Original Research Article

Antimicrobial Activities and Phytochemical Screening of the Solvent fractions of the Leaves and Stems of *Leea guineensis* G. Don

ABSTRACT

Aim

We evaluated the antibacterial activities and phytochemical distribution of the solvent fractions of the leaf and stem of *Leea guineensis G.Don*.

Methodology: The antibacterial activities and phytochemical distribution of the solvent fractions of the leaf and stem of *L. guineensis* were determined using standard procedures.

Results: Methanol fraction of the leaves at 100 mg/mL showed activity against *Acinetobacter baumammii* while both ethyl acetate and methanol fractions of the leaves at 100 mg/mL have activities against *Acinetobacter baumammii**.*** The methanol fraction of *LGS* leaves ***at*** 25-100 mg/mL was potent against *Escherichia coli,* while the ethyl acetate fraction of the stem at 100 mg/mL was inhibitory to *Escherichia coli.* Both the ethyl acetate and methanol fractions of the leaf of *LGS* possess antibacterial activity against *Staphylococcus aureus, however, the* methanol fractions of the stem alone inhibit *Staphylococcus aureus*. Ethyl acetate and methanol fractions of the leaf were inhibitory to *Pseudomonas aeruginosa while* ethyl acetate fraction of the stem was highly efficacious on *Pseudomonas aeruginosa*. Only the methanol fraction of the leaf was potent against *Proteus mirabilis*, while both ethyl acetate and methanol fraction of the stem were potent against *Proteus mirabilis*. Both ethyl acetate and methanol fractions of the stem at 100 µg/mL were potent against *Klebsiella pneumoniae* but, only the methanol fraction of the stem was potent on *Klebsiella pneumoniae*. The methanol fraction of the leaves was potent against *Salmonella Typhi* however, both ethyl acetate and methanol fraction of the stem were potent against *Salmonella Typhi*. More phytochemicals were found in ethyl acetate and methanol fractions of both the leaves and stems.

Conclusion: The ethyl acetate and methanol fractions of the leaves and stem of *L. guineensis* seem to possess some antibacterial properties courtesy of the abundant phytochemicals found in these fractions.

Keywords: Antimicrobial, Medicinal plant, Leea guineensis, Phytochemical

1. INTRODUCTION

The increase in the prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from alternative sources [1]. An important way to prevent antibiotic resistance is by using new compounds that are not based on the existing synthetic antimicrobial agents. Medicinal plants have been in use for centuries because they are found to contain chemical compounds, usually secondary metabolites that are used as natural medicines to treat common bacterial infections. They have been regularly used in developing countries because of minimal side effects and cost-effectiveness [2]. There are more than 35, 000 plant species being used in various human cultures around the world for medicinal purposes [3]. A large number of purified constituents of medicinal plants have shown beneficial therapeutic potentials [4]. To promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their uses.

Plant phytochemicals are a potential source for new antimicrobial drugs. Numerous methods have been utilized to acquire compounds from medicinal plants and other natural sources for drug discovery [5,6]. Also, the distribution of these phytochemicals is related to their biological activities and has been associated with the type of solvent used for extraction or fractionation [7].

Leea guineensisis (Yoruba name: "Alugbokita") *is* a shrub with evergreen leaf which can be found in tropical Asia and some parts of Africa including Nigeria [8]. In folk medicine, the plant has been claimed to be efficacious for management of enlarged spleen in children, pregnancy detection, purgative, toothache, gonorrhea, general weakness, skin lesions, skin rash, ulcer, diarrhea, dysentery, as a diuretic; oral treatments, as a pain killer, paralysis, epileptic fits (juice of fresh leaves used as an enema), convulsions, spasm, stomach troubles, herpes and boils. It is also claimed to have fungistatic and bacteriostatic efficacies [9]. Based on the medicinal claim of this botanical, it is important to study the antimicrobial potential of the plant. This, therefore, evaluated the phytochemical distribution and antimicrobial activities of the solvent fractions of leaf and stem of *L. guineensis*.

