Phytochemical and Phytomineral Status of Spigelia anthelmia Linn Leaves

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Authors’ contributions
This work was carried out in collaboration among all authors. Author OLA designed the work and conduct the laboratory analysis. Author POO performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OSA managed some of the analyses of the study. Author EPC managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aims: Spigelia anthelmia is a medicinal plant used in the treatment of diverse diseases and contain plant-based natural bio-active constituents.

Study Design: Phytochemical and mineral analysis of Spigelia anthelmia leaves using standard analytical procedures.

Place and Duration of Study: Forestry Research Institute of Nigeria, between May 2019 and July 2019.

Methodology: Phytochemical and phytomineral status of Spigelia anthelmia leaves were investigated using standard analytical procedures. Phytochemicals screening/analysis examined include; alkaloids, flavonoids, saponin, tannin, phenolics, cardiac glycosides, phlobatannin and terpenoids. The atomic absorption spectrophotometer (AAS) was used to determine the minerals which are calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu), while flame photometer was used in determining potassium (K) and sodium (Na). Data were presented using descriptive statistics (mean and standard deviation).

Results: Result indicates the presence of phytochemicals in the leaves of S. anthelmia, the quantitative analysis shows: alkaloid (2.34mg/100g), flavonoid (6.13mg/100g), saponin...
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(18.12mg/100g), tannin (9.61mg/100g), phenol (4.61mg/100g), cardiac glycosides (1.89mg/100g), terpenoids (0.98mg/100g) and phlobatannin (0.32mg/100g). The macro elements reveals Na (0.022%), K (0.23%), Ca (0.48%), Mg (0.15%), P (0.035%), while the micro element shows that Fe (0.19%), Mn (0.003%), Cu (0.001%), Zn (0.004%).

Conclusion: The result suggests that S. anthelmia has a strong pharmacological prowess and could be used in the prevention and treatment of various health-related diseases.

Keywords: Bioactive; constituents; phytochemical; Spigelia anthelmia; oxidative.

1. INTRODUCTION

Herbal medicine is the use of herbs for their therapeutic or medicinal value [1]. The medicinal and pharmaceutical properties of medicinal plants are due to the type of chemical substance they produce and possess. These include primary metabolites that are utilized as food and give nutritional benefits and also secondary metabolites that exert physiological effects and give therapeutic benefits to plants. The term used for different chemical substances is called "constituents". The plant’s constituents that have pharmacological properties are "active constituents". The chemical constituents possessed by plants is very essential and widely studied because it helps in the production of most drugs used as medicine [2]. Secondary metabolites are the chemical components that are of medicinal benefits. The analysis of the plants chemical constituents can reveal the accumulation of specific compounds in some plants organ. The abundance and deficiency of such constituents largely depend on the specificity of plants, the extent of accumulation and the analytical method employed [3]. Phytochemicals are large chemical compounds naturally derived from plants, vegetables and fruits and are responsible for disease protection [4]. Based on their chemical structure, phytochemicals can fall into the following groups; flavonoids, saponin, alkaloids, tannin, steroids, phenols, terpenoids, cardiac glycosides [5]. Spigelia anthelmia is an annual weed that belongs to the genus of flowering plants in the Loganiaceae family. It is also known as pinkroot. It has a small purple flower with a feather lobed leaves [6]. The plant is mostly used for the expulsion of tapeworm and roundworm in the body. Also, it is used to treat people with chronic catarh, difficulty in breathing, headache, throbbing pain, vermifuge and common cold [7]; Previous studies on different solvent extracts of S. anthelmia revealed the presence of varying presence of phytochemicals [8]. Studies on plant-based chemicals such as phytochemicals and minerals present in S. anthelmia, some of which have been demonstrated to possess health-boosting properties. However little study has been done reporting the mineral status of S. anthelmia, and in view of this, this study aims at reporting the phytochemical status (both qualitative and quantitative) and the phytomineral composition of S. anthelmia.

2. METHODOLOGY

2.1 Collection and Preparation of Plant Materials

The leaf samples of S. anthelmia were collected within the premises of Forestry Research Institute of Nigeria, identified and authenticated by a taxonomist at the Forest Herbarium Ibadan and a voucher specimen (FHI 112462) was deposited. The leaves were air-dried at room temperature after which it was milled to powdered using a milling machine (Arthur H. Thomas Co. Phila, P.A. S.A). The fine powder was then packed into a tight container and kept for further use.

