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HPLC Evaluation of Phenolic Compounds in *Physalis angulata* Linn. and *Physalis micrantha* Linn. (Solanaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author CE designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors CE and CAO managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Physalis angulata Linn. and *Physalis micrantha* Linn. (Solanaceae) used for different medicinal purposes in Nigeria and other parts of the world were subjected to high performance liquid chromatography (HPLC) analysis to determine their phytochemical profiles. Forty five (45) alkaloids, 36 flavonoids, 18 glycosides and 44 phenolic acids were observed in the plants. *P. angulata* had the highest concentrations of flavonoids, phenolic acids and alkaloids while *P. micarantha* had the highest concentration of glycosides. The presence and concentration of these chemicals in these studied species confirm their medicinal and ethno-botanical uses and make them potential sources of raw materials for the pharmaceutical industries.

Keywords: Alkaloids; flavonoids; glycosides; phenolic acids; Physalis; phytochemicals.

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1. INTRODUCTION

Physalis Linn. are medicinal, edible and are used in different parts of the world for one purpose or the other [1-5]. In India, *P. alkekengi* is used as a diuretic, applied in urinary and skin diseases, can help to cure worm infections and has abortifacient properties [1,2] while *P. angulata* is used as diuretic and to cure stomach troubles [1].

Tropane and alkaloids with anti-muscarinic activity which block the activity of the neurotransmitter acetylcholine by binding to the muscarinic receptors of the parasympathetic nervous system and are useful in treating gastrointestinal and muscular spasms and Parkinson's disease have been extracted from *Physalis* [1]. Recently, Physalins a compound found in *Physalis* are under investigation due to their anti-tumour and cytotoxic activity [6,7], furthermore these compounds have anti-bacterial, antiseptic and abortifacient activity [8,9]. In Nigeria, there is need to intensify research on our indigenous plant species to enhance and widen the sources of raw materials for our pharmaceutical industries and thereby boost our domestic income.

Considerable information exists on the West African species of *Physalis* (*P. angulata, P. micrantha* and *P. peruviana*) as food items (leaves and fruits), as medicinal plants and as sources of various secondary plant products [10]. There is little or no report on the chemical or phytochemical profile of these species. Therefore, this work is carried out to determine the alkaloid, glycoside, flavonoid and phenolic acid compositions of *P. angulata* and *P. micrantha* to promote the economic importance and pharmaceutical potentials of these species.

2. MATERIALS SAND METHODS

2.1 Plant Materials

The plant materials were collected from University of Port Harcourt Botanic Garden. They were authenticated by the Curator in the Department of Plant Science and Biotechnology Herbarium (UPH), processed and voucher specimen deposited in the Herbarium for reference purpose (Table 1). Fresh leaves were removed from the plants, washed with distilled water and used for the phytochemical analysis using high performance liquid chromatography (HPLC), anatomical and epidermal studies.

2.2 Phytochemical Methods

2.2.1 Alkaloids determination

Five (5 g) of the de-fated was weighed into a flask. 100 ml of 12% alcohol was added, shake and filtered. This was thereafter washed with 20 ml of industrial alcohol. The extracted residue was washed into flasks with 50 ml of ammonia-water (i.e. use ultrapure water), heated in boiling water for 20 minutes and cooled. 0.1 g of diastase (+ water) was added and the temperature maintained at 50-55°C for 2 hrs. At the expiration of 2 hrs, the sample was allowed cool and made up to 250 ml with ultrapure water, swirled and filtered. 200 ml of the filtrate was mixed with 20 ml hydrochloric acid (sp.g. 1.125), heated in boiling water for 3 hrs, cooled, neutralise with sodium hydroxide solution, made up to 250 ml, shake, centrifuge and decanted. The supernatant was used for alkaloid determination using water 616/626 HPLC with the nitrogen gas flow rate of 40 ml/mins, detector temperature of 170°C, injection port temperature of 190°C and column temperature of 125°C [11].

