***Original Research Article***

# Extraction of Phytochemical Compounds of *Leea guineensis* (G. Don) leaves using Non-Polar and Polar solvents


## Abstract

**Aims:** Selection of a suitable solvent is important and utilized in the extraction of desirable chemical components in medicinal plants.

**Study design:** Chemical analysis of various extracts of *Leea guineensis* leaves using standard analytical procedures.

**Place and duration of study:** Forestry Research Institute of Nigeria, between March 2019 and August 2019.

**Methodology:** Leaves of *Leea guineensis* were extracted with six solvents categorized into polar (Acetone, Methanol and Aqueous) and non-polar (Ethyl acetate, Hexane and Chloroform) types using cold maceration method, the qualitative and quantitative phytochemical assay was done on the respective extracts using the standard methods.

**Results:** Phytochemical screening of the non-polar solvent extract revealed the presence of flavonoids, alkaloids and saponins for all the solvents, while tannin was present only in ethyl acetate extract. For the polar solvent extracts, all the secondary metabolites determined were present except terpenoids and phlobatanins. In the quantitative test, alkaloid levels ranged from 1.31-38.25 mg/100g, saponin: 2.01-14.35 mg/100g, flavonoids: 1.10-6.25 mg/100g, Tannin: ND-4.62 mg/100g, terpenoids: ND-1.02 mg/100g, cardiac glycosides: ND-0.84 mg/100g, Anthraquinone: ND-2.58 mg/100g and phlobatanins: ND-0.95. The results obtained for each of the phytochemicals are significantly different ($p<0.05$) across all the solvent extracts, while phytochemicals such as terpenoids, cardiac glycosides, anthraquinones and phlobatanins were not detected in the non-polar solvent extract of *L. guineensis*.

**Conclusion:** The phytochemical constituents detected in varying quantities depend on the polarity of the substances, *L. guineensis* could be exploited and extracted very well using a polar solvent like methanol, acetone and aqueous.

Key Words: *Leea guineensis,* Non-polar Solvent, Phytochemicals, Polar Solvent

## 1. INTRODUCTION

Phytochemicals also known as phytonutrients are naturally occurring substances found in plant [1]. Any plant where one or more of its part contains substances that can be utilized for medicinal purposes or serves as starting materials for the creation of functional medication is called medicinal plant [2]. Medicinal plants are considered as such when they have been purported and established to have restorative action [3]. The bioactive substances which are in a specific part of plants are synthesized as secondary metabolites; every living thing, from one cell bacterium to million cell plants, possesses diverse chemical compounds for their survival and subsistence. Secondary metabolites describe compound class other than primary metabolites reputed to help the plant to increase their general capacity to survive and surmount environmental challenges by allowing them to relate with their environment. They are made by plants majorly as a result of primary metabolism and also as part of their innate mechanism for defence, phytochemicals such as tannins, flavonoids and alkaloids are some of the secondary metabolites produced by plants and from which medicinal plants obtained their therapeutic activities [4]. Individual need of various species, as well as evolutional adaptation needs determines the production of secondary metabolites in different species; these substances have found use among humans as flavours, medicines and recreational drugs. Therefore, the processing of raw plant materials for phytochemical extraction is needed to enhance their levels and also to preserve their activities [5]. Extraction is a vital activity in the process of phytochemical isolation for the detection of pharmacologically active components in plant materials [6, 7]. The selection of an appropriate solvent system for extraction is vital for medicinal product standardization as it is used in the isolation of the required constituents while excluding the unwanted matrix. *Leea guineensis* commonly called Red tree vine is an evergreen shrub or small tree that belongs to the family Leeaceae. It is locally called Alugbokita, Sasamura among the Yoruba people and usually propagated by seed or stem cutting; the seed germinates in 14-21 days at 70° F and can grow up to 20ft high; the plant is widely distributed in moist, intermediate temperate zones in tropical Africa [8]. The plant is reported to exhibit potential in-vivo anti-tumour and antioxidant activity [9]. It is also used in the treatment of toothache, gonorrhoea, general weakness, diarrhoea, skin rash, paralysis, spasm, ulcer, epileptic fits, dysentery, general weakness, as a purgative, as a diuretic, as a pain killer and a host of other ailments [10]. Some medicinal plants including *L. guineensis* exploited for medicinal purposes have to undergo phytochemical screening and bioassay as steps towards drug developments [11]. Therefore, the objective of this study is to comparatively assess the extraction efficiency of six solvents type in terms of the qualitative and quantitative phytochemical assay with a view to provide information on the best solvent type for the extraction of phytocompounds from the leaves of *L. guineensis*, a Nigerian medicinal plant.

