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Research Article

Phytochemical Screening and Antimicrobial Activity of *Lagenaria siceraria* Seeds Extracts

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ABSTRACT

The phytochemical and antimicrobial activity of seeds extracts obtained from the mature Nigerian grown short-hybrid bottle gourd (*Lagenaria siceraria*) was examined in this study. The different concentrations of *L. siceraria* seeds extracts (diethyl ether, chloroform, ethyl acetate, *n*- butanol, methanol and water) exhibited potent antibacterial and antifungal activity against selected pathogens (*Staphylococcus aureus, Pseudomonas sp., Escherichia coli, Bacillus subtilis, Candida sp. and Aspergillus niger*) using agar well diffusion method and the minimum inhibitory concentrations (MIC) varied between 0.002 and 0.100g/ml. The phytotchemical screening of the different seeds extracts revealed the presence of phlobatannins, saponins, cardiac glycosides, phenols, alkaloids, flavonoids, terpenoids, deoxy-sugar, carbohydrates and reducing sugars in varying quantities. *Lagenaria siceraria* seeds exhibit proven potential to contain antimicrobial agents of pharmacological interest.

Key words: Curcubitaceae, *Lagenaria siceraria*, Seed extracts, Phytochemicals, Antimicrobial

INTRODUCTION

Phytochemicals are natural bioactive compounds produced by plants as secondary metabolites that work with nutrients to protect against pathogenic attack. The most important phytochemicals are saponins, alkaloids, polyphenols and terpenoids[1]. Plants have provided a good source of anti-infectious agents; ementine, quinine and berberine remain highly effective instruments in the fight against microbial infections[2]. Although pharmaceutical industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased and has become a global concern[3]. The increasing failures of chemotherapeutics and antibiotics resistance exhibited by pathogenic microbials infectious agents have lead to the screening of several medicinal plants for their potential antimicrobial activity[4]. Selecting the right scientific and systematic approach to biological evaluation of plant products, based on their use in traditional medicine is the key to ideal development of new drugs from plants[2].

Lagenaria siceraria commonly known as bottle gourd belongs to Cucurbitaceae family and is a large pubescent climbing herb. The plant has been used in traditional system of medicine in India[5]. The seed is diuretic, anthelmentic and is also used to reduce inflammation and pain. It is in addition used in treatment of boils, aching teeth and gums, diabetes mellitus, cough, fever and skin diseases[6,7]. A number of phytochemical and pharmacological studies have been reported for the fresh fruit, leaves and root of *L. siceraria* from some parts of Asia[8]. Lagenin - a novel ribosome protein was isolated from the lyophilized water extract of *L. siceraria* seeds and shown to possess antitumour, immunosuppressive, antiviral, antiproliferative and anti-HIV activities[9]. Seeds extract of the Indian cultivated bottle gourd was also shown to exhibit antimicrobial activity against selected pathogens[10].

However, the Nigerian grown *L. siceraria* have been under-utilized. The known use of the mature fruits as containers is obsolete and their cultivation is no longer popular. Hence, there has been a paucity of scientific information on their chemical constituents and biological activity. The present study was undertaken to determine the phytochemical constituents and antimicrobial activity of the seeds extracts of *L. siceraria*.

MATERIALS AND METHODS

Sample Collection and Authentication

The mature fruits of *L. siceraria* (short hybrid bottle gourd) were collected from a farmland in September, 2012 at Ikono Local Government Area of Akwa Ibom State, Nigeria. The plant was identified and authenticated by a taxonomist, Dr. M. E. Bassey of the Botany and Ecological Studies Department, University of Uyo, where voucher specimens were deposited.

Sample Preparation and Extraction

The fruits were cut open to expose the seeds inside. The seeds were carefully collected, washed, shade-dried for three days and pulverized with the husk. The pulverized seeds (804.40g) were soaked in 1.2 L of dichloromethane for 72 hours under frequent agitation at room temperature to remove the seed oil. The dry residue (630.80g) was

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Table 1: Phytochemical screening of *L. siceraria* seeds extracts