2.2.2 Phenolics determination

Two (2 g) of the sample was weighed into a set of test tubes. 3 ml of 70% acetone and water were added to the test tube, placed in an ultrasonic water bath at 10°C for 5 minutes. The sample was stirred occasionally with a glass rod and filtered through a 50-60µ Gooch crucible into a 50 ml Erlenmeyer flask. Steps (ii) and (iii) were repeated 3 times and the test tubes with the final rinsed with 3 ml portion of 70% acetone in water and emptied into the test tubes. 2 ml of 0.1M acetate and 15 ml of 0.1M TEA reagent were added into the filtrate. Thereafter the contents of the test tube were transferred into volumetric flask, closed with rubber stopper, swirled, shake for 20 minutes and allowed to settle for 4 hrs. The supernatants were collected for analysis using HPLC (Water 616/626) with the argon gas flow rate of 60 ml/mins, detector temperature of 120°C, injection port temperature of 155°C and column temperature of 117°C [11-13].

2.2.3 Glycosides determination

0.5 g of sample each was weighed into a set of digestive tubes. 5 ml of 0.1M HCl was added and warm gently for 15 minutes at 105°C and transferred into a 50 ml volumetric flask. Steps (i) and (ii) above were repeated twice, rinsed with two to three additional aliquot, allowed for

complete filtration and the filtrate made up to 100 ml mark with the extractant solution and mixed thoroughly. 5 ml of extract solution from the 100 ml flask was purified by running it through a 2 cm layer (the resin is packed on a macro pipette tip) cation exchange resin (CEC). The glycoside compounds were eluded with 10 ml of absolute ethanol, the ethanol washed from the column with ultrapure water (10 ml), supernatant transferred to a sample vial and ran on HPLC (Water 616/626) with the nitrogen gas flow rate of 38 ml/mins, detector temperature of 167°C, injection port temperature of 183°C and column temperature of 130°C [11].

2.2.4 Flavonoids determination

1.5 g of sample was weighed into a set of extraction tube. 20 ml of boiled ultra-pure water dispensed into each extraction tubes, allowed to stand for 1½ hours, vertex for 5 minutes and transferred to a set of centrifuge tubes, shake for 15 minutes and centrifuged for 5 minutes at 3000 rpm. Thereafter, the supernatant was transferred to set of vials and determined on water 616/626 HPLC with the nitrogen gas flow rate of 60 ml/mins, detector temperature of 147°C, injection port temperature of 166°C and column temperature of 115°C [11,13].

3. RESULTS

The results of the phytochemical screening of the *P. angulata* and *P. micrantha* showed the presence of different alkaloids, flavonoids, glycosides and phenolic acids were extracted from the plant species (Figs. 1-5).

3.1 Phytochemical Constituents

Four groups of phenolic compounds namely alkaloids, glycosides, phenolic acids and flavonoids were observed in the species studied (Figs. 1-5). The highest percentage composition of flavonoids, alkaloids and phenolic acids were found in *P. angulata* while the highest percentage composition of glycosides was found in *P. micrantha* (Fig. 1).

Table 1. List of voucher specimens studied

Species name	Locality/date of collection	Collector(s) name	Accession number
P. angulata L.	Rumudike Road, Alakahia, Obio-Akpor, LGA, Rivers State/April 10, 2016	Ekeke, C.	Ekeke 0300
P. micrantha L	Beside University of Port Harcourt Bottling Company, Abuja Park/ November 10, 2017	Ekeke and Ogazie	Ekeke and Ogazie 014

