTS AND DISCUSSION

The result of the phytochemical screening of the hexane, ethylacetate and methanol extracts of *A. barteri* stem was presented in Table 1.

Table 1: Phytochemical constituents of the hexane, ethylacetate and methanol extracts of *Alafia barteri* stem

Secondary metabolites	Extracts (whole plant)		
	Hexane	Ethylacetate	Methanol
Alkaloids	-	-	-
Saponins	-	-	++
Tannins	-	-	-
Reducing sugars	++	++	++
Steroids	-	++	++
Glycosides	++	++	++
Flavonoids	++	++	++
Anthraquinones	++	++	++

Key: - : Absent; ++: Present

Preliminary phytochemical studies of the three extracts indicated the presence of reducing sugar, steroids, glycosides, flavonoids and anthraquinones except in hexane extract which showed the absence of steroids. There was also presence of saponins in ethylacetate and methanol extracts of *A. barteri* stem, but was found absent in the hexane extract of the plant. Meanwhile, both tannins and alkaloids were not found in all the extracts of *A. barteri* stem in our study.

The antibacterial activities of the hexane, ethylacetate and methanol extracts at concentrations ranging from 6.25 to 200mg/ml were presented in Table 2. The bacteria used were clinical strains of *Staphyloccocus aureus and Bacillus subtilis* (gram positive), *Escherichia coli, Pseudomonas aeruginosa, Klebsiellae pneumonae and salmonellae typhii* (gram negative). Methanol extract inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa* at concentrations ranging from 25 to 200mg/ml while hexane and ethylacetate extracts of *A. barteri* stem showed inhibition on *Escherichia coli* and *Pseudomonas aeruginosa* at concentrations ranging from 50 to 200mg/ml.

Further, hexane and ethylacetate extracts showed lower inhibition on *Staphyloccocus aureus and Bacillus subtilis* (gram positive) and *Klebsiellae pneumonae* (gram negative), while methanol extract of the plant exhibited antibacterial properties on *Staphyloccocus aureus* at concentrations between 50 and 200mg/ml and *Bacillus subitlis, Klebsiellae pneumonae* and *salmonellae typhii* at concentractions between 100 and 200mg/ml. Meanwhile, the ethylacetate extract of *A. barteri* stem also inhibited the growth of *Salmonellae typhii* at concentrations ranging from 50 to 200mg/ml.

The result of the antifungal activities of the hexane, ethylacetate and methanol extracts of *A. barteri* stem at concentrations between 6.25 and 200mg/ml was presented in Table 3.

Table 2: Antibacterial activities of the hexane, ethylacetate and methanol extracts of *Alafia barteri* stem

Extracts	Extract conc./Ref./ Control (mg/ml)	Diameter of well = 8 mm Diameter of zone of inhibition of bacteria(mm)					
		S. a	***E. c.***	***B. s.***	***P. a***	***K. p.***	***S. t.***
Hexane	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	-	-	-	-	-	-
	50	-	10	-	10	-	-
	100	-	12	-	12	-	-
	200	12	14	12	14	12	10
	Hexane	-	-	-	-	-	-
	Gentamycin	36	34	34	34	36	32
Ethylacetate	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	-	-	-	-	-	-
	50	-	12	-	10	-	10
	100	-	14	-	12	-	12
	200	12	16	10	14	12	14
	Ethylacetate	-	-	-	-	-	-
	Gentamycin	36	34	36	32	34	34
Methanol	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	-	10	-	10	-	-
	50	10	12	-	11	-	-
	100	11	13	10	13	10	10
	200	14	15	13	15	12	12
	Methanol	-	-	-	-	-	-
	Gentamycin	36	34	36	34	32	34

Key: S. a: Staphylococcus aureus; E. c.: Escherichia coli; B. s.: Bacillus subtilis; P. a: Pseudomonas aeruginosa; K. p.: Klebsiellae pneumonae; S. t.: Salmonellae typhi;

Six clinical strains of fungi were used; *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum.* All the extracts effectively exhibited intrinsic antifungal properties on the six test fungi at concentrations between 25 and 200mg/ml except hexane extract which showed antifungal activities on *Penicillum notatum* and *Tricophyton rubrum* only at higher concentrations between 100 and 200mg/ml. The activities of the three extracts were comparable to that of the reference drug tioconazole troysd against *Candida albicans and Tricophyton rubrum* for methanol extract and *Candida albicans* for hexane and ethylacetate extracts of *A. barteri* stem. However, the sensitivities of the test bacteria and fungi on the three extracts were concentration dependent, activity being higher at higher concentrations of the extracts.

Table 3: Anti-fungal activities of the hexane, ethylacetate and methanol extracts of *Alafia barteri* stem

Extracts	Extract conc./Ref. /Control (mg/ml)	Diameter of well = 8 mm Diameter of zone of inhibition of bacteria(mm)					
		C. a.	***A. n.***	***R. s.***	***P. n.***	***T. r.***	***E. f.***
Hexane	6.25	-	-	-	-	-	-
	12.5	12	-	-	-	-	-
	25	14	-	-	-	-	10
	50	16	10	10	-	-	12
	100	18	12	12	12	12	14
	200	22	16	16	14	16	18
	Ethylacetate	-	-	-	-	-	-
	Tioconazole	22	24	24	22	20	24
Ethylacetate	6.25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	25	12	-	-	-	-	10
	50	16	10	12	10	10	14
	100	20	12	14	14	12	16
	200	24	16	16	16	16	18
	Hexane	-	-	-	-	-	-
	Tioconazole	20	22	24	22	20	24
Methanol	6.25	-	-	-	-	-	-
	12.5	10	-	-	-	10	-
	25	13	-	-	-	12	10
	50	17	11	11	10	13	12
	100	20	14	13	12	15	15
	200	25	17	18	14	18	20
	Methanol	-	-	-	-	-	-
	Tioconazole	24	24	25	22	22	24

Key: C. a: Candidas albicans; A. n: Aspergillus niger; R. s.: Rhizopus stolon; P. n.: Penicillum notatum; T. r: Tricophyton rubrum; E. f.: Epidermophyton floccosum

4. CONCLUSION

The stem extracts of *A. barteri* possess higher antifungal activities than antibacterial activities. The higher sensitivity of fungi on hexane, ethylacetate and methanol extracts of *A. barteri* stem suggests the application of the plant in alternative traditional medicine for the treatment of fungi infections in addition to its uses previously reported. The need for development of new antifungal drugs and more importantly from natural sources cannot be overemphasized. *Alafia barteri* provides a good opportunity for drug development in this area. The continuation of study on the plant is essential to isolate, identify, characterize and elucidate the structure of bioactive compounds responsible for the observed pharmacological activities.

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