2. MATERIAL AND METHODS

2.1 Chemicals, drugs, and reagents

All chemical reagents used in this research were of analytical grades. All essential chemicals were procured from recognized suppliers like Hi media laboratories, SRL, qualigens, and Sigma Aldrich Inc., USA.

2.2 Sample preparation

Fresh leaf and stem of *Leea guineensis* were collected in September 2019. The plant was identified and authenticated with a voucher number. The plant samples were then transported to the Laboratory of the Department of Microbiology, Lead City University, Ibadan. The fresh leaves and stems were rinsed in distilled water, dried in vacuum air, and pulverized into powder using Emel EM242 blender.

2.3 Preparation of solvent fractions of *L. guineensis*

The fractions of the plant were collected in the order of solvent increase in polarity. One hundred and fifty grams (150 g) of the pulverized leaf and stem samples were weighed, transferred into a container, and 600 mL of n-hexane was added, the extraction proceeds for 24 hours on a rotatory shaker (Hati rotamixer) at 220 rpm. The extract was filtered, and the solvent was evaporated on a rotary evaporator (Stuart RE300) at 40°C to obtain the n-hexane fraction. The residue collected was dried weighed and soaked in ethyl acetate (1g: 4mL), extraction in ethyl acetate was allowed to continue for 24 hours on a mechanical shaker at 220 rpm. The extract was also filtered, the solvent was evaporated to obtain ethyl acetate fraction, the residue was collected and the process of extraction was repeated for methanol and water consecutively.

2.4 Test for antibacterial properties solvent fractions of *L. guineensis*

2.4.1 Procurement of microorganisms

Strains of *Acinetobacter baumanni* (ATCC 17978, MTCC 1425), *Escherichia coli* (ATCC 25922, *MTCC1698*), *Klebsiella pneumonia* (*ATCC* 35657, *MTCC* 432), *Salmolena typhi (ATCC 19430, MTCC 98), Staphylococcus aureus* (ATCC 25923, *MTCC3160*), *Pseudomonas aeroginosa* (ATCC 27853, MTCC 6458), *Proteus mirabilis* (***ATCC*** 7002*, MTCC425*), were obtained from the Department of Parasitology and Medical Microbiology, University College Hospital (UCH), Ibadan, Nigeria.

2.4.2 Preparation and maintenance of stock cultures

All bacterial strains were inoculated on nutrient agar slants and incubated overnight at 37°C fungal strain was inoculated on an SDA slant and incubated at 30°C for 24-48 hours. These cultures were stored in a refrigerator at 4°C.

2.4.3 **Culture Media**

Nutrient Broth, Nutrient Agar, and Muller Hinton Agar (M.H.A) were prepared using the method described by Qayyum et al., (2020) [10]. Bacterial strains were inoculated on nutrient agar slants and incubated overnight at 37°C while fungal strain was inoculated on SDA slant and incubated at 30°C for 24-48 hours. These cultures were stored in a refrigerator at 4°C.

2.4.4 Antimicrobial activity screening test.

The antimicrobial activity of the solvent fractions of *Leea guineensis* eight selected microorganisms were screened by using the agar well diffusion method [11]. An inoculum suspension was swabbed uniformly to solidified Muller Hinton agar (MHA) plates and the inoculum was allowed to dry for 5 min. Holes of 6mm in diameter were made in the seeded MHA plates using a sterile cork borer. Aliquots of 20 µL from different concentrations (100, 50, 25, 12.5, and 6.25 µg/mL) of each fraction were added to each well on the seeded medium, and culture plates were allowed to stand on the bench for 1hour for proper diffusion and thereafter incubated at 37°C for 24 hrs. The resulting inhibition zones were measured in millimeters (mm). Gentamycin was used as the positive control.

