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Phytochemical Screening and Antiimicrobial Activity of *Leea Guineensis* Seed

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Abstract: ***The phytochemical screening and antimicrobial activity of leea guineensis seed was carried out using qualitative, quantitative and broth dilution methods. The extracts were obtained using cold and soxhlet extraction processes. The phytochemical screening revealed the presence of tannins (1.233 ± 0.06%), alkaloids (4.028 ± 0.05%), flavonoids (5.245 ± 0.02%), Phenols (6.319 ± 0.12%), steroids (8.698 ± 0.34%), saponins (2.120 ± 0.03%) and cardiac glycosides (3.247 ±0.11%). The methanolic extract of the seed exhibit a high antimicrobial potency against staphylococcus aureus with a Minimum Inhibitory Concentration of 12.5µg/ml and Minimum Bactericidal Concentration of 25µg/ml, pseudomonas aeruginosa with Minimum Inhibitory Concentration of 3.13µg/ml and Minimum Bactericidal Concentration of 6.25µg/ml and candida albicans with Minimum Inhibitory Concentration of 6.25µg/ml and Minimum Bactericidal Concentration of 12.5µg/ml. The presence of these phytochemicals makes leea guineensis seed fungistatic and bacteriostatic.***

Keywords: Phytochemical screening, antimicrobial activity, *Leea guineensis*, Fungistatic, Bacteriostatic.

1. Introduction

In recent time, plants constitute a major economic resource of most countries of the world. Plant parts such as stem, leaves, roots and seeds are employed in pharmaceutical research and in the manufacture of new drugs. Herbal medicines have become more popular in the treatment of diseases due to the belief that herbs are safer, cheaper and have fewer side effects. Medicinal plants contain organic compounds with physiological action potency on human body [1]

Studies has shown that leea guineensis leaves contain phytochemicals such as alkaloids, flavonoids, tannins, saponins and cardiac glycosides [11]. The leave extract of the plant exhibited a high efficacy against a number of microbes [11] and L. guineensis has been shown to contain significant amount of Vitamin A, C, D and E [21].

This present study is tailored to check the presence and quantity of the phytochemical constituents in the seed of leea guineensis, its antimicrobial potential and to enrich the scientific data on the phytochemistry and antimicrobial activity of leea guineensis seed.

2. Samplying and Extraction

Freshly matured reddish seeds of *Leea guineensis* were collected from Crospil Estate in Akpabuyo Local Government of Cross River State, Nigeria and were authenticated by a Herbarium, University of Calabar, Calabar. The seeds were removed from the endocarp and sun dried for three days, grinded to powdered form. Two methods of extraction were employed (Cold and Soxhlet extraction). The cold extraction was done by weighing exactly 200g of powdered sample into a container together with 250ml of the various solvents (ethanol, methanol, acetone, distilled water, n-hexane, chlorofoam and ethylacetate) used in the extraction and allowed to stand for 96 hours after which decantation was carried out with a Whatmann No.1 filter paper. The extract was concentrated by simple evaporation in a water bath at 100^0C.

Soxhlet extraction was carried out with the use of a soxhlet apparatus. 500ml of n-hexane, acetone, methanol (3:1:1) mixture were measured and poured into a round bottom flask, followed by weighing exactly 200g of powdered sample into a soxhlet extractor was then equipped with a condenser and place on a heating mantle. The solvent was heated to reflux which travels up the distillation arm and flows into the chamber containing the sample. This cycle repeats itself for over 8 hours until the extraction process was completed. During the cycle, the non-volatile compound dissolves in the solvent and after many cycles the desired compound was concentrated on the distillation flask after which the solvent was removed by rotary **e**vaporation leaving only the extracted compound.

3. Phytochemical Screening

Phytochemical examination was carried out for all the extracts using standard analytical procedures to identify the constituents of the seed. Chemical tests were carried out on both aqueous extract and the powdered sample using standard analytical methods as described by [9], [4] and [22]. The procedures are described as follows;

3.1. Test for Tannins

1.0ml of the sample extract (*leea guinensis* seed) was boiled in 100ml of water (H_20) in a test tube & then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue black precipitate which indicates the presence of Tannins [4]. The quantitative analysis was carried out using UV spectrophotometer and the result calculated thus;

$$\frac{\text{Abs. sample} - \text{Abs. Blank X Standard Conc. X 100}}{\text{Abs. Standard}}$$

3.2. Test for Alkaloids

An aliquot of aqueous extracts (2ml) was first put in a test tube and treated with 10ml of 1% HCl and heated in a water bath for 10minutes. 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second; 1 ml portion was treated with Dragendroff reagent. Turbidity or reddish brown precipitate with either of the reagent was taken as evidence for the presence of alkaloids. [9].