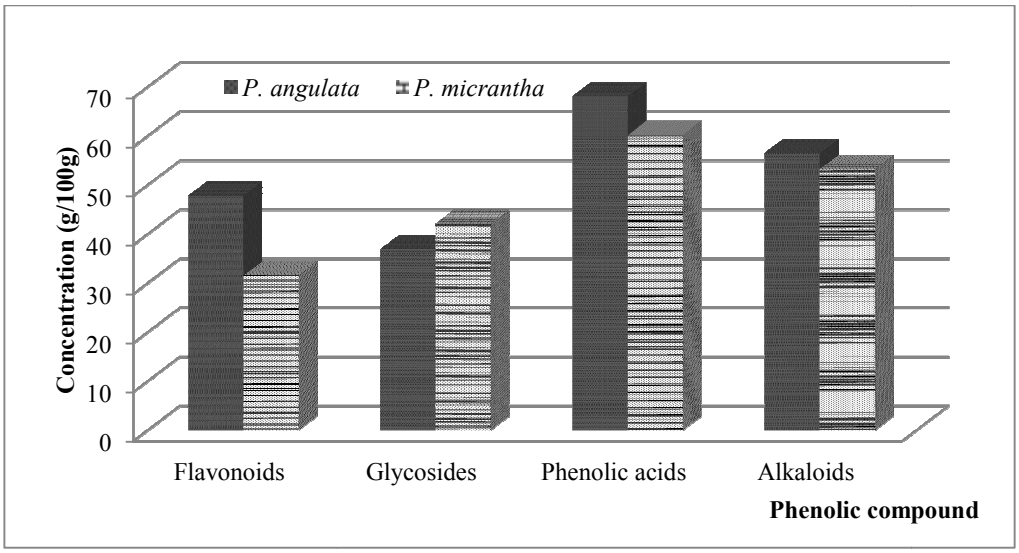

Fig. 1. Percentage composition of different phenolic compounds in *Physalis* species

3.2 Alkaloids

Forty five (45) alkaloid types were identified in the *Physalis* species studied (Fig. 2). The total concentration (g/100 g) of alkaloids in *P. angulata* and *P. micrantha* is 56.602 and 53.274 respectively. These alkaloids can be grouped in ten groups namely; Acridine, Colchicine, Imidazole, Indole, Isoguinoline, Phenylethlamine, Piperidine, Purine, Pyridine, Quinoline and Tropane. In *P. micrantha,* Nicotine (7.216 g/100 g) had the maximum concentration. This followed by Psychotrine (6.595 g/100 g), Acridine (5.097 g/100 g), Morphine (3.175 g/100 g), Quinine (2.735 g/100 g), Quinidine (2.685 g/100 g), Theobromine (2.539 g/100 g), B-Carboline (2.150 g/100 g) and Berberine (1.889 g/100 g) while Nornicotine (0.101 g/100 g) was the least concentration. In *P. angulata,* the concentration (g/100 g) of the alkaloids ranged from Psychotrine (6.353) to Apoatropine (0.083). This is followed by Acridine (5.630 g/100 g), Nicotine (5.381 g/100 g), Morphine (4.073 g/100 g), Quinine (3.576 g/100 g), Quinidine (3.284 g/100 g), Theobromine (2.709 g/100 g), Ephedrine (2.408 g/100 g) and Vinblastine (2.190 g/100 g).

3.3 Glycosides

Eighteen (18) glycoside types were observed in these species of *Physalis.* Among these glycosides, Metoprolol acid had the least concentration in both *P. angulata and P. micrantha* (Fig. 3). The decreasing concentration sequence in *P. angulata* is Captopril acid (8.205 g/100 g), E-strophanthin acid (7.978 g/100 g), Glycyrhizic acid (6.435 g/100 g), Digitoxin acid (3.626 g/100 g) and Digoxin acid (2.655 g/100 g) while in *P. micrantha* the decreasing concentration sequence is Hydrochlorathiazide acid (12.727 g/100 g), Captopril acid (8.682 g/100 g), Digoxin acid (3.864 g/100 g), Glycyrhizic acid (3.592 g/100 g), E-strophanthin acid (3.568 g/100 g) and Furosemide acid (2.806 g/100 g).