2.5 Phytochemical screening of solvent fractions of *Leea guineensis*

Qualitative screening of phytochemicals in the plant fractions was done using procedures described by Harborne (1973) [12], Sofowora (1993) [13], and Evans (2009) [14]

3. RESULTS

This study revealed that the n-hexane fraction and aqueous fraction did not produce an inhibitory effect on the selected strains. Methanol fraction of the leaf at 100 mg/mL showed activity against *Acinetobacter baumammii* while both ethyl acetate and methanol fraction of the stem at 100 mg/mL have activities against *Acinetobacter baumammii* (Table 2 and 4)**.** Methanol fraction of *Leea guineensis* leaves at 25-100 mg/mL possess higher activity against *E. coli (Table 3),* 100 mg/mL ethyl acetate fraction of the leaf also shows activity on the organism while only ethyl acetate fraction of the stem possesses antimicrobial activity against *E. coli* (Table 1 and 2).

In Tables 1 and 3*,* both the ethyl acetate and methanol fractions of the leaf of *Leea guineensis* possess antimicrobial activity against *S. aureus* at 50 and 25 mg/mL respectively*.* While methanol fractions of the stem of *Leea guineensis* at 50 mg/mL possess antimicrobial activity against *S. aureus* (Table 4). Both ethyl acetate and methanol fractions of the leaf of *Leea guineensis* at 25 mg/mL possess antimicrobial activity against *P. aeruginosa* (Tables 1 and 3). Ethyl acetate fraction of the stem, even at a concentration of 12.5 mg/mL, shows antimicrobial activity on *P. aeruginosa* compared to the methanol fractions of the stem while the methanol fraction of the stem shows inhibits the organism at 100 mg/mL (Table 2 and 4). Only the methanol fraction of the leaf was potent against *P. mirabilis*. While both ethyl acetate and methanol fractions of the stem were potent against P. mirabilis and K. pneumonia (see tables 2 and 4). The methanol fraction of the leaves at 50 mg/mL produced inhibition of *S. typhi.* Both ethyl acetate and methanol fractions of the stem were potent against *S. typhi* with the ethyl acetate fraction exhibiting more potency on S. typhi with a lower concentration of 50 mg/mL

Table 5 showed the phytochemical content in solvent fractions of *Leea guineensis* leaves. Steroid, terpenoids, alkaloid, and phlobatinins were phytochemicals detected in the n-hexane fraction of the leaves, while flavonoids, saponin, anthraquinone, glycosides, and tannin were not detected. The listed phytochemicals were observed to be present in the ethyl acetate fraction, while steroids and glycosides were absent in ethyl acetate fractions. The water fraction contains flavonoids, saponin, terpenoids, anthraquinone and alkaloids, and phlobatinins.

In the solvent fractions of the stem (Table 4.5), the n-hexane fraction contains saponin, steroid, and terpenoids while all phytochemicals were found in the ethyl acetate fraction, the steroid was absent in the methanol fraction while the water fraction of the stem of *Leea guineensis* was observed to contain flavonoids, saponin, terpenoids, anthraquinones, and phlobatinins.

Table 1: Antimicrobial susceptibility testing of ethyl acetate fraction of the leaf of *Leea guineensis*

Ethyl acetate(leaf)	***A. baumannii***	***E.coli***	***S.aureus***	***P.aeruginosa***	***P.mirabilis***	***K. Pneumoniae***	***S.typhi***
100 mg/mL	-	15±0.00	11.0±1.00	13.5±2.12		11.00±1.00	-
50 mg/mL	-	-	13.67±1.53	10.5±4.95	-	-	-
25 mg/mL	-	-	-	9.00±4.24	-	-	-
12.5 mg/mL	-	-	-	-	-	-	-
6.25 mg/mL	-	-	-	-	-	-	-
CN	16.67±0.58	16.67±0.58	16.67±0.58	16.67±0.58	6.00±0.00	6.00±0.00	16.00±1.00

Results were presented as mean ± standard deviation of triplicate determination. – represent no zone of inhibition. CN: Gentamycin at 30 µg/mL