## 2. MATERIALS AND METHODS

### 2. 1 Identification, Authentication and Plant Sample Preparation

Fresh samples of *L. guineensis* were collected from the herbal garden of Forestry Research Institute of Nigeria, the plant samples were identified and authenticated at the taxonomy unit of the institute and a voucher specimen (FHI 112460) was deposited at the forest herbarium Ibadan. The leave samples were cleaned and washed with water, air-dried on a cabinet drier at room temperature, it was then pulverized using a milling machine. The powdered sample was preserved in a clean airtight containers, kept away from light, heat and moisture until further use.

### 2.2 Method of Extraction

The leave extract was obtained by maceration. 50g of the plant sample was soaked with 250ml of each solvent – Methanol (95%, b. pt 64.6 $^{o}$C), Acetone (99.5%, b. pt 56.2 $^{o}$C), Ethylacetate (99.5%, b. pt 77 $^{o}$C), Hexane (99.0%, b. pt 69 $^{o}$C), Chloroform (98.5%, b. pt 61 .2 $^{o}$C) - for three days during which time, it was agitated on a mechanical shaker at 220 rpm, then, the resulting mixture was filtered and the filtrate concentrated under vacuum using the rotary evaporator, and the crude extract recovered in a petri dish, it was then kept in a desiccator at room temperature to remove residual solvent [12].

### 2.3 Phytochemical Assay

Qualitative phytochemical screening of the leave extracts of the respective polar and non-polar solvent was determined using the standard methods described by Boye *et al.,* [13] and Omoruyi *et al.,* [14], while the quantitative phytochemical assay was equally carried out for all the crude extracts obtained from each solvent using standard procedures described by Ushie *et al.,* [15]. The phytochemical constituents determined are Alkaloids, Saponin, Flavonoids, Tannin, Terpenoids, Cardiac glycosides, Anthraquinone and Phlobatanins.

### 2.4 Statistical analysis

Quantitative data were expressed as Mean ±SD of triplicate measurement; analysis of variance (ANOVA) was used to test significant difference between the mean of phytochemicals from each extract, while specific differences were identified using Duncan Multiple Range Test, where $p<0.05$ was considered significant. IBM SPSS version 20 was used for the statistical analysis.

## 3. RESULTS AND DISCUSSION

Table 1. shows the results of the qualitative phytochemical screening of the crude extract of *Leea guineensis* leaves using non-polar (Ethyl Acetate, Chloroform and Hexane) and polar (Methanol, Acetone and Aqueous) solvents. Extracting adequate quantities of chemical compounds rely majorly on the type of solvent used during the extraction process; during this process, the solvents percolate into the matrix of the plant material where phytochemicals that are of the same polarity with the solvent are dissolved. The phytochemical screening shows the presence and absence of some phytochemicals determined. Alkaloids, Saponin, Flavonoids are present in all the non-polar solvents, with tannin also present in the ethyl acetate crude extract solvent and absent in both chloroform and hexane. However, phytochemicals such as terpenoids, cardiac glycosides, anthraquinoone, phlobatanins were absent in the screening test of the non-polar extracts. Thus, ethylacetate, chloroform and hexane extracts of *L. guineensis* were good sources of alkaloids, saponin and flavonoids. This may suggest that these solvents are selective in the isolation of bioactive compounds due to their non-polar nature. In the polar solvents, the presence of all the bioactive compounds evaluated except for Terpenoids and Phlobatanins that was absent in the acetone extract was observed in the phytochemical screening. This same trend was previously reported for aqueous extract of *L. guineensis* [16]. Neji *et al.,* [17] also reported variation in the phytochemical screening of different solvent extracts of *L. guineensis* stem bark where like this study, the presence of alkaloid, tannin, flavonoids and saponin were detected in the hexane extract while in contrast, only flavonoids and cardiac glycoside were present chloroform extract. Gul *et al.,* [18] reported the presence of alkaloid, cardiac glycosides and flavonoids in the methanolic extract of the leaves of *E. intermedia*, while saponin and tannin were not indicated in the extract. Saponin, flavonoids, cardiac glycosides, terpenoids and tannin were extracted from the aqueous extract of *Tulbaghia violacea* leaves as reported by Madike *et al.,* [19]. The trend observed in this study is similar to that observed in the study by Senguttuvan *et al.*, [20], where methanolic extract of *Hypochaeris radicata* leaves contain all the bioactive compounds. Alkaloids was observed to be present in the crude extract of all the non-polar solvent; it has been reported for its analgesic, antispasmodic and bactericidal, and antimalarial activities [21, 22]. Flavonoids were detected in all the non-polar solvents, which is following the same observation in the result obtained by Khanam *et al.,* [23] on the stem and root of *E. longifolia* using ethylacetate, chloroform and methanol as solvent. Flavonoids belong to the group of polyphenolic compounds and are characteristically recognized for health promoting activities such as anti-inflammatory, anti-cancer, anti-allergic, antioxidant, and antimicrobial properties [24]. They are commonly found in many plants; a positive correlation between ingestion of plants rich in flavonoids and reduced risk of cardiovascular diseases and cancer have been reported [25].