3.4 Phenolic Acids

Forty four (44) phenolic acids were identified in the *Physalis* species studied (Fig. 4). The total concentration (g/100 g) of alkaloids in *P. angulata* and *P. micrantha* is 68.178 and 60.269 respectively. The decreasing concentration sequence In *P. angulata* is Astringin acid (9.293 g/100 g), Mendelic acid (7.484 g/100 g), Catechin acid (5.179 g/100 g), Caffeic acid (4.362 g/100 g), Castarinol C3 acid (3.609 g/100 g), Cafein acid (3.575 g/100 g), Aesculetin acid (2.918 g/100 g), Pyrogallic acid (2.872 g/100 g), Garlic acid (2.755 g/100 g) and Genticitic acid (2.459 g/100 g) with Veratoc acid (0.094 g/100 g) as the least concentration. Astringin acid (5.825 g/100 g) had the maximum concentration in *P. micrantha* followed by Genticitic acid (4.821 g/100 g), Cafein acid (4.042 g/100 g), Caffeic acid (3.600 g/100 g), Pyrogallic acid (3.396 g/100 g), Castarinol C3 acid (3.104 g/100 g), Catechin acid (2.899 g/100 g), Valnilic acid (2.641 g/100 g), Aesculetin acid (2.574 g/100 g), Mendelic acid (2.484 g/100 g) and Homovanilic acid (2.011 g/100 g) with Singlic acid (0.099 g/100 g) as the least concentration.