Fig. 1. Spigelia anthelmia leaves

2.2 Phytochemical Evaluation

Qualitative screening and Quantitative phytochemical analysis were done to identify and quantify the chemical components present in the leaves of S. anthelmia. The bioactive constituents assayed includes; alkaloids,
saponin, flavonoids, tannin, phenolics, terpenoids, cardiac glycosides and phlobatannin. The phytochemical screening was evaluated on the powdered sample using simple standard procedures as described by Harborne [3], while the quantitative phytochemical analysis was done according to the methods described by Mbaebie [9] using spectrophotometric and gravimetric principle.

2.3 Mineral Evaluation

1 g of the powdered sample was weighed in porcelain crucibles which were ignited in a muffle furnace at 550°C. The ash was dissolved in 3 ml of 3 M Hydrochloric (HCl) acid, transferred to 100 ml volumetric flask and diluted to the 100 ml mark. Potassium and sodium were analyzed using Flame Photometer while, iron, zinc, copper, manganese, and magnesium were analyzed using a Buck Scientific 210 atomic absorption spectrophotometer, phosphorus was determined using UV-Vis Spectrophotometer [10].

2.4 Data Analysis

Quantitative data were expressed as Mean ±SD of triplicate measurement; analysis of variance (ANOVA) was used to detect significant difference between mean of measured parameters of different species, while specific differences were identified using Least Significant Difference (LSD) statistical test at 5% level of probability. SPSS version 20 was used for the statistical analysis.

3. RESULTS

3.1 Qualitative Screening

The result presented in Table 1 shows the phytochemical screening of S. anthelmia. The result reveals the presence of a variety of secondary metabolites like saponin, tannin, flavonoid, terpenoids, phenols, alkaloids, phlobatannin and cardiac glycosides. The indication of these phytochemicals suggests that the plant S. anthelmia is a very potent plant that could be used in the treatment and prevention of various diseases.

3.2 Quantitative Analysis

The quantitative phytochemical analysis of S. anthelmia, as expressed in Table 2 reveals the levels of phytochemicals present in the leaves of S. anthelmia. The saponin content obtained in the study was the highest with a value of (18.12mg/100g), followed by tannin (9.61 mg/100g), flavonoid (6.13mg/100g), Phenol (4.16mg/100g), Alkaloids (2.34mg/100g), Cardiac glycosides (1.89 mg/100g), Phlobatannin (0.32mg/100g) and Terpenoids (0.98mg/100g). Phytochemicals usually possess pharmacological activities that can reduce or prevent the risk of human diseases.

3.3 Mineral Composition

Table 3 presents the result obtained for mineral analysis of the leaves of S. anthelmia.

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Table 1. Phytochemical screening of the leaves of Spigelia anthelmia

| Phytochemical test                          | Observation         | Inference |
|---------------------------------------------|---------------------|-----------|
| Saponins (Frothing test)                    | Persistence foaming | +         |
| Tannins (Ferric chloride)                   | Greenish-brown precipitate | + |
| Flavonoids (Ethyl-acetate test)             | Intense yellow coloration | + |
| Terpenoids (Chloroform and acetic anhydride test) | A grey coloration  | +         |
| Phenols (Distilled water test)              | Blue-Greenish coloration | +       |
| Alkaloid                                    |                      |           |
| • Dragendorff’s reagent                     | Red precipitate     | +         |
| • Mayer’s reagent                           | Creamy-white precipitate | + |
| • Wagners reagent                           | Reddish-brown precipitate | + |
| Cardiac Glycoside (Sulphuric acid test)     | Brick-red precipitate | +         |
| Phlobatannin                                | Red precipitate     | +         |

Source: Original + Present, - Absent
Higher amounts were obtained for the macro elements, while lower amounts were indicated for micro-elements. The highest concentrations for macro-elements were observed for Calcium (2.48%), Magnesium (0.41%) and potassium (0.29%). While the highest concentration reported for micro-elements was Iron (Fe).