The quantitative analysis was carried out using UV spectrophotometer and the result calculated thus;

$$\frac{\text{Abs. sample} - \text{Abs. Blank X Standard Conc. X 100}}{\text{Abs. Standard}}$$

3.3. Test for Flavonoid

1.0ml of the extract of the sample (*leea guinensis* seed) was measured into a test tube, 1.0ml of 10% lead acetate was added and shaken for 30 seconds and left standing, formation of yellow precipitate indicted the presence of flavonoids [7]

The quantitative test was carried out using UV spectrophotometer and the result was obtained using;

$$\frac{\text{Abs. sample} - \text{Abs. Blank X Standard Conc. X 100}}{\text{Abs. Standard}}$$

3.4. Test for Saponins

1.0ml of methanolic extract of *leea guinensis* seed was boiled with 5.0ml of distilled water in test tube and the solution was shaken vigorously and observed for a stable persistent froth. Stable froth was observed for 3 minutes then mixed with 3 drops of olive oil & shaken vigorously after which the formation of an emulsion shows the presence of saponins [8].

Gravimetric method was used to analyze for the quantity of saponins present in the sample using;

$$\frac{\text{Final weight} - \text{Initial weight}}{\text{Weight of sample used}} \text{ X } 100$$

3.5. Test for Phenol

1.0ml of 10% ferric chloride was added to 1.0ml of the extracted sample and shaken. The formation of a greenish-brown coloration precipitates indicated the presence of phenol nucleus. The quantitative test was carried out using UV spectrophotometer and the result computed using

$$\frac{\text{Abs. sample} - \text{Abs. Blank X Standard Conc. X 100}}{\text{Abs. Standard}}$$

3.6. Test for Steroids (Salkowski Test)

1.0ml of the extract was dissolved in 2.0ml of chloroform in test tube before 1.0ml of concentrated H_2S0_4 was carefully added at the side of the test tube. The observance of red or reddish brown color indicates the presence of steroid nucleus in the sample [5].

The quantitative test was carried out using UV spectrophotometer and the result calculated using;

$$\frac{\text{Abs. sample} - \text{Abs. Blank X Standard Conc. X 100}}{\text{Abs. Standard}}$$

3.7. Test for Cardiac Glycoside

2ml of aqueous extracts (water and petroleum ether) were separately dissolved in 2ml of chloroform. Conc. H_2SO_4 was carefully added to form a colored layer. A brown ring obtained at the interface indicated the presence of a deoxy sugar, a characteristics of cardiac glycosides [9].

Keller – Kition Test was used for the quantitative analysis;

$$\frac{\text{Final wt} - \text{Initial wt}}{\text{Wt of sample used}} \text{ X } 100$$

4. Antimicrobial Activity of Leea Guinensis Seed

The methanol, acetone & n-hexane extracts were used for this analysis because they gave higher concentration of phytochemicals. The microbes used were *staphylococcus aureus*, *pseudomonas aeruginosa* and *candida albicans* with amphoteric B and amphilicin drugs gotten from the Teaching Hospital of University of Calabar, Calabar.

4.1. Preparation of the Whatsman Filter Paper Discs

The drug was first diluted by placing 1ml (100µg) in 5ml of distilled water. Next a 1:9 dilution was carried out to reduce its concentration to 100µg/ml. Then a double dilution was carried out by placing 2ml of 100µg/ml dilution in the first tube while each of the other 3 tubes were severed with 1ml each of distilled water, subsequently 1ml was transferred from tube 1 to 2, 1ml from tube 2 to tube 3, and finally 1ml from tube four, giving concentrations of 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml respectively. Each transfer was followed by thorough and gentle shaking to allow for homogeneous mixing of contents. Next filter paper discs already prepared were suspended or soaked in each of the tubes and allowed to stay for 24-48hours, to enable the discs absorb the ingredients of the drug. At the end of this treatment the discs were brought out on trays and dried in the oven at 60^0C for 10 minutes. After the drying, they were brought out of the oven, into their respective sterile container. The discs were then transferred into already prepared seeded agar plates for the sensitivity test.

4.2. Cultivation of Fungal Isolates:

7.8g of the dextrose agar power was weighed using a digital weighing balance. Next the agar was dissolved in 200ml distilled water contained in a 500ml flat bottom flask. The contents of the flask were shaken continuously for the agar to dissolve properly. Then the flask and its contents were further placed in a water bath maintained at

100^0C for 30 minutes with vigorous shaking intermittently to enable the agar dissolve completely in the solution. Finally the flask and its content were sterilized in the autoclave at 15 pounds per square inch (PPI) giving a temperature equivalence of 121^0C for 15 minutes. After sterilization, the flask was removed from the autoclave and allowed to stand for 30-45 minutes in order for the agar to cool. As the agar cooled, nine well labeled Petri-dishes were served with 20ml each of the agar and the plates with their lids in place were allowed to cool further for 30 minutes in order to solidify. After solidification of the agar, with the help of a sterile forceps, grown mycelia of the fungal isolates were transferred from stale food on to the agar plates and then incubated at room temperature for 1-5 days. At the end of incubation, the isolates were identified using 1% Sodium Hydroxide solution or Lactophenol blue.