**Table 1. Results of Phytochemical Screening of Non-Polar and Polar Solvent Crude Extract of *L. guineensis***

| Phytochemicals | Non-Polar Solvents | | | Polar Solvents | | |
|---|---|---|---|---|---|---|
| | Ethyl Acetate | Chloroform | Hexane | Methanol | Acetone | Aqueous (Water) |
| Alkaloids | + | + | + | + | + | + |
| Saponin | + | + | + | + | + | + |
| Flavonoids | + | + | + | + | + | + |
| Tannin | + | - | - | + | + | + |
| Terpenoids | - | - | - | + | - | + |
| Cardiac glycosides | - | - | - | + | + | + |
| Anthraquinone | - | - | - | + | + | + |
| Phlobatanins | - | - | - | + | - | + |

$^{+}$ Present, $^{-}$ Absent

Table 2. presents the result obtained for the quantitative phytochemical composition of extracts using polar and non-polar solvents. Alkaloid levels ranged from 1.31-38.25 mg/100g, saponin: 2.01-14.35 mg/100g, flavonoids: 1.10-6.25 mg/100g, Tannin: ND-4.62 mg/100g, terpenoids: ND-1.02 mg/100g, cardiac glycosides: ND-0.84 mg/100g, Anthraquinone: ND-2.58 mg/100g and phlobatanins: ND-0.95. Ethyl acetate, Chloroform and Hexane successfully extracted little quantity of alkaloids, saponin and flavonoids in varying proportions. The results obtained for each of the phytochemicals are significantly different ($p<0.05$) across all the solvent extracts, while phytochemicals such as terpenoids, cardiac glycosides, anthraquinones and phlobatanins were not detected in the non-polar solvent extract of *L. guineensis*.

**Table 2. Results of Quantitative Phytochemical Composition of Non-Polar and Polar Solvent Crude Extract of *L. guineensis***

| Phytochemicals (mg/100g) | Non-Polar Solvents | | | Polar Solvents | | |
|---|---|---|---|---|---|---|
| | Ethyl Acetate | Chloroform | Hexane | Methanol | Acetone | Aqueous(Water) |
| Alkaloids | 2.71±0.15a | 1.31±0.55a | 1.79±0.41a | 38.25±1.67c | 22.28±01.23b | 20.81±1.12b |
| Saponin | 3.83±0.64a | 2.01±0.62a | 2.54±0.71a | 12.09±0.99bc | 10.94±0.73b | 14.37±0.55c |
| Flavonoids | 2.91±0.61a | 1.10±0.72a | 2.00±0.02a | 6.25±0.61b | 5.62±0.91b | 5.02±0.34b |
| Tannin | 1.82±0.81a | ND | ND | 4.62±0.24b | 3.02±0.31b | 3.55±0.51b |
| Terpenoids | ND | ND | ND | 1.02±0.02a | ND | 0.95±0.08a |
| Cardiac glycosides | ND | ND | ND | 0.71±0.03a | 0.84±0.07a | 0.72±0.09a |
| Anthraquinone | ND | ND | ND | 1.53±0.78a | 1.50±0.90a | 2.58±0.93b |
| Phlobatanins | ND | ND | ND | 0.09±0.006a | ND | 0.95±0.67a |

ND = Not detected. Values are expressed as mean and SD of triplicate measurements. Means with the same alphabets in the same row are not significantly different at $p<0.05$

