PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *Leea guineensis*

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Abstract: The phytochemical screening and antimicrobial properties of the leaf extracts of *Leea guineensis* was studied. A number of solvents and solvent-mixtures were used to assess the most effective solvent type that is recommended for the extraction of the active components of this plant. Two extraction methods were employed: cold and Soxhlet extraction. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides in all the samples of ethanol and hexane/acetone/methanol mixture extracts. The antimicrobial test results revealed that the plant extracts exhibited efficacy against a number of microbes (*Eschericha coli*, *Staphylococcus aureus*, *Baccillus substillus*, *Streptococus pneumonia*, *Psudomonas aeroginosa* and *Candidas alblican*) that were used in the study by exhibiting clear zones of inhibition against these microbes. The finding of this study recommends the use of either ethanol as a single solvent or a solvent system with a mixture of hexane/acetone/methanol with increasing polarities.

Keywords: Phytochemicals, antimicrobial, activity, extracts, *Leea guineensis*

Introduction

Given the rate of resistance of disease organisms to synthetic drugs, the need to find new drugs that can stand the test of time cannot be over emphasized. It is reported that about 80% of the world population make use of medicinal plants in the treatment of diseases with an even much higher rate in most African countries (WHO, 2001); while an estimated 90% of the population in developing countries depend on medicinal plants as a means of primary healthcare (WHO, 2002). Jimoh (2006) believes that the use of traditional medicine is not synonymous with developing countries alone. Well over 50% of the entire modern clinical drugs the world over are of natural products origin (Stuffness & Douros, 1982), while Baker *et al*. (1995) highlight the fact that drug development programmes in the pharmaceutical industry depend largely on natural products. It has been reported that not less than 25% of drugs employed in pharmacopeia are plant-based (FAO, 2000).

The plant, *L. guineensis* has been shown to contain appreciable amounts of vitamin A, C, D and E (Ajiboye *et al*. 2014). The treatment with aqueous seed extract of *L. guineensis* (200 and 400 mg/kg), particularly 400 mg/kg could ameliorate the biochemical indices related to liver toxicity in the animals (Ajiboye *et al*., 2014). Fadolun *et al*. (2007) examined the anti-edematogenic activity of the aqueous extract of *L. guineensis.* The phytochemical screening revealed the presence of saponins and reducing sugars. It also confirmed the presence of steroidal saponins. The aqueous extract was found to be partially non-toxic at the doses range of 1 – 5 mg/kg since pharmacological evaluation was also carried out. This present study has the main aim of extraction, preliminary phytochemical screening and antimicrobial activities of the crude extracts of the leaf of *Leea guineensis*.

Materials and Methods

Sample collection and preservation

A fresh leaves *Leea guineensis* was collected from Akparabong village in Ikom Local Government Area of Central Cross River State and was authenticated by Mr. Apejoye at the Department of Botany, University of Calabar. A voucher specimen of the plant has been placed in the official herbarium of the Department.

Sample preparation and extraction

The fresh parts of the leaves were air-dried under shade for three (3) weeks followed by oven-drying at a temperature of 60^{o}C until all the moisture content was completely expelled. The samples were then crushed into a powdery form with the help of blender. Two methods of extraction were used namely; cold and sohxlet extractions.

The cold extraction was done by carefully weighing-out 200 g each of the powdered leaves into separate containers together with 250 ml of the various solvents (ethanol, methanol, acetone, chloroform and hexane) used in the extraction. After allowing the sample to stand for a period of 4-days, it was then decanted with the use of a Whatmann No. 1 filter paper. The extract was then concentrated by simple evaporation in a water bath at 100^{o} C. The continuous extraction on the other hand involved the use of a Sohxlet apparatus. 500 mL of hexane/acetone/methanol (3:1:1) mixture was carefully measured with a measuring cylinder and poured into a round bottom flask. This was followed by weighing out 200 g of the sample into a Sohxlet apparatus, already lagged with wool so as to prevent particles of the sample falling into the solvent from the top. The extractor was then fitted with the round bottom flask (greased with petroleum gel) and the condenser connected to it, with an outlet and the inlet end connected to a water tap. The extraction ran for 6 h. The extract was then concentrated in a water bath at 100^{o}C before subjecting it to phytochemical examination.

Phytochemical screening

The qualitative test was carried out to determine the presence of secondary metabolites as described by Harborne (1973) and Trease & Evans (1989). The procedures are described briefly as;

Test for alkaloids

0.5 g of the extract was weighed into a 100 mL conical flask containing 2 mL of 5% H_2SO_4 in ethanol. The mixture was heated to boiling in a water bath, and was

allowed to cool and then tested for the presence alkaloids. 2 mL of the filtrate of the heated samples were then used to test for colour change using 2 drops of Mayer's reagent for a yellow precipitate and 2 drops of Wagner's reagent for reddish-brown precipitate.