Table 2: **Antimicrobial susceptibility testing of ethyl acetate fraction of the stem of *Leea guineensis***

Ethyl acetate(stem)	***A. baumannii***	***E.coli***	***S.aureus***	***P.aeruginosa***	***P.mirabilis***	***K.Pneumoniae***	***S.typhi***
100 mg/mL	10.5 ±4.95	16.00±0.00	-	13.5±2.12	12+0.00	-	12.00±0.00
50 mg/mL	-	16.00±0.00	-	14±0.00	12+0.00	-	12.00±0.00
25 mg/mL	-	-	-	12±0.00	10.67±0.95	-	-
12.5 mg/mL	-	-	-	11.00±1.00	9.00±4.24	-	-
6.25 mg/mL	-	-	-	-	9.00±4.24	-	-
CN	16.67±0.58	16.67±0.58	16.67±0.58	16.67±0.58	6.00±0.00	6.00±0.00	16.00±1.00

Results were presented as mean ± standard deviation of triplicate determination. – represent no zone of inhibition. CN: Gentamycin at 30 µg/mL

Table 3: **Antimicrobial susceptibility testing of methanol fraction of the leaf of *Leea guineensis***

Methanol (leaf)	***A. baumannii***	***E.coli***	***S.aureus***	***P.aeruginosa***	***P.mirabilis***	***K. Pneumoniae***	***S.typhi***
100 mg/mL	9.00 ±4.24	16.67+0.58	13.00±1.00	14±0.00	14.00±0.00	13.00±1.00	13.00±1.00
50 mg/mL	-	13.67±1.53	13.00±1.00	13.00±1.00	5.00±0.00	-	7.5±1.54
25 mg/mL	-	12.67±2.52	10.50±0.95	10.00+0.00	5.00±0.00	-	-

12.5 mg/mL	-	-	-	-	-	-	-
6.25 mg/mL	-	-	-	-	-	-	-
CN	16.67±0.58	16.67±0.58	16.67±0.58	16.67±0.58	6.00±0.00	6.00±0.00	16.00±1.00

Results were presented as mean ± standard deviation of triplicate determination. – represent no zone of inhibition. CN: Gentamycin at 30 µg/mL

Table 4: **Antimicrobial susceptibility testing of methanol fraction of the stem of *Leea guineensis***

Methanol (stem)	***A. baumannii***	***E. coli***	***S. aureus***	***P. Aeruginosa***	***P. mirabilis***	***K. Pneumoniae***	***S. typhi***
At 100 mg/mL	9.0 ±4.24	-	13.67±0.58	14±0.00	14+0.00	13.00±1.00	13.00±1.00
At 50 mg/mL	-	-	10.5±0.95	-	9.00±2.65	-	-
At 25 mg/mL	-	-	-	-	8.33±2.89	-	-
At 12.5 mg/mL	-	-	-	-	9.33±3.06	-	-
At 6.25 mg/mL	-	-	-	-	-	-	-
CN	16.67±0.58	16.67±0.58	16.67±0.58	16.67±0.58	6.00±0.00	6.00±0.00	16.00±1.00

Results were presented as mean ± standard deviation of triplicate determination. – represent no zone of inhibition. CN: Gentamycin at 30 µg/mL

Table 5: Phytochemical screening of the solvent fractions of the stems and leaves of *Leea guineensis*

PHYTO CHEMICAL	**N-HEXANE**		**ETHYL ACETATE**		**METHANOL**		**WATER**	
	STEM	Leaf	STEM	LEAVES	STEM	LEAVES	STEM	LEAVES
Flavonoids	-	-	+	+	+	+	+	+
Saponin	+	-	+	+	-	+	+	+
Steroids	+	+	+	+	+	-	-	-
Terpenoids	+	+	-	+	+	+	+	+
Anthraquinone	-	-	+	-	+	-	+	-
Alkaloids	-	-	+	-	+	-	-	-
Cardiac glycosides	-	-	+	-	+	-	-	-
Tannins	-	-	+	-	+	-	-	-
Phlobatinins	-	-	+	-	+	-	+	-

The experiments were performed in triplicate. + represent present, - represent not detected.

