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RESEARCH ARTICLE

Phytochemical Analysis and Elemental Contents of Varieties *of Polyalthia longifolia* (Sonn.) Thwaites

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Abstract

Polyalthia longifolia (Sonn.) is a medicinal plant that belongs to the family Annonaceae, and it is distributed in the tropics. This plant is widely grown in West Africa for its ornamental and medicinal purposes. There are two varieties of *P. longifolia* which are commonly distinguishable by the direction of their branches. One has spreading perpendicular branches, and the other has drooping pendulous branches. Traditional herbal practitioners believe that one variety (*P. longifolia* cv. *pendula*) is more medicinal than the other. This study, therefore, sought to investigate the phytochemical components of the two varieties of *P. longifolia* by HPTLC, UPLC, and elemental analysis by ICP-EOS. No observable differences were found in the phytochemical and elemental profiles of these varieties that could help distinguish one from the other or could account for its supposed differences in medicinal properties. A total of 22 elements were detected in the samples of the two varieties of the plant. Qualitatively, the elemental content of both varieties was similar. Only Iridium was not detected in all samples. Heavy metals including As, Pb, Cd, and Hg had their levels above the recommended limits.

Keywords: Elemental Content, Fingerprint, Phytochemical Analysis, *Polyalthia longifolia*

1. Introduction

Polyalthia longifolia (Sonn.) Thwaite is a flowering plant that belongs to the genus *Polyalthia*, and the Annonaceae family which falls within the Magnoliales order and Magnolids clade accordingly[1]. This plant originates from India, but it is grown in various parts of the tropics, including Ghana, Nigeria, and across West Africa for both ornamental and medicinal purposes. The plant is lofty, evergreen, and is mostly planted because of its effectiveness in alleviating noise[2]. The Annonaceae family belongs to the sub-class of Dialypetalae, which are primitive plants characterized by unclear boundaries between sepals, petals, and parts of fruit. Morphological features characteristic of the Annonaceae family include distichous leaf arrangement, cross-section of stem being striate, stems or twigs when exfoliated will produce a unique aroma, leaves having no stipules, aestivation of petal being valvate, ruminate endosperm, berries or drupe fruits, arillus seeds, and vessels with simple perforated fields[3].

Several plants species from the genus *Polyalthia* have been reported to possess various medicinal properties[4,5], *P. longifolia* cv. *pendula*, grown in India, is known to be the most commonly used in traditional indigenous medicine[6]. Two distinct varieties of *P. longifolia* are known. One of them has spreading perpendicular

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branches and is generally known as the typical variety. The other variety which has drooping pendulous branches, is also commonly used as an avenue tree and is sometimes also known as *P. longifolia* cv. *pendula*[6].

This plant is used in traditional medicine across several Asian and African countries, with Ghana inclusive[7,8] for the management of diseases such as malaria, fever, skin diseases, diabetes, hypertension and helminthiasis[2,4,6,9,10]. *P. longifolia* aqueous extract has been shown to lower the blood pressure, rate of respiration and glucose level in animal models[9]. The plant is reported to possess antibacterial, antifungal, antitumor, anti-ulcer and antioxidant properties, cytotoxic function and hypotensive effects[2,10], hepatoprotective and anti-inflammatory, antimicrobial, hypoglycemic and anti-ulcer activities[4,9,11].

Polyalthia species have been shown to have various kinds of secondary metabolites, such as alkaloids, terpenes, and chalcone[5], aporphine and azafluorene alkaloids, proanthocyanidins, β-sitosterol, and leukocyanidin, clerodane, and ent-helimane, diterpenoids. These compounds were isolated from the leaves, stem, and stem bark[6]. The clerodane diterpenoids and the alkaloids are known to contribute to its medicinal importance[6].

The aqueous leaf extract is known to contain terpenes, non-reducing sugars, flavonoids, resin, gums and mucilages, carbohydrates and fibre, low fat and phenols, as well as rich quantities of minerals[10,12]. The young leaves have been shown to contain 4% ash, 0.21% lipid, 25% fibre, 54% carbohydrate 9% protein, and 8% moisture while the mature leaves contained 10% protein, 5% ash, 0.26% lipid, 19% fibre, 9% moisture and 57% carbohydrates[12]. Quantitative analysis revealed that the young leaves contained slightly higher quantities of tannins and phenols but lower content of flavonoids as compared to the matured leaves. Both young and mature leaves showed appreciable quantities of minerals with the mature samples having higher concentrations of Na, K, Ca and Mg[10]. The essential oils of the leaf and stem bark of *P. longifolia* is exclusively composed of sesquiterpene derivatives, with allo-aromadendrene being in the highest content with 19.7%, followed by caryophyllene oxide, β-caryophyllene, β-selinene, α-humulene and ar-curcumene[6].

In Ghana, the two varieties of the plant are available. However, anecdotal reports have it that *P. longifolia* cv. *pendula* is more medicinally