Test for tannins

About 0.5 g of the extract was boiled in 20 mL distilled water in a water bath. On cooling, a drop of ferric chloride was added and observed for a brownish-green or blue-black colouration.

Test for flavonoids

5 mL of dilute water was added to 5 ml of aqueous filtrate of each sample. To this mixture, about 2 drops of H_2SO_4 was added and observed for a yellow colouration which would disappear on storage.

Test for saponins

About 2 g of the sample was boiled in 20 mL distilled water in a water bath. After cooling, the boiled mixture was filtered. 10 mL of the filtrate was mixed with 5 ml distilled water and shaken vigorously for stable froth formation. Three (3) drops of olive oil were added to the frothing solution, and the formation of an emulsion confirmed the presence of saponins.

Test for cardiac glycosides

A small portion of the extract was boiled in 5 ml of 70% of ethyl alcohol for 2 min. The mixture was filtered and 10ml of water and 5 mL of chloroform was added to the filtrate and shaken. The lower chloroform layer was separated off and evaporated to dryness in a water bath. The cooled chloroform residue was dissolved in 3 mL of glacial acetic acid containing 0.1 mL of $FeCl_3$. The solution was carefully transferred to the surface of 2 ml of sulphuric acid (H_2SO_4) and observed for a reddish-brown layer formed at the interface and also observed for the formation of a bluish-green colouration at the upper layer.

Bioassay

The term bioassay is used to describe the study of antimicrobial activity of the crude or purified extracts of a plant against microorganisms. The test microbes were obtained from the University of Calabar Teaching Hospital, Calabar. The bacterial assay procedures of Water Worth (1978) and Perez *et al*. (1990) were employed with small modification. The clinical isolates (*Escherichia coli, Staphylococcus aureus, Bacillus substillus, Candida albilcan, Pseudomonas aeruginosa* and *Streptococcus pneumonia*) used in this study were subjected to antimicrobial susceptibility testing, using the convectional agar disc diffusion method on Muller Hinton agar. The antimicrobial herbal extracts (stem and leaves) were used and their disc concentrations with 0.6 mg/ml, 1.0 mg and 1.3 mg/ml and the positive control (Ciprofloxacin) concentration 30 µg.

Standardization of inoculums

The six test organisms were sub-cultured with nutrient broth using a wire loop (done aseptically) and incubated for 24 h at 35 ^oC for bacteria and 48 h at 25^oC for fungi. The growth of the microorganisms in the broth by the turbidity produced was adjusted to match 0.5 McFarland standards (10^8 cfu/mL), which was further adjusted to 10^5 cfu/mL and 10^3 cfu/mL for bacteria and fungi respectively.

Innoculation of the plates and application of the extracts

The agar plates NA (nutrient agar) and MEA (Malt extract agar) were inoculated by spreading a small volume (0.05 mL to 0.10 mL) of the liquid inoculums (sub-cultured nutrient broth) by means of an L-shaped glass rod in such a way that the surface of the agar in the plates were covered with microbes. One microbe was inoculated to one plate making a total of six plates for six microbes. The plant extracts are diluted using dilution method and in each of the appropriately labeled well diluted plant extract was introduced. Ciprofloxacin and fulcin were also introduced in the other two wells (holes) as control. The inoculated plates were left on the bench for about an hour to allow the extracts diffuse into the agar. The NA (nutrient agar) and MEA (malt extract agar) were aerobically incubated at 37^oC for 23 h for the bacteria and 48 h for the fungi. The diameter of zones of inhibition was measured in millimeter.

Results and Discussion

Phytochemical assay

The phytochemical composition of *L. guineensis* revealed the presence of the five phytochemicals screened for across an array of solvents used in the extraction (Tables 1 and 2). The results revealed that reveals the presence of alkaloids, tannins, flavonoids, saponnins and cardiac glycosides in the leaf extracts in the hexane/acetone/methanol mixture. The results revealed the presence of alkaloids in all the extracts, saponnins was detected in hexane, acetone and ethanol extracts and absent in chloroform and methanol extracts. Alkaloids, which are considered to be the most important of all phytochemicals, possessing pharmacologically active components whose actions have been noticed in blood vessels, respiratory tract, malaria, malignant diseases, vervous system, uterus, etc. (Trease & Evans, 1989). Alkaloids have been shown to exihibit analgesic and bactericidal effects (Stary, 1998).