The strength of medicinal plants is ascribed to the activity of the phytochemicals present in them [26]. These substances, some of which are biologically active compounds usually occur in low concentration in plants, therefore, an extraction system that is able to obtain extract with a high yield of phytochemicals and also, with minimal changes to the inherent functional properties is required [27]. Several studies have reported variations in the phytochemical composition as well as the biological activities of extracts

prepared using different extraction solvents [28-31], therefore, selection of appropriate solvent for extraction of phytochemicals from the plant is very essential based on some characteristics such as chemical properties of the analytes, matrix analyte interaction, sample matrix properties, efficiency and desired properties [32-33]. The phytochemical evaluation of *L. guineensis* extracted using different solvents showed that alkaloids, flavonoids, saponin, tannins, anthraquinone, cardiac glycosides are present in all the leaf extracts of the polar solvents, except for terpenoids and Phlobatanins that was not detected sufficiently in the acetone extract. These classes of secondary metabolites are known to show medicinal activity, against several organisms and it is not surprising that these plant extracts are used traditionally to cure bacteria related ill-health among other common diseases [34-35]. Compounds such as flavonoids, saponins and tannins have been shown to have therapeutic properties against most disease-causing organisms [23]. The properties elicited by these compounds include antioxidant activity, anti-allergic, anti-inflammatory and many others. All the polar solvents were able to extract the phytochemical contents present in the leaves of *L. guineensis*. Flavonoid compounds found in plants have antioxidant powers that may provide important health benefits. Consuming medicinal plants rich in flavonoids have been associated with reduced risk of a variety of diseases. The presence of flavonoids in all the extracts mirrors that observed in the study by Garg and Garg, [36] where its presence was reported in both the chloroform and methanol extract of *T. cordifolia*; as well as in the chloroform and ethylacetate extracts of *Boerhaavia diffusa, Terminalia bellerica, Tribulus terrestris* [37]. On the contrary, Gurupriya *et al*., [38] reported its total absence in all the non-polar solvents extracts of *Simarouba Glauca*. There was a significant difference ($P<0.05$) in the mean phytochemicals of all the solvent extracts. Among the polar solvent, methanol was found to extract higher concentration of flavonoid compound. Comparable levels of phytochemicals ($P<0.05$) are obtained among the three non-polar solvent extracts and also among the three polar extracts of *L. guineensis*. Plants produce saponins to fight infection by parasites. When ingested by humans, saponins also seem to help our immune system and to protect against viruses and bacteria. Some studies have shown that saponins can cause apoptosis of leukaemia cells by inducing mitotic arrest. Saponin was detected and quantified in all the solvents both non-polar and polar, while on the contrary, it was reportedly present only in the aqueous (Water) and methanol extract in the work reported by Ibrahim *et al.,* [39] for *Mimosa pudica*. For the quantitative analysis, the saponin content obtained in this study was significantly different ($P<0.05$) for both non-polar solvents: ethylacetate, chloroform and hexane (3.83, 2.01 and 2.54 mg/100g respectively) and polar; Methanol, Acetone, Aqueous (12.09, 10.94, 14.37 mg/100g respectively) solvents for all the phytochemical constituents. The result shows that the Aqueous extract of the leaf (14.37 mg/100g) had the highest quantity of saponin, followed by Methanol (12.09 mg/100g) extract while chloroform (2.01 mg/100g) had the least phytochemical content. Saponins in seeds have been known to possess both beneficial and deleterious properties depending on its concentration in the sample [40]. The result indicated for alkaloid compound (38.25 mg/100g) by methanol solvent is very high compared to other polar solvents, they are all significantly different ($P<0.05$) from each other. The values reported for tannin in this study for the

polar solvents Methanol (4.62 mg/100g), Acetone (3.02 mg/100g), Aqueous (3.55 mg/100g), which is comparably higher than that reported by Senguttuvan *et al.*, [20] for the leaves of *Hypochaeris radicata*. It has been reported that tannins possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorated inflamed mucous membrane. They have important roles such as stable and potent anti-oxidants [41]. In summary, investigations on the phytochemical constituents of methanol, acetone and aqueous and the non-polar solvent extracts of leaves of *L. guineensis* revealed the presence of alkaloids, flavonoids, saponin, anthraquinone, phlobatanins, cardiac glycosides, and tannins in all extracts. These compounds are described as potent biologically active compounds found in medicinal plants parts which are precursors for clinically useful drugs.

## 4. CONCLUSION

The presence of bioactive compounds and the quantitative determination of the phytochemical constituents of different solvent studied showed that the leaf is rich in alkaloids, tannins, flavonoids, saponins, anthraquinone, phlobatanins, cardiac glycosides and terpenoids; this enables the plant to have the tendency to be useful in the treatment of varieties of ailments traditionally and can also be used as precursors in the manufacturing of new drugs for treatment of various diseases. Results from these studies also suggest that the efficiency of extraction for different phytochemicals may not only depend on the solvent type but also, it is dependent on the type of plant. This position was arrived going by the variation observed in these studies and other similar studies using different plants. However, *L. guineensis* could be exploited and extracted very well using a polar solvent like methanol, acetone and aqueous (Water).

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