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Phytochemical Screening and Antimicrobial Studies of the Aerial Part of *Aeschynomene uniflora* Mey.

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Abstract

The aim of the study was to investigate the phytochemical composition and antimicrobial activities of extracts of aerial part of *Aeschynomene uniflora* Mey used in folklore medicine in order to scientifically validate some of its ethnomedicinal claims. The dried and pulverized aerial part of *Aeschynomene uniflora* was extracted using petroleum ether, chloroform, ethyl acetate and methanol by Soxhlet extraction and each concentrated *in vacuo* to yield four extracts. The extracts were then subject to preliminary phytochemical screening and antimicrobial studies using standard method. Carbohydrates, cardiac glycoside, flavonoids, saponins, steroids, triterpenes and tannins were present in the crude extracts. The highest zone of inhibition of 25 mm was exhibited by the ethyl acetated extract against *Staphylococcus aureus*, while the petroleum ether extract show the lowest zone of inhibition of 16 mm against *Streptococcus pyogenes*. The chloroform and ethyl acetate extracts exhibited a minimum inhibitory concentration (MIC) of 7 mg/mL against, *Bacillus subtilis and Candida stellatoidea* respectively. Both the methanol and petroleum ether extracts had a MIC of 30 mg/mL against *Candida stellatoidea*. The minimum bactericidal/fungicidal concentration (MBC/MFC) of the extracts ranged between 15 mg/mL to 60 mg/mL. In conclusion, the aerial parts of *Aeschynomene uniflora* showed significant antimicrobial properties. This justifies the use of the in the treatment of human and animal infectious disease.

Keywords: *Aeschynomene uniflora*; Aerial part; Antimicrobial activity; Phytochemical screening; Fabaceae

Introduction

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from plants are easily available, less expensive, safe, efficient and rarely have side effects [1]. There has been an increasing interest in the study of medicinal plants as natural products in different parts of the world [2]. Medicinal plants represent rich sources of antimicrobial agents used medicinally in different countries and are a source of many potent drugs used for traditional medicine. They contain a wide range of substances that can be used to treat chronic as well as infectious diseases [3]. Medicinal plants play important role in meeting the basic health needs in many developing countries and the industrialized countries. The use of medicinal plants has been based on the extraction which has led to the development of several drugs traditionally used in folk medicine [4]. Various drugs with medicinal properties have been attributed to natural herbs and medicinal plants which constitute the main source of new pharmaceuticals and health-care products [5]. Plants are used in the traditional medicine for treatment of various types of ailments, including microbial infections [6,7]. Many plant species are reputed to possess antimicrobial activities. *Aeschynomene uniflora* is an erect shrub, it is rarely almost prostrate and a short-lived (2-4 years) perennial shrub. It is 0.5-2 m tall [8,9]. The plant is used in the treatment of psychotic disorders, tuberculosis, skin infections, menstrual disorders, small pox and antidote to snake venom. The aqueous extract of the whole plant is administered topically over the whole body to cure small pox in northern Nigeria. The plant is eaten as vegetable to cure fever symptoms and cough in Benue State Nigeria. The main aim of this study was to carry out the phytochemical and antimicrobial screening of *Aeschynomene uniflora* in order to scientifically justify the ethnomedicinal usage of the plant in folklore medicine.

The *Aeschynomene* genus belongs to the family Papilionaceae which are marshy erect herbs. In traditional medicinal system, this plant is used to treat body pain and swellings [10,11]. It is also used to treat mumps [12]. The *Aeschynomene* genus is comprised of about 60 species which are widely distributed throughout tropical and subtropical regions. Most species are annual herbs or shrubs, but a few are small trees [13]. *A. aspera* aerial part juice is administered to cure cold, cough, and fever. The Dried young shoot powder with half tea spoon powdered candy is given to increase the consistency of semen; local herbalists used it for urinary troubles [14]. *A. aspera* is also recognized as leafy vegetable [15]. *Aeschynomene indica* is a swampy medicinal plant used to treat kidney stones and urinary disorders by local herbalists [16]. The leaves of *A. granilflora* are used as tonic, diuretic, laxative, antipyretic, chewed to disinfect mouth and throat. The flower in headache, dimness of vision catarrh, headache, cooling and improving appetite, bitter, astringent, acrid, antipyretic [17]. It is used as a bitter tonic, anthelmintic, astringent febrifuge, and for curing diarrhea, small pox [18]. The fruits are bitter and acrid, used as a laxative and to cure fever, pain, bronchitis, anemia, tumors, colic, jaundice, poisoning. Root used in rheumatism, expectorant, painful swelling, catarrh [19]. The hepatoprotective activity of benzene and alcoholic extracts of root of *Aeschynomene aspera* was investigated in rats for carbon tetrachloride induced hepatotoxicity and reported by Ref. [20].