Table 1: Results of phytochemical screening of *Leea guineensis* Leaf extracts using various solvents (cold extraction)

S/N	Phytochemicals	HE	CE	AE	EE	ME
1	Alkaloids	+	+	+	+	+
2	Tannins	+	+	-	+	+
3	Flavonoids	+	-	+	+	+
4	Saponins	+	-	+	+	-
5	Cardiac glycosides	+	-	+	+	+

HE = Hexane extract, CE = Chloroform, AE = Acetone extract, EE = Ethanol extract, ME = Methanol extract, L: Leaves, +: Present, - = Not present

Table 2: Results of phytochemical screening leaf extracts of *Leea guineensis* using hexane/acetone/methanol mixture (soxhlet extraction)

S/N	Phytochemicals	Hexane/acetone/methanol
1	Alkaloids	+
2	Tannins	+
3	Flavonoids	+
4	Saponins	+
5	Cardiac glycosides	+

+ = Present

Tannins were not present in leaves extract of acetone but were present in all other extracts. Tannins which are non-toxic have the ability to revive physiologies in animals that ingest them (Scalbert 1991), though they are poisonous to filamentous fungi, bacteria and yeast. The presence of tannins attests to its role in antihemorrhoidal, antifungal and antioxidant agents (Asquit & Butter, 1986). Flavonoids were present in all the extracts except leaves extract of chloroform. The presence of flavonoids in this plant accounts for its antitumor, anti-oxidant, anticarcinogenic, antiradical properties. Because of these properties exhibited by flavonoids, this plant is recommended for health challenges such as cancer,

inhibition of heart diseases as well as against a number of microbial infections (Harborne, 1973; Kandaswami *et al*., 1994; Nakayoma & Yamada, 1995; Manikandan *et al*., 2009). The anti-inflammatory and antimicrobial activities of flavonoids have also been documented by Cushnie & Lamb (2005).

Saponins were not detected in leaf extracts of chloroform and methanol. A study by Falodun *et al.* (2007) showed marked inhibition of up to 73% of the oedema level in rat paw, even greater than the control drug at a dose of 400 mg/kg, and attributed it to the abundant level of saponins in *L. guineensis* extract used in the study. Saponins have been reported to have anti-inflammatory and cardiac depressants properties (Trease & Evans, 1985) and seemingly inhibit the growth of carcinogenic cells but without necessarily killing the normal cells in the process (Lewis & Elvis-Lewis, 1995). Cardiac glycosides were present in all the extracts except extract of chloroform. Their clinical effects in cases of congestive heart failure have been reported (Brian *et al.*, 1985), and the potency against cardiac arest is demonstrated by acting on the heart muscles as well as increases the renal flow (Olaleye *et al*., 2007). Cardiac glycoside is highly recognized stimulant that has been used over a number of years in cases of cardiac failure and diseases (Trease & Evans, 1978; Olayinka *et al*., 1992).

Antimicrobial test results of the leaves extracts of L. guineensis

Activity of five (5) different solvents (hexane, acetone, chloroform, ethanol and methanol) and a combined mixture of three (3) solvents (hexane/acetone/methanol) extract from the leaves of *L. guineensis* were tested on five (5) clinical isolates with measured zones of clearance of the pathogens are presented in Table 3.

The application of the leaf extracts on *E. coli* showed that only the higher concentrations had susceptibility against this microbe. It therefore tells that the high concentrations (1.0 mg/mL and above) of the stem and leaves of *L. guineensis* may be used in the treatment of diarrhea, urinary tract infection (UTI), anaemia (shortage of blood) as well as correct kidney failuire since *E. coli* is behind these diseases. Extracts of the leaf exhibited appreciable potency against *S. aureus* meaning that it may be useful in the treatment of staphylococcus infection. Similarly, the extracts of the leaf demonstrated considerable sensitivity on *B. substillus* even when the negative reference sample was not expected to show any inhibitive properties. *S. pneumonia* was found to be inhibited by the extracts as seen from the marked zones of inhibition recorded in Table 3. Further, of the three concentrations (0.6 mg/mL, 1.0 mg/mL and 1.3 mg/mL) of extracts used for the antimicrobial test, the leaves extracts were more potent in order of increasing concentrations.

Test Organisms	Concentration of Extracts				
	0.6 mg/ml	1.0 mg/ml	1.3 mg/ml	Positive control	Negative control
E. coli	00	14	20	25	00
S. aureus	13	15	15	14	00
B. substillus	16	18	21	19	00
S. pneumonia	10	17	26	22	00
P.aeroginosa	11	13	14	40	00
C. alblican	13	15	16	31	00

The leaf extracts were also active against *P. aeroginosa.* The positive control here clearly shows higher sensitivity against this microorganism albeit the extracts also showed signs of inhibition. This could mean that concentrations of the extract higher than those used for the study may give better results. High concentrations of both the leaf extracts showed appreciable levels of inhibition of *C. alblican.* This finding reveals that extracts from (stem and leaves) of this plant may possibly be active in the treatment of candidiasis which is caused by *C. alblican.*

Conclusion

The study shows that this plant contains a number of phytochemicals. It also reveals that the plant extracts used for the study possess bioactive constituents and exhibit antibacterial and antifungal properties.

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