Test	Diethyl ether	Chloroform	Ethyl acetate	n-Butanol	Methanol	Water
Phlobatannins	-	-	-	-	++	+
Anthroquinone	-	-	-	-	-	-
Saponins	+++	+	+++	++	+++	++
Tannins	-	-	-	-	-	-
Alkaloids	+++	_	-	++	++	+
Cadiac Glycoside	+++	+	+	++	+	+
Terpenes	+++	++	++	++	+++	+
De-oxy sugar	+++	+	+	+++	+++	+
Flavonoids	-	-	-	-	++	+
Phenols	++	-	++	-	+++	+
Carbohydrate	+++	+	+	+	++	++

+++: High, ++: Moderate, +: Trace, - : Not detected

Table 2a: Zones of inhibition of *L. siceraria* seeds extracts in millimeters (mm)

Organisms	Diethyl ether (g/ml)			Ethyl acetate (g/ml)			Chloroform (g/ml)			n-Butanol (g/ml)		
	0.05	0.10	0.15	0.05	0.10	0.15	0.05	0.10	0.15	0.05	0.10	0.15
B. subtilis	15	20	25	-	-	-	-	-	-	11	12	15
S. aureus	-	10	12	10	13	19	-	11	13	6	10	12
Pseudomonas sp.	-	15	20	-	-	-	-	5	10	-	-	-
E. coli	-	10	12	-	-	-	-	11	20	-	-	-
Candida sp.	10	15	18	-	5	12	-	14	20	-	10	12
A. niger	6	9	12	-	-	4	-	7	9	-	-	-

-: No observed inhibition

Table 2b: Zones of inhibition of *L. siceraria* seeds extracts in millimeters (mm)

Organisms	Methanol (g/ml)			Water (g/ml)			Gentamycin	Nystatin
	0.05	0.10	0.15	0.05	0.10	0.15	0.15	0.15
B. subtilis	-	-	-	-	-	-	16	NT
S. aureus	-	11	15	-	-	-	30	NT
Pseudomonas sp.	-	-	15	-	-	-	20	NT
E. coli	-	-	-	-	-	5	22	NT
Candida sp.	-	11	200	-	6	9	NT	30
A. niger	-	10	12	-	-	-	NT	18

-: No observed inhibition; NT: Not tested

Table 3: Minimum inhibitory concentration (g/ml) of *L. siceraria* seed extracts

Organisms	Diethyl ether	Ethyl acetate	Chloroform	n- Butanol	Methanol	Water
B. subtilis	0.002	0.040	0.040	0.004	0.060	0.080
S. aureus	0.006	0.020	0.020	0.004	0.020	0.100
Pseudomonas sp.	0.002	0.080	0.020	0.080	0.020	0.080
E. coli	0.006	0.060	0.020	0.020	0.060	0.020
Candida sp.	0.002	0.004	0.020	0.020	0.020	0.008
A. niger	0.002	0.008	0.020	0.060	0.020	0.100

further soaked with methanol for 72 hours under frequent agitation, filtered and concentrated. The aqueous extract was prepared by soaking the dry residue (403g) obtained after treatment with methanol in distilled water and was filtered and concentrated using a rotatory evaporator. The total methanolic extract (41.90g) was further partitioned with solvents of increasing polarity: diethyl ether, ethyl acetate, chloroform and *n*-butanol. The extracts/ fractions were concentrated at 40^0C in the oven. All chemicals and solvents used were of analytical grade from Sigma-Aldrich GmbH, Sternheim, Germany.

Phytochemical Screening

Standard methods for phytochemical screening (alkaloids, flavonoids, saponins, tannins, carbohydrates, sterols and triterpenes) were employed. Alkaloids determination was done using Mayer's and Dragendoff's reagents following the methods of Sofowora[11]; tannins and phlobatannins[12]. The methods described by Harborne[13] and Trease and

Evans (1989)[12] were used for determining flavonoids, phenol and cardiac glycosides. The persistent frothing, sodium bicarbonate and carbonate tests, as described by Trease and Evans[12] and Safowora[11] were used for saponins. Carbohydrates, sterols and triterpenes determination were done using Fehling's reagent following the method described by Harbone[13].