Saravana *et al.* (2012) [21] reported the preliminary phytochemical screening of methanolic extract of *Aeschynomene gradiflora* to contain alkaloids, flavonoids, tannins, triterpenes, gums and mucilage. Phytochemical investigation of *Aeschynomene fluminensis* leaves and

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branches led to isolation of the flavonoidglycosides kaempferol 3,7-di-*O*-α-L-rhamnopyranoside, kaempferol 7-*O*-α-L-rhamnopyranoside, kaempferol 3-*O*-apiofuranosil- 7-*O*- rhamnopyranoside, quercitin 3-*O*-α-L-rhamnopyranoside, quercitin 3-*O*-arabinofuranoside, 8-β-D-glucopyranosyl 4',5,7 trihydroxyflavanone, the isoflavonoid 4',7-di-hydroxy-isoflavone, the dimer epicatechin-(2β, 4β)+- epicatechin, the polyol 3-*O*-methyl-*chiro*-inositol and two steroids in sitosterol and stigmasterol mixture [22]. In 2011, Chen et *al.* [23] reported novel compound monotetracontane from the dry leaves of *Aeschynomene indica* Linn.

Materials and Methods

Collection of plant materials

The aerial part of *A. uniflora* was collected during the rainy season in September, 2012 from Makurdi, Benue State, Nigeria. The plant was identified in the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria; and a Voucher specimen, number 2408, was deposited in the Herbarium. The plant materials were air-dried, pulverized and stored in air-tight containers until needed for further investigation.

Extraction procedure

The air-dried pulverized plant material (200 g) was placed in a Soxhlet extractor, where it was exhaustively and successively extracted using petroleum ether (60-80°C), chloroform, ethyl acetate and methanol. The crude extracts of the plant were concentrated *in vacuo* at 40°C using a rotary evaporator. The crude extracts were subjected to phytochemical and antimicrobial studies.

Phytochemical screening

Phytochemical screening of the extracts was carried out using the standard procedures [7,24].

The anti-microbial screening

The anti-microbial activity of the petroleum ether, chloroform, ethyl acetate and the methanol extracts was determined using some pathogenic microbes, obtained from the Department of Medical Microbiology Ahmadu Bello University Teaching Hospital, Zaria. Each extracts (0.6 g) was weighed and dissolved in 10 ml dimethyl sulphoxide (DMSO) to obtain a concentration of 60 mg/ml. This initial concentration was used to determine the antimicrobial activity of the extracts. Mueller Hinton agar was the growth medium used for the microbes. The medium was prepared, sterilized at 121°C for 15 minutes and the sterilized medium was poured into sterile Petri dishes. The plates were allowed to cool and solidify. Agar diffusion method was used for screening of the extracts. The sterilized medium was seeded with 0.1 ml of the standard inoculum of the test microorganism; the inoculum was spread evenly over the surface of the medium with a sterile swab. Using a standard cork borer of 6 mm in diameter a well was cut at the center of each inoculated medium. Solution (0.1 ml) of each extracts of concentration of 60 mg/ml was then introduced into each well on the medium. The inoculated medium was then incubated at 37°C for 24 h after which each plate was observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result recorded in millimeters [25].