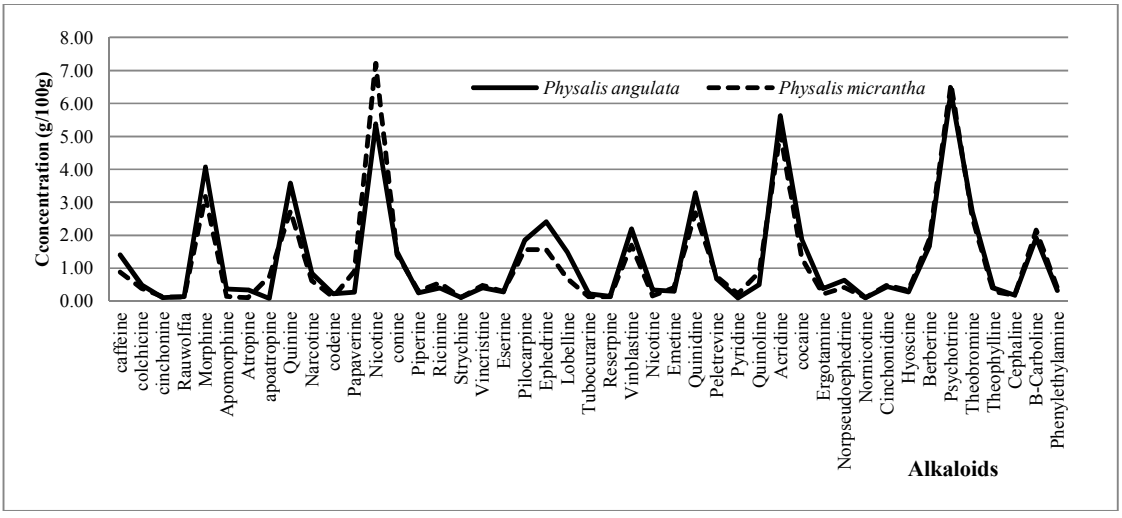

Fig. 2. Comparative concentration of alkaloids in *Physalis* species

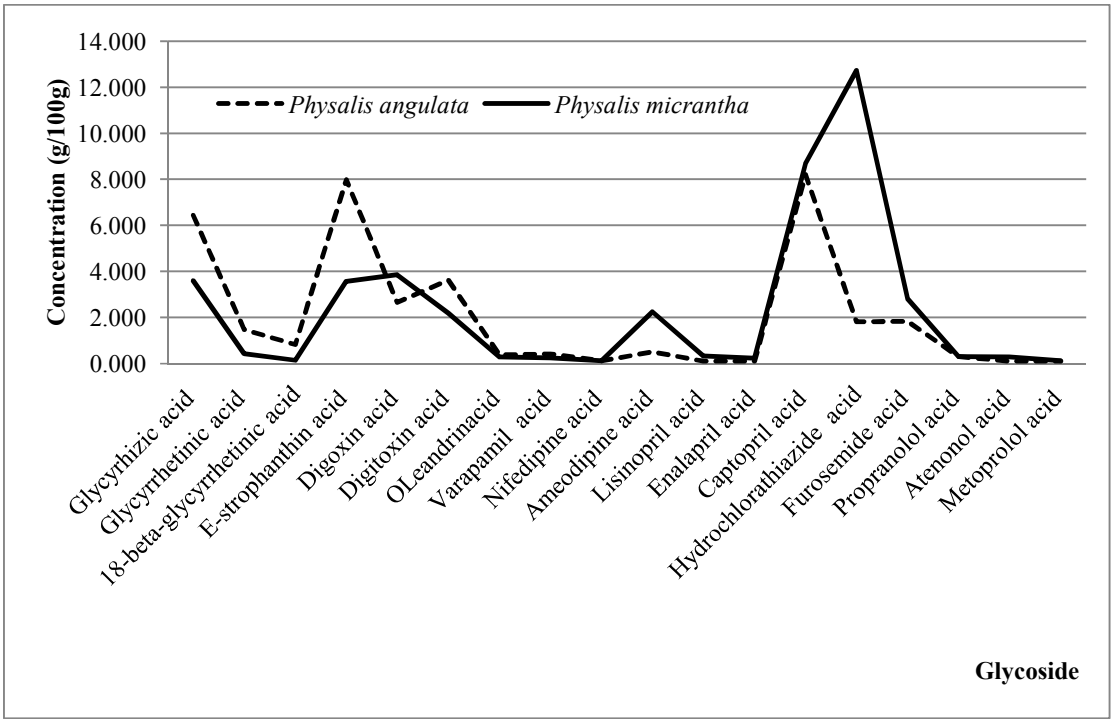

Fig. 3. Comparative concentration of glycosides in *Physalis* species

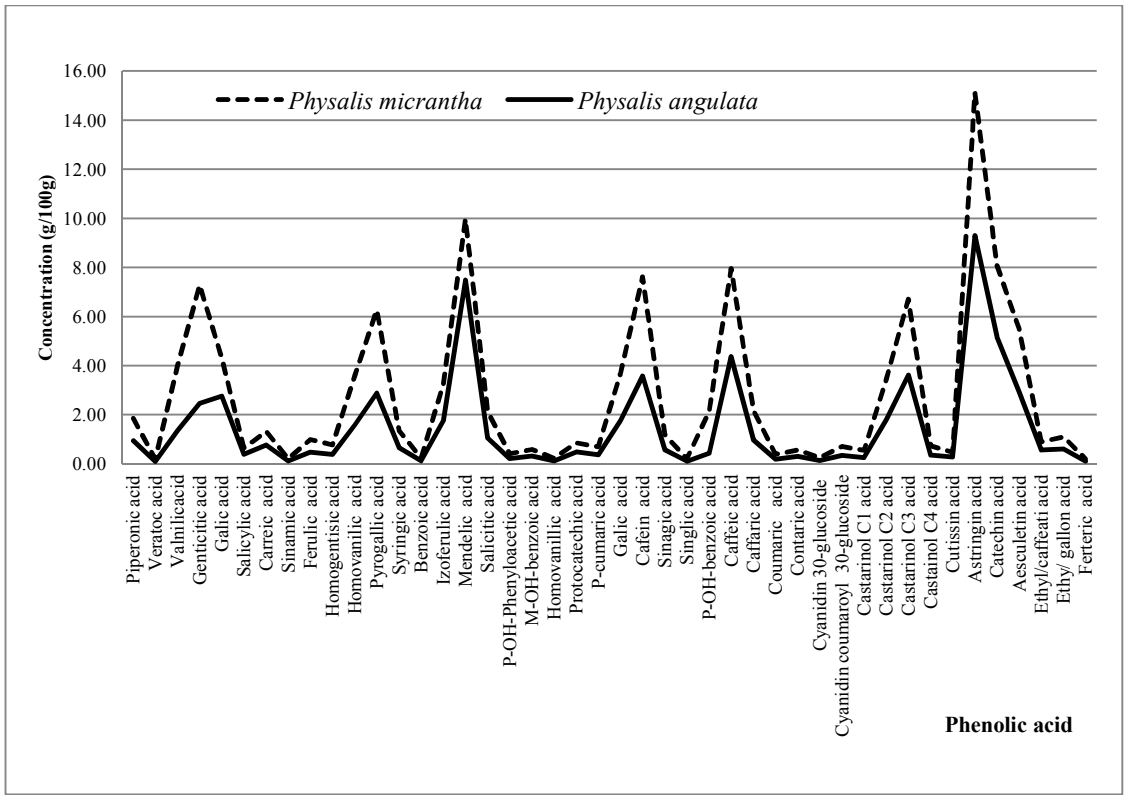

Fig. 4. Comparative concentration of phenolic acids in *Physalis* species

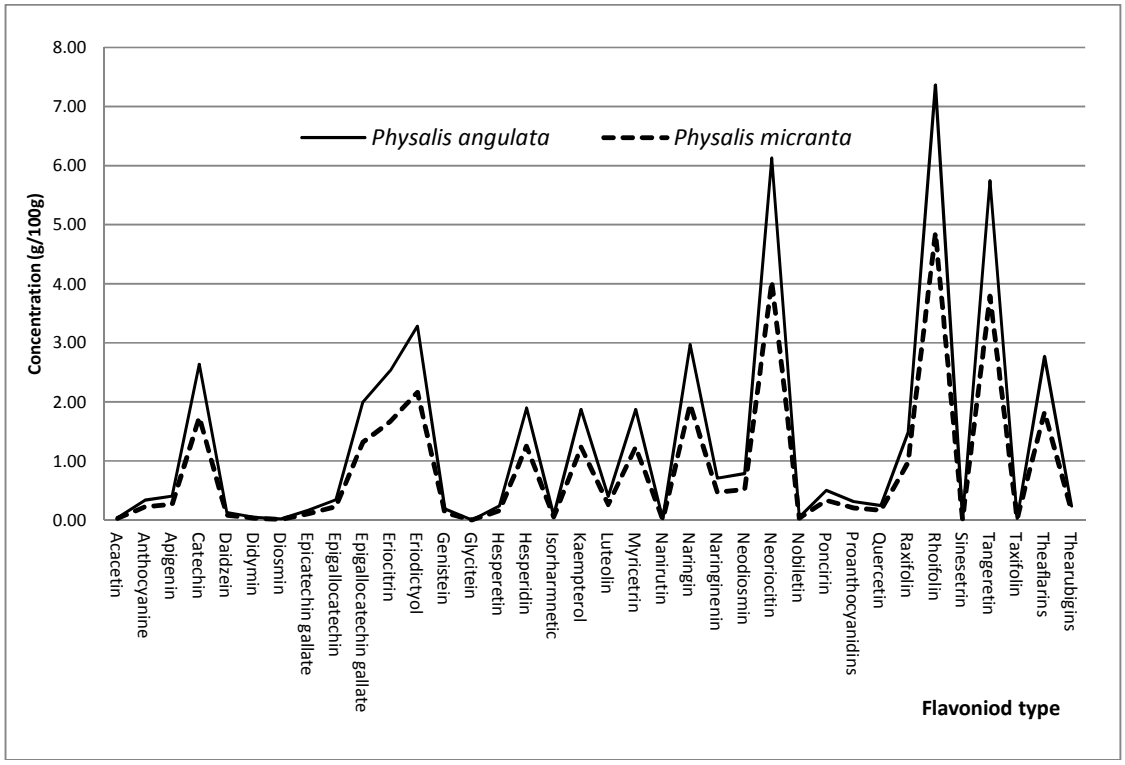

Fig. 5. Comparative concentration of flavonoids in *Physalis* species

3.5 Flavonoids

We found 36 flavonoids in the *Physalis* species which could be grouped into seven groups namely; anthocyanins, flavan-3-ols, flavanones, flavone, flavonols, isoflavone and flavanonols (Fig. 5). The flavonoid concentration varied from Rhoifolin (7.368 g/100 g) in *P. angulata* to Glycitein (0.003 g/100 g) in *P. micrantha* (Fig. 5). This is followed by Rhoifolin, Neoriocitin, Tangeretin, Eriodictyol, Naringin, Theaflarins, Catechin, Eriocitrin and Epigallocatechin gallate.