4. DISCUSSION

In folklore medicine, *L. guineensis* has been claimed to be efficacious against several infectious related diseases such as toothache, gonorrhea, general weakness, skin lesions, skin rash, ulcer, diarrhea, and dysentery [9]. Plant fractionation could separate the bioactive compound in plants and increase the purity and distribution of bioactive compound to phase [15].

From our study, Ethyl acetate and methanol fractions were fractions with antibacterial activities. These fractions possess a wide range of antibacterial activities due to the presence of a wide variety of bioactive phytochemicals. Ethyl acetate and methanol which are semipolar solvents are capable of extracting several bioactive polar compounds such as flavonoids and tannins. Studies have shown that the flavonoid isolate from the ethyl acetate fraction of a plant exhibits antibacterial properties against *E. faecalis [16]*. Ethyl acetate and methanol could dissolve tannins that possess antibacterial activity. Tannins inhibit the synthesis of bacterial cell walls and cell membranes through the hydrolysis of ester bonds [17]. Catechin, a subclass of tannins has been reported to have the capability of penetrating and interacting with lipid bilayers and causing membrane fusion [6]. Meanwhile, terpenoids can inhibit lipid formation and change the structure of cell membranes by inhibiting ergosterol synthesis [10].

The mechanism through which bioactive compounds exhibit their antibacterial activity is multifaceted [18]. Antibacterial properties are also attributed to the presence of flavonoid, tannin, and triterpenoid compounds which could inhibit the growth of the organism [19]. Flavonoid content in plant fractions has been noticed to be an inhibitor of nucleic acid synthesis (caused by topoisomerase inhibition), cytoplasmic membrane function (membrane permeability and leakage), and inhibition of energy metabolism [20,6]. Flavonoids such as rutin inhibit bacterial growth by topoisomerase inhibition, which is important for DNA synthesis; quercetin could reduce bacterial membrane permeability and antibacterial resistance [7].

A wide range of fractionation solvents (water, methanol, ethyl acetate, and hexane) result in various distributions of Chemical structures based on polarities [21]. From the current study, most bioactive compounds were dissolved in ethyl acetate fraction followed by methanol in the leaves and stem of *L. guineensis*, this indicates that most semipolar and polar bioactive compounds were distributed to this solvent phases while the least bioactive compounds were dissolved in the non-polar hexane water fractions. Our finding is similar to report by Kuswandani et al [17] and Widyawati et al [22] who reported more distribution of phytochemicals in ethyl acetate fraction of *M. pendens*

Concerning our finding, some previous studies reported that methanol can dissolve polar compounds, such as sugar, amino acid, glycoside compounds, phenolic compounds with low and medium molecular weights and medium polarity, aglycon flavonoids, anthocyanins, terpenoids, saponins, tannins, xantoxilin, totarol, quacinoid, lactones, flavones, phenones, and polyphenols. Ethyl acetate is also effective to extract alkaloids, aglycons, glycoside compounds, sterols, terpenoids, and flavonoids. Hexanes can solve non-polar compounds or lipophilic compounds, which are generally lignin, wax, lipids, aglycon, sterols, and terpenoids [5].

5. CONCLUSION

This study revealed that ethyl acetate and methanol fractions obtained from *L. guineensis* leaves and stem shows activity against a wide range of microbes, this activity is attributable to the abundant spectrum of bioactive phytochemicals distributed in the fractions.

DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

REFERENCES

1. Shah P. The need for new therapeutic agents: what is in the pipeline? Clinical Microbiology and infection. 2005;11:36-42

2. Rajlakshmi D, Banerjee S, Sood S, Maulik S. In-vitro and in-vivo antioxidant activity of different extracts of the leaves of Clerodendron colebrookianum Walp in the rat. Journal of Pharmacy and Pharmacology, 2003; 55 (12):1681-1686

3. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. Nature reviews Drug discovery, 2004; 3 (6):509-520

4. Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z, Farah H. Antioxidant activity of some common plants. Turkish Journal of Biology 2008; 32 (1):51-55

5. Ley SV, Baxendale IR. New tools and concepts for modern organic synthesis. Nature Reviews Drug Discovery. 2002; 1 (8):573-586

6. Lombardino JG, Lowe JA. The role of the medicinal chemist in drug discovery—then and now. Nature Reviews Drug Discovery, 2004; 3 (10):853-862

7. Azadbakhta M, Safapour S, Ahmadi A, Ghasemi M, Shokrzadeh M. Anti-diabetic effects of aqueous fruits extract of Diospyros lotus L. on streptozotocin-induced diabetic rats and the possible morphologic changes in the liver, kidney and heart. Journal of Pharmacognosy and Phytotherapy 2010; 2 (2):010-016

8. Ajiboye B, Oso A, Kobomoje O. Chemical composition and nutritional evaluation of Leea guineensis seed. International Journal of Food Science, Nutrition and Dietetics. 2014; 3 (2):1-7

9. Neji P, Neji H, Ushie O, Ojong O PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF Leea guineensis.

10. Qayyum S, Basharat S, Mian AH, Qayum S, Ali M, Changsheng P, Shahzad M, Sultan F Isolation, identification and antibacterial study of pigmented bacteria. Appl Nanosci. 2020; https://doi org/101007/s1320:4-020

11. Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial susceptibility testing protocols. Crc Press, 2007.

12. Harborne J. Flavonoids. Phytochemistry. 1973; 2:344-380

13. Sofowora A. Recent trends in research into African medicinal plants. Journal of ethnopharmacology. 1993; 38 (2-3):197-208

14. Evans WC. Trease and evans' pharmacognosy E-book. Elsevier Health Sciences, 2009.

15. Justino CIL, Duarte K, Freitas AC, Duarte AC, T. R-s. Classical methodologies for preparation of extracts and fractions. Anal Mar Samples Search Bioact Compd. 2014; 65:35–57.

16. Kurnia D, Apriyanti E, Soraya C, Satari MH. Antibacterial flavonoids against oral bacteria of Enterococcus faecalis ATCC 29212 from sarang semut (Myrmecodia pendans) and its inhibitor activity against Enzyme MurA. Current Drug Discovery Technologies. 2019; 16 (3):290-296

17. Kuswandani F, Satari MH, Maskoen AM. Antimicrobial Efficacy of Myrmecodia pendens Extract and Fraction Combination against Enterococcus faecalis ATCC 29212. Journal of Dentistry Indonesia 2019; 26 (3):119-125

18. Slobodníková L, Fialová S, Rendeková K, Kováč J, Mučaji P. Antibiofilm activity of plant polyphenols. Molecules 2016; 21 (12):1717

19. Soraya C, Dharsono HDA, Aripin D, Satari MH, Kurnia D, Hilmanto D Effects of sarang semut (Myrmecodia Pendens Merr. & Perry) extracts on Enterococcus faecalis sensitivity. Dental Journal (Majalah Kedokteran Gigi). 2016; 49 (4):175-180

20. Cushnie TT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. International journal of antimicrobial agents. 2011; 38 (2):99-107

21. Thouri A, Chahdoura H, El Arem A, Hichri AO, Hassin RB, Achour L. Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). BMC complementary and alternative medicine. 2017; 17 (1):248

22. Widyawati PS, Budianta TDW, Kusuma FA, Wijaya EL Difference of solvent polarity to phytochemical content and antioxidant activity of Pluchea indicia less leaves extracts. International Journal of Pharmacognosy and Phytochemical Research. 2014; 6 (4):850-855