Minimum inhibition concentration of extracts

The minimum inhibition concentration (MIC) of the extracts was determined using broth dilution method. Mueller Hinton broth was prepared; 10 ml was dispensed into test tubes and was sterilized at 121°C for 15 minutes. The broth was allowed to cool. Mc- Farland turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared and 10 ml was dispensed into sterile test tubes. The test microbes were inoculated incubated at 37°C for 6 hours. Dilution of the test microbes was done in the normal saline until the turbidity matched that of the Mc - Farlands scale by visual comparison. At this point the concentration of the microbes was about 1.5×10^8 cfu/ml. Two-fold serial dilution of the extracts in the sterile broth was made to obtain different concentration (60 mg/ml, 30 mg/ml, 15 mg/ml, 7.5 mg/ml and 3.25 mg/ml). The initial concentration was obtained by dissolving 0.6 g of the extracts in 10 mL of the sterile broth. The broths were incubated at 37°C for 24 hours. The results were recorded after 24 hours. Thereafter 0.1 ml of the test microorganism in the normal saline was inoculated in to the different concentrations, incubations was made at 37°C for 24 h for bacteria and at 30°C for 48 h for fungi, after which each test tube of the broth was observed for turbidity (growth). The MIC was the value recorded from the test tube with the lowest concentration of the extract in the broth which showed no growth [25,26].

Minimum bactericidal concentration/Minimum fungicidal concentration

The minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) is the concentration that determines if the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared and sterilized at 121°C for 15 minutes, poured into Petri dishes and allowed to cool and solidify. The content of the MIC in the serial dilution was then sub-cultured onto the prepared medium and incubation was done at 37°C for 24 h. Thereafter each plate of the medium was observed for colony growth. The value obtained in the plate with the lowest concentration of the extract without colony growth was recorded as the MBC/MFC [7,25,26].

Results

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [27]. The presence of steroids and triterpenes in all the four extracts were observed: cardiac glycosides were present in all the extracts except in petroleum ether extracts. Carbohydrates, cardiac glycoside, tannins, flavonoids, steroids and triterpenes were present in the ethyl acetate, chloroform, and methanol extracts. Saponins were present only in the methanol extract while alkaloids and anthraquinones were absent in all the four extracts (Table 1). The result of the antimicrobial screening is summarized in Tables 2 and 3. From the results of the antimicrobial screening (Table 2), the extracts showed activity against *S. pyogenes, B. subtilis, C. albicans and S aureus.* The petroleum ether extract was effective against *S. pyogenes, B. subtilis, C. stellatoidea, K. pneumoniae, S aureus* and *C. albicans* with a zone of inhibition of 16, 17, 17, 18 and 18 mm respectively. The MIC showed that the petroleum ether extract inhibited the growth of all the pathogenic microorganisms at a concentration of 30 mg/ml. The MBC/MFC was found to be 60 mg/ml for the petroleum ether extract against all the test microorganism (Table 3). The chloroform extract showed activity against *S. pyogenes, C. albicans, C. stallatoidea, S. aureus and B. subtilis,* with zones of inhibition of 22, 22, 23, 24 and 27 mm, respectively. At the MIC of 15 mg/ml the chloroform extract inhibited the growth of *S. aureus, S. pyogenes, K. pneumoniae, C. albicans* and *C. stallatoidea*, while at 7.5 mg/ml the growth of *B. subtilis* was inhibited. The MBC/MFC was found to be 30 mg/ml and at this concentration the extract exhibited activity against all the test microorganisms;

Test	AUPE	AUCH	AUET	AUME
Carbohydrates	+	+	+	+
Cardiac glycosides	-	+	+	+
Tannins	-	-	+	+
Saponins	-	-	-	+
Flavonoids	-	-	+	+
Anthraquinones	-	-	-	-
Steroids	+	+	+	+
Triterpenes	+	+	+	+
Alkaloids	-	-	-	-

Key: +=present; -=absent; AUPE=Petroleum ether extract; AUCH=Chloroform extract; AUET=Ethyl acetate extract; AUME=Methanol extract

Table 1: Phytochemical screening of the extracts of *A. uniflora.*

Test organism	Diameter of zones of inhibition (mm)					
	AUPE	AUCH	AUET	AUME	Ciprofloxacin	Fluconazole
S. aureus	18	24	25	21	35	ND
S. pyogenes	16	22	22	20	30	ND
B. subtilis	17	27	28	22	42	ND
K. pneumonia	18	24	23	20	44	ND
C. albicans	18	22	24	20	ND	40
C. stellatoidea	17	23	26	19	ND	32

Key: AUPE=Petroleum ether extract; AUCH=Chloroform extract; AUET=Ethyl acetate extract; AUME=Methanol extract; ND=Not determine

Table 2: Zone of Inhibition of extracts of *A. uniflora* (mm).