**Table 2. Phytochemical analysis of the leaves of S. anthelmia**

| Phytochemical        | Concentration (mg/100g) |
|----------------------|-------------------------|
| Alkaloid             | 2.34±0.21               |
| Flavonoid            | 6.13±1.08               |
| Saponin              | 18.12±1.06              |
| Tannin               | 9.61±0.45               |
| Phenol               | 4.61±0.01               |
| Cardiac glycosides   | 1.89±0.23               |
| Terpenoids           | 0.98±0.51               |
| Phlobatannin         | 0.32±0.27               |

Source: Original Mean ±SD, n=3

**Table 3. Mineral analysis of S. anthelmia leaves**

| Minerals          | Concentration (%) |
|-------------------|-------------------|
| Sodium (Na)       | 0.022±0.03        |
| Potassium (K)     | 0.23±0.12         |
| Calcium (Ca)      | 0.48±0.01         |
| Magnesium (Mg)    | 0.15±0.02         |
| Phosphorus (P)    | 0.035±0.23        |
| Iron (Fe)         | 0.19±0.02         |
| Manganese (Mn)    | 0.003±0.02        |
| Copper (Cu)       | 0.001±0.01        |
| Zinc (Zn)         | 0.004±0.01        |

Source: Original Mean ±SD, n=3

4. DISCUSSION

Naturally occurring plant chemicals accumulate in different plant parts, such as leaves, roots, stem, bark, fruits, and seeds [11-12]. However, these plant chemicals, apart from the fact that they help in the prevention of diseases in human, they also protect the plant [13]. Apparently, all the phytochemicals examined in these studies, gave a positive result. Fagbohun et al. [14] in their findings reveal a similar result for all the phytochemical compound examined also in their study. The presence of all the phytochemicals was similarly reported by Awotedu et al. [15] in their findings on Azadirachta indica, Leea guineensis and Parkia biglobosa leaves. Alkaloids have been observed to have a microbial effect, anti-diarrheal effect, anti-hypertensive, anti-fungal, anti-inflammatory and anti-fibrogenic effect [16] Alkaloid presence was confirmed in this study, however, the result obtained compares favourably with that reported by Awoyinka et al. [17] for alkaloid in Cnidoscolus aconitifolius. The presence of tannin was observed also in this study, meanwhile, tannin in medicinal plants shows the ability of these plants to play an essential role as anti-fungal, anti-diarrheal, antioxidant and anti-hemorrhoidal agent [18]. In similar research, saponin, cardiac glycosides, flavonoids, phenolics are all present too in the seeds of Pilostigma thonningii as described by Jimoh and Oladiji [19]. A contrary result was obtained for Garg and Garg [20] where all the phytochemicals examined for Tinospora cordifolia leaves using methanolic extract gave a negative result including; alkaloids, cardiac glycosides, phenolics, and saponin except flavonoid that was present. However, the result obtained in the findings of Anyasor et al. [21] reveals the absence of some phytochemicals like alkaloids, saponin and tannin for Costus afer. The result for the quantitative phytochemical analysis revealed some amount of all the phytochemicals present in the leaves of S. anthelmia. The saponin content of the leaves presented the highest amount of 18.12mg/100g, followed by tannin (9.61mg/100g), while the lowest value was reported for terpenoids (0.98mg/100g). Infections from parasites are usually prevented by saponins when consumed by humans. The saponin content in this study it was found to be higher compared to the values reported for Azadirachta indica, Parkia biglobosa and Leea guineensis whose values gave 15.35mg/100g, 10.47 mg/100g, 7.53mg/100g respectively as reported by Awotedu et al. [15]. The presence of saponin usually attract foamy characteristics and possess wound healing properties [22]. The values reported in these studies are all on the lower side compared to that reported by Nduke et al. [23] and Harry-Asobara and Samson [24]. The leaves of S. anthelmia contains a low content of alkaloids (2.34mg/100g) compared to some values reported by different authors. The values reported in the study conducted by Okeke and Harry-Asobara [25] for Oleome ciliata leaves, stems and roots are lower for all the phytochemicals examined compared to that reported in these studies. Terpenoids and flavonoids are present in considerable amount while alkaloids are present in very high concentration in Psidium guajava [26]. Terpenoids possess various pharmacological activities like anti-malarial, anti-inflammatory, anti-cancer, anti-viral, anti-bacterial and also inhibits cholesterol synthesis [27]. The presence of phenols in the study suggests that