4. DISCUSSION

Phenolic compounds are the most important class of phytochemicals present in plant-based food sources. These compounds, including hydroxyl benzoic acid and their derivatives, flavonoids, flavanones, flavonols, flavones, catechols, anthocyanins and anthraquinones are the fundamental constituents of the food plants [14]. The phytochemical constituents and the medicinal uses of some members of *Physalis* have been recognized [15-18]. Hsua et al. [15], Leong and Shui [16]. Jin [17] and Hsu et al. [18] opined that *Physalis* species contain several phytochemicals including anti-proliferative withanolides which have anti-microbial and antioxidant properties and are used in the treatment of different diseases. Alkaloids are effective in the treatment of gastrointestinal, muscular spasms and Parkinson's disease [5] and have anti-microbial properties. Flavonoids are used as antioxidant [19], protect cell from degradation, stress, act as signalling molecules, phytoalexins, detoxifying agents, reduce toxic effects and stimulants [20], triggers the production of naturalenzymes that fight disease, hence reduce the risk of certain cancers, heart disease, and age-related degenerative diseases and play chemopreventive role in cancer [20] while phenolic acids have been observed to have inhibitory properties against cancer and antioxidant capacity [21] including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, and vasodilatory actions [22].

In our study, we identified 45 alkaloids, 36 flavonoids, 18 glycosides and 44 phenolic acids in *P. micrantha* and *P. angulata. P. angulata* had the highest concentrations of flavonoids, phenolic acids and alkaloids while *P. micarantha* had the highest concentration of glycosides. Among the glycosides, the concentration of glycyrhizic acid,

glycyrrhetinic acid, 18-beta-glycyrrhetinic acid, E-strophanthin acid and digitoxin acid were higher in *P. angulata* than *P. micrantha* while digoxin acid, ameodipine acid and hydrochorathiazide acid and furosemide acid were higher in *P. micrantha*. The concentration of phenolic acids (genticitic acid, mendelic acid, astringin acid and catechin acid) are higher in *P. angulata* than *P. micrantha* while the concentration of the alkaloids in the two species are relatively the same but the concentration of all the flavonoids are higher in *P. angulata* than *P. micrantha.*

In other members of this genus like *P. solanaceus* chemical studies of roots have shown that withasteroids -compounds with an ergostane skeleton [23,24] as well as pyrrolidine and *nor*-tropane alkaloids [25,26] are the main secondary metabolites isolated from it. Also, withasteroids and flavonoids are the main compounds isolated from the calyxes [27,28] while in *P. solanaceus* physalins which are a group of *seco*-withasteroids with a high degree of oxidation were isolated from the stems, leaves and fruits [29] including sucrose esters. The food and medicinal uses of *P. longifolia,* and other related *Physalis* species found north of Mexico have been reported [24] including anti-cancer properties and ethnobotanical uses [24]. Withanolides were also isolated from *P. longifolia* [20] and exhibit significant biological activities, specifically antimicrobial, antitumor, anti-inflammatory, immunomodulatory, and insect-antifeedant activities [30-32] and suppress the growth of different tumor cells, including breast, pancreatic, prostate, lung, leukemia, and head and neck squamous cell carcinoma, by inducing programmed cell death [12].

Other recent anti-cancer researchers have shown that *P. angulata* has anti-metastatic and anti-angiogenic activity [15] and contain anti-proliferative withanolides, cyto-toxic against prostate cancer cells [17], and as well as Physalin B, which has anti-melanoma activity [18]. In addition, *P. minima* have been shown to have significant cytotoxic activity on human lung cancer cells [16].

5. CONCLUSION

The presence and concentration of these chemicals in these species studied confirm their medicinal and ethno-botanical uses and make them potential sources of raw materials for the pharmaceutical industries in Nigeria.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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