After the preparation of the Whatsman filter paper discs, the identification of the isolates, the susceptibility test was carried out using broth dilution methods.

5. Results and Discussion

Table 1 shows result of qualitative phytochemical analysis of *Lees guineensis* seed. From the result obtained, tannins were detected in all the extract except that of water, n-hexane and chloroform. It was also detected in the leaves [10] and stem bark [11] of this same plant. Alkaloids were found in all the extracts except that of ethylacetate and chlorform. This phytochemical was also detected in the stem bark [11] and the leaves [10] of this same plant. Alkaloids have been adjudged the most potent and therapeutically important of all substances extracted from plants and this may explain its use in fighting stomach ache, dysentery, diarrhea, vomiting, constipation and intestinal worms, [12]. Flavonoids were detected in all the extracts which corresponds with the work of [11] for *leea guinensis* stem bark and [10] for the leaves. Flavonoids are a large group of naturally occurring phenols. This phytochemical is not without pharmaceutical interest and remains a current area of research [4] Saponins were detected in all the extracts except in that of chloroform, this agrees with the work done by [11] on the stem bark of *leea* guinensis and [10] on the leaves. Saponins have been reported to have anti-inflammatory and cardiac depressant properties [13] and seemingly inhabit growth of carcinogenic cells but without necessary killing the normal cells in the process [14]. Phenols were detected in all the extract except in n-hexane and chlorofoam. Phenol possesses antibacteriocidal and antimicrobial properties which are known to exert preventive activity against infectious and degenerative diseases, inflammation and allergies through antioxidant, antimicrobial and proteins. Steroids were detected in all the extracts; this corresponds with the work done by [10] on the leaves. Steroids have great importance in the pharmaceutical industry due to its relationship with such compounds as sex hormones [15]. Cardiac glycosides were found in all the extracts which agree with the work done by [11] on the stem bark of *leea* guinensis. Their chemical effects in cases of congestive heart failure have been reported and the potency against cardiac arrest is demonstrated by acting on the heart muscles as well as increases the renal flow [16]

Table 1: Results of Qualitative Phytochemical analysis of *Leea guineensis* seed Extracts using various solvents (Cold Extraction)

S/no	Reagents	Tannins	Alkaloid	Flavonoids	Saponins	Phenol	Steriods	Cardiac glycosides
1.	Ethanol	+	+	++	+	+	+	++
2.	Methanol	+	+	+	+++	+	+	+
3.	Water	-	+	+	++	+	+	+
4.	Acetone	+	+	+	++	+	++	+
5.	Ethylacetate	+	-	+	++	+	+	+
6.	n-Hexane	-	+	+	++	-	+	+
7.	Chloroform	-	-	+	-	-	+	+

Where; – Represent Absence, + Represent Low Concentration, ++ Represent Moderate Concentration and +++ Represent High Concentration

Table 2: Results of quantitative phytochemical screening of Leea guineensis seed Extracts Using hexane/Acetone/Methanol Mixture (Soxhlet Extraction)

S/No	Solvent	Tannins (%)	Alkaloid (%)	Flavonoid (%)	Phenols (%)	Steriods (%)
1.	Methanol	1.066 ± 0.05	1.662 ± 0.02	3.768 ± 0.12	2.417± 0.08	4.350 ± 0.07
2.	Acetone	1.233 ± 0.06	1.310 ± 0.06	0.416 ± 0.01	6.319± 0.12	8.698 ± 0.34
3.	Hexane	0.961 ± 0.02	4.028 ± 0.05	5.245 ± 0.02	2.591± 0.06	5.395 ± 0.11

Table 3: Saponins and Cardiac Glycosides Content Determination of *Leea guineensis* seed Using Gravimetric Method