	MIC				MBC/MFC			
Test organism	AUPE	AUCH	AUET	AUME	AUPE	AUCH	AUET	AUME
S. aureus	30	15	15	15	60	30	30	60
S. pyogenes	30	15	15	15	60	60	60	60
B. subtilis	30	7.5	7.5	15	60	30	30	30
K. pneumonia	30	15	15	15	60	30	30	60
C. albicans	30	15	15	15	60	30	30	60
C. stellatoidea	30	15	7.5	30	60	30	30	60

Key: AUPE=Petroleum ether extract; AUCH=Chloroform extract; AUET=Ethyl acetate extract; AUME=Methanol extract

Table 3: Minimum inhibitory concentration and minimum bactericidal/fungicidal concentration of extracts of *A. uniflora* (mg/ml).

except *S. pyogenes* which had 60 mg/ml. The MIC showed that at lower concentration of 7.5 mg/ml the ethyl acetate extract inhibited the growth of *B. subtilis* and *C. stellatoidea,* but the inhibitions of *S. aureus, S. pyogenes,* and *C. albicans* occurred at 15 mg/ml. MBC/MFC (30 mg/ml) of the ethyl acetate extract was active against *S. aureus, B. subtilis, K. pneumniae, C. albicans* and *C. stallatoidea,* and the value for *S. pyogenes* was 60 mg/ml. The methanol extract induced significant zone of inhibition for *C. stallatoidea, C. albicans, S. pyogenes, S. aureus* and *B. subtilis,* with values of 19, 20, 20 and 21 mg/ml, respectively. At MIC of 15 mg/ml for *S. aureus*, *S. pyogenes*, *B. subtilis* and *C. albicans.* At MIC of 30 mg/ml, the growth of *C. stellatoidea* was inhibited, while other microbes were inhibited at a value of 15 mg/ml. At MBC/MFC of 60 mg/ml, the methanol extract exhibited activity against all the microbes with the exception of *B. subtilis* inhibited at 30 mg/ml.

Discussion

The presence of the secondary metabolites in the crude extracts of this plant may be responsible for some of the biological activities observed [28]. Phenolic compounds such as flavonoids and tannins which are presents in this plant are one of the largest and most ubiquitous groups of plant metabolites [29]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [30]. This could explain the vast usage of this plant to manage infectious disease in folklore medicine. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [31,32]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. This therefore implies that this plant could possess antiaging, anticarcinogenic properties [33]. Saponins are known to produce inhibitory effect on inflammation and as such, the presence of saponins in the crude extracts of this plant shows that this plant could be used as an anti-inflammatory agent [34]. The presence of saponins in the crude extracts may be responsible for the significant anti-bacteria activity exhibited, as these bacteria are responsible for inflammations. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [35,36]. The presence of steroids in all the crude extracts account for the significant antibacterial activity observed especially in the ethyl acetate extracts. Steroids have been reported to have antibacterial properties [37], and they are very important compounds especially due to their relationship with compounds such as sex hormones [38]. Glycosides are known to lower the blood pressure according to many reports [39]. The results obtained in this study suggest that, the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit [40].

Of the four extracts tested, the ethyl acetate extract demonstrated the highest antimicrobial activity against all the microorganisms. The result demonstrated that the aerial part of the plant may contain potent active constituent which are beneficial in the treatment and prevention of microbial diseases. Based on the finding of this present study, the application of the decoction of the aerial part of *A. uniflora* in ethnomedicine is justified.

In conclusion, the extracts of *Aeschynomene uniflora* demonstrated significant antibiotic potential may be used for the development of novel antimicrobial agents for the treatment of several diseases caused by microorganisms.

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