**S. anthelmia** as an important plant which is responsible for the protection of tissue membranes and proteins against harmful free radicals [28-29]. Phenolic compounds are one among plant secondary metabolites that are known to act as natural antioxidants [30]. Flavonoids tend to be most commonly known with regards to antioxidant nature. They also possess anticancer, anti-inflammatory, antibacterial and anti-allergic characters [31]. They serve as potent antioxidants which can protect the human body from free radicals and reactive oxygen species. Different authors (Oluwasina et al. [32] and Dewick [33] have reported high alkaloid content in the roots other than leaves, and this is in accordance with the report of Benbott et al. [34] that high alkaloids constituent of the seed and root may arise because those organs serve as storage point of the plant. Alkaloids, apart from the fact that they are naturally derived from plants, are also produced by a large variety of organisms including bacteria, fungi, plants and animals. Alkaloids possess diverse pharmacological properties asephetrine (for antiasthma), quinine (for anti-malarial), vincamine (for vasodilatorial [35]. Alkaloids are usually known for evoking bitter taste in plants and they usually act on various metabolic systems in humans and animals [36]. Alkaloids tend to be poisonous when taken in bulk amount due to their stimulatory effects producing excitation associated with cell and nerve disorders [37]. Tannin content in the leaves of *S. anthelmia* was reported to be 9.61mg/100g and this is comparably higher than those reported previously in other similar studies by Awotedu et al., [15]; Senguttuvan et al., [38]; Ekwueme et al., [39]; Ajuru et al., [40]. Plants containing tannins are used for healing of wounds, antidotes for poisons, varicose ulcers, haemorrhoids, frostbite and burns Hence, herbs containing tannins can be used as mouthwashes, eyewashes, snuff and even as vaginal douches and also treat rectal disorders [41-42]. The results for the mineral composition of *S. anthelmia* leaves (Table 3) revealed that the leaves contain minerals in different degrees as evidenced by the values obtained. The result showed that calcium had the highest value of 0.48%, while sodium gave the lowest value (0.022%) for macro elements. In microelements, iron had the highest concentrations and all other micro elements indicate a low value. The calcium values (0.48%) obtained in this study is higher than that reported by Olaniyi et al., [43] for *Crescentia cujete* leaves (6.13mg/100g). Calcium is vital in the formation of bones and teeth as well as regulation of nerve and muscle functions and blood functions [44]. Hassan et al. [45] also report that calcium is important and very safe for cancer patients. The phosphorus content (0.035%) is lower compared to the value (0.99%) reported in the findings of Awotedu et al. [15] in the leaves of *Azadirachta indica* which had the highest levels among the three medicinal plant examined. Phosphorus helps in the normal kidney functioning, transfer of nerve impulse and ionic balance in the body Ahmed and Chandhary [46]. However, the range (1.33-1.62) reported by Chavan et al. [47] for Phosphorus in the leaves of *Artemisia nilagirica*, *Cytocline purpurea* and *Sphaeranthus indicus* are very high compared to that reported in the study. A higher concentration of magnesium was observed for *S. anthelmia* (0.15%) compared to the value reported by Fagbohun et al. [14] for the three species examined. Magnesium is an important enzyme activator in carbohydrates and protein metabolism as claimed by Vormann [48]. The values reported for sodium and potassium are (0.022% and 0.23% respectively). The ratio of sodium to potassium should be less than 1 according to the recommendation of FND [49]. The ratio of Na: K calculated for *S. anthelmia* gives (0.13) which suggest that the plant would reduce high blood pressure. Ujowundu et al. [50] also reported very low values for sodium and potassium in the seeds of *Dacryodes edulis*. However, all the values reported the micro elements in this study are very low and are comparably similar to other results obtained by different authors.

## 5. CONCLUSION

The medicinal attributes of *S. anthelmia* leaves as evident from its ethnobotanical and folkloric uses, could be attributed to the presence of these secondary metabolites; Saponin, Tannin, flavonoids, alkaloids, phenols, cardiac glycosides, terpenoids, phlobatannin. Thus, from our findings, we can suggest that *S. anthelmia* is a rich source of phytochemicals which in no small measure can help in the treatment, prevention and management of diverse medical complications. Also, the phytomineral status of the plant shows a considerable amount of minerals that can be beneficial to human health.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.
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