S/No	Saponins (%)	Cardiac glycoside (%)
1	2.120 ± 0.03	3.247 ± 0.11

Table 2 and 3 shows the quantitative phytochemical analysis of *Leea guineensis* seed using UV spectrophotometer and Gravimetric method respectively. From the results obtained, tannins content using acetone gave a higher yield of 1.233 ± 0.06% which close to 5.81% of the leaves [10] but lower than 10.19% of *chysophyllum albidun* [17] and 26.57% of *elaeis guinensis* leaves [18]. The alkaloid in *leea guinensis* seed was higher using the combination of three solvents (n-hexane, acetone and methanol) giving 4.028 ± 0.05% which agrees favorably with 5.81 of the leaves [10] but higher than 0.81 of the leaves of *elaeis guinesis* [18] and lower than 25.80

of *chrysophyllum albidun* fruits [17]. The flavonoids content is higher in n-hexane extract (5.245 ±0.02) but comparatively lower than 15.11 ± 0.07% of *chysophyllum albidun* fruits [17] and 41.38 of *elaeis guinensis* leaves [18] however, it is higher than 0.36 of *leea guinensis* leaves [10]. The Saponin content was 2.120 ± 0.03% which is closely related to 3.86% of the leaves of *elaeis gunensis* [18] and higher than 0.09% of *chrysophyllum albidun* fruits [17]. This compound has been reported to have autohyper-cholesterol, anti-inflammatory, cardiac depressant properties [6]. The acetone extract also gave a higher content of 6.319 ± 0.12% of phenol which is lower when compared with 11.28% of the leaves of *elaeis guinensis* [18]. The acetone extract gave a higher yield of 8.698 ±0.34% of steroid. Steroids are of great importance in the pharmaceutical industry due to their relationship with such compounds such as sex hormones [15]. The cardiac glycoside content was 3.247±0.11%. [10] And [11] identify it in the leaves and stem bark respectively but did not quantify it. This compound has been used for over two centuries as stimulant in cases of cardiac failure and disease [19] & [20].

Table 4: Antimicrobial Susceptibility Test of Metholic Extract of *Leea Guinensis* Seed

Isolates	Concentration of the drugs Control						
	100µg/ml	50µg/ml	25µg/ml	15.67mm	6.25µg/ml	3.13µg/ml	10µg/ml
SA	15.67mm	16.00mm	13.93mm	9.33 mm	13.33 mm	13.00 mm	≥ 10mm
PA	43.00 mm	38.67mm	37.00mm	33.33 mm	37.33 mm	18.67 mm	≥ 14mm
CA	20.00 mm	24.67mm	23.33mm	19.33 mm	12.33 mm	13.67 mm	≥15mm

Where SA = *Staphylococus aureus*, PA = *Pseudomonas aeruginosa* and CA = *Candida albicans*

Table 5: MIC, MBC/MFC of Isolates

ISOLATES	MIC (µg/ml)	MBC/MFC (µg/ml)
Staphylococus aureus	12.5	25
Pseudomonas aeruginosa	3.13	6.25
Candida albicans	6.25	12.5

Where MIC represents Minimum Inhibitory Concentration, MBC represent Minimum Bactericidal Concentration and MFC represent Minimum Fungicidal Concentration.

Table 4 and 5, shows result of methanol extract of *leea guinensis* seed on *stayphylococus aureus*, *Pseudomonas aeruginosa* and *Candida albicans.* The extract exhibit some level of sensitivity against this microbes with a Minimum Bacteriocidal Concentration (MBC) of 25µg/ml and Minimum Inhibitory Concentration (MIC) of 12.5µg/ml giving a ratio of above 1:1 for MBC:MIC making the seed bacteriosatic and bactericidal since it has the ability to inhibit and kill microorganisms. This implies that the seed can be used in the treatment of staphylococcus and other related bacteria. The potency of this seed agrees with the stem bark recorded by [11]. Also the use of the extract on *Pseudomonas aeruginosa* shows high appreciable sensitivity against this microbe with a Minimum Bacteriocidal Concentration (MBC) of 6.25µg/ml and Minimum Inhibitory Concentration (MIC) of 3.13µg/ml. This implies that the ratio of MBC: MIC is 2:1 which makes the seed bacteriosatic and bactericidal. This indicates that *leea guinensis* seed could be used in the treatment of urinary tract infection, respiratory system infection, joint infection etc. This result agrees with that of the stem bark done by [11]. The extract was also used on *candida albicans* and was sensitive giving a Minimum Fungicidal Concentration (MFC) of 12.5µg/ml and Minimum Inhibitory Concentration (MIC) of 6.25µg/ml giving a ratio of above 1:1 for MFC: MIC making *leea guinensis* seed fungistatic and fungicidal. From the analysis, *leea guinensis* seed could be used in the treatment of *candidiasis* and other related fungi and preservation of food just like *solanum lycopersicium* [11].

6. Conclusion

From the study, it was revealed that *leea guinensis* seed contains some phytochemical namely, flavonoid, alkaloid, saponin, tannins, phenol, steroid and cardiac glycosides which makes it resistance to some bacteria and fungi similar to that of the leaves and stem bark and can be used in the treatment of *staphylococcus, candidiasis* and other related diseases.

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