Antimicrobial Activity of the Extracts

Collection of Bacterial and Fungal Isolates

Clinical bacterial and fungal isolates were collected from St. Lukes Hospital, Anua, Uyo and University of Uyo Teaching Hospital, Uyo, Akwa Ibom State. These isolates were transported on slants to Microbiology Laboratory, University of Uyo. The test organisms were sub-cultured into nutrient broth and incubated for 24 hrs at 37°C. All Gram positive (*S. aureus and B. subtilis.*) and fungal isolates (*Candida sp*. and *A. niger*) were serially diluted to factor three using 10 fold dilution. Gram negative isolates (*Pseudomonas sp.* and *E. coli*) were serially diluted to factor five using 10 fold dilution. The isolates were sub-cultured into their selective medium based on their exhibited morphological characteristics. They were preserved at 4°C and later used for this work.

Evaluation of Antimicrobial Activity

Antimicrobial activity was performed by Agar well diffusion method[14]. Each crude extract (0.1g, 0.2g, 0.3g, 0.4g) was dissolved in 5ml of the stock solution. With a sterile cork-borer of 5mm, holes of equidistant diameter was made on the surface of the seeded plates and different concentrations of each extract were aseptically made to fill the holes such that each isolate was tested on different concentrations of the extract. Plates were incubated at 37°C for 24 hrs.

Antimicrobial activity of each extract against test organisms was determined by measuring their zones of inhibition in millimeters. Control experiment was carried out using commercial antibiotics, antifungal and solvent (stock). These were set up alongside with extracts. Gentamicin antibiotic (80 mg) was used for bacterial isolates and Nystatin (1000 iu) for fungal isolates; plates were inoculated for 24 hrs and 48 hrs respectively. Zones of inhibition were measured and recorded.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of each crude extract was determined using tube dilution method[15]. Different concentrations of crude extracts ranging from 0.1-0.5mg/ml were prepared and standard volume of each diluted isolate (0.1ml) was aseptically inoculated into different concentrations of the extract. Control experiment was carried out without the crude extracts. All tubes were incubated at 37°C for 24 hrs. Minimum inhibitory concentrations were determined as the lowest concentration without turbidity.

RESULTS AND DISCUSSION

The results of phytochemical screening of diethyl ether, ethyl acetate, chloroform, n-butanol, methanol and aqueous extracts of *L. siceraria* seeds are presented in Table 1. The phytochemical screening indicated varying quantity of saponins, cardiac glycosides, alkaloids, terpenoids, de-oxysugars, phenols, flavonoids, reducing sugars, phlobatannins and carbohydrates in the seeds extracts. The phytochemical profile revealed that the diethyl ether extract contains high amount of saponins, alkaloids, cardiac glycosides, terpenoids, de-oxy sugars, and reducing sugars; methanol extract contained saponins, terpenes, de-oxy sugars and phenols; saponins and de-oxy sugars were abundant in ethyl acetate and n-butanol extracts respectively. Tannins and anthraquinones were not detected in any of the extracts. The degree of extraction of phytochemicals in the seeds of the studied *L. siceraria* cultivar followed the order: Diethyl ether > methanol > n-butanol > ethyl acetate > water > chloroform. The variation in the presence and concentration of phytochemicals in seeds extracts may be attributed to the difference in polarity of the solvent medium and the nature of the constituents. Rodge and Biradar[16] reported the presence of tannins in leaves extract of *L. siceraria*; triterpenoids and carbohydrates have also been characterized from fruits, leaves and roots[8].

The antimicrobial activity of the seeds extracts of *L. siceraria* against pathogenic bacteria and fungi at varied concentrations (0.05, 0.10, 0.15 g/ml) is presented in Table 2a & Table 2b. The results revealed that all the extracts tested showed varying degree of antimicrobial activities against the test microbial strains. The zones of inhibition varied with the extract and the organism tested. It was observed that the zones of inhibition increased with increase in concentration as improved antimicrobial activity is concentration dependent. The diethyl ether extract (0.15g/ml) demonstrated highest antibacterial activity against *B. subtilis* (25 mm) and *Pseudomonas sp.* (20mm). *n*- Butanol extract was active against *B. subtilis*, *S. aureus*, *E. coli*, *Candida sp.* with highest zone inhibitory diameter for *E. coli* (20 mm). Water extract showed weak activities against *E-coli*, *Candida sp.* and showed no inhibitory effect against all other organisms. *Bacillus subtilis*, *Pseudomonas sp*. and *A. niger* were resistant to the phytochemicals in ethyl acetate, *n*- butanol and water extracts.

Generally, the diethyl ether extract (inhibition zone 6 - 25mm) was found to be most effective with broad spectrum antibacterial and antifungal activity; the water extract (inhibition zone 5 - 9 mm) was least active against the entire organisms. At 0.15 g/ml, diethyl ether extract (25mm) exhibited better antimicrobial activity when compared with the standard drugs gentimycin (16mm). Chloroform, ethyl acetate, n-butanol, methanol and water extracts did not have better activity when compared to the standard drugs (gentamicin and nystatin) and were found to show selectivity in antimicrobial action. The degree of selective resistance increased in the order: chloroform < methanol < butanol < ethyl acetate < aqueous extracts. Methanol extract showed better antifungal potency than diethyl ether extract, followed by chloroform, ethyl acetate and butanol extracts. The antimicrobial profile of diethyl ether extract indicates better susceptibility of *B. subtilis* (Gram positive bacteria) than *E. coli* (Gram negative bacteria). It has been suggested that Gram-

positive bacteria are more sensitive to chemical compounds than Gram negative bacteria due to the relative thickness of their cell walls.[17] Sood *et al.*[10] showed that *L. siceraria* seed extract was effective against *E. coli*, but no inhibition against *A. niger* and *C. albicans*. Goji *et al.*[18] also evaluated antimicrobial activity of methanolic extracts of the leaves, seeds, and fruit-flesh of *L. siceraria* using the agar-well diffusion method. Results revealed that *L. siceraria* extract demonstrated activity against *P. aeruginosa* and *Streptococcus pyogenes*, but not against clinical isolates of *S. aureus* and *E. coli*.

Low activity of the water extract against most bacterial and fungal strains investigated in this study is in congruence with its relative least phytochemical profile (Table 1) and consistent with previous work which reveal that aqueous extracts of plant generally showed little or no antibacterial and antifungal activities.[19] Joseph *et al.*[20] stated that the medicinal properties and biological activities of plants are usually due to their chemical profile. This suggests that the saponins, alkaloids or terpenoids in the potent diethyl ether extract of *L. siceraria* in this study may be implicated for its pronounced antimicrobial effects shown. Triterpenoids, saponins and flavanoids have been isolated and characterized from the fresh fruits of this plant.[7,21] However, no work has been reported on the characterization of compounds from the seeds and antimicrobial activity. The antibacterial and antifungal activities of *L. siceraria* seeds (Table 2a & Table 2b) are found to be more potent than assay reported for the fruits.[16,19] These differences in activity may be due to the source of the microbial strains, plant part utilized in the studies or environmental factors. The minimum inhibitory concentration (MIC) values of *L. siceraria* extracts on different microorganisms are reported in Table 3. Antimicrobial agents with low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC. The MIC, the lowest concentration of an extract that will inhibit the visible growth of a microorganism after overnight incubation ranges from 0.002-0.100 g/ml on tested bacteria and fungi for the seeds extracts. The *L. siceraria* seeds extracts showed positive inhibitory activity against all the tested microorganisms. The diethyl ether extract had least MIC (0.002g/ml) against *B. subtilis*, P*seudomonas sp.*, *Candida sp.* and *A. niger*; ethyl acetate exhibited the least MIC against *C. albicans* (0.004/ml); *A. niger* (0.008g/ml) and n-butanol demonstrated least MIC (0.004g/ml) against *B. subtilis* and *S. aureus*. The MIC values (10- 50mg/ml) were reported by Rodge and Biradar (2012) for leaves extracts of *L. siceraria*. This work lends credence to the fact that *L. siceraria* seeds could be employed in the treatment of various infectious disorders implicated with the test pathogens highlighted in Table 2 and Table 3. The phytochemical screening revealed that *L. siceraria* seed extracts contained alkaloids, cardiac glycosides, saponins, terpenoids, phenols, carbohydrate and reducing sugars. Diethyl ether and methanol extracts contained high amount of these phytochemicals. These compounds are known to be biologically active and therefore suggestive of their implicated antimicrobial activities. Generally, the diethyl ether extract was found to be most effective with broad spectrum antibacterial and antifungal activity. The inhibitory effect of the extracts of *L. siceraria* against pathogenic bacterial and fungal strains (*B. subtilis, E. coli, Pseudomonas sp., S. aureus, A. niger*, and *C. albicans*) lends credence to the plant as a potential candidate for bioprospecting for antibiotic drug development for the treatment of ailments caused by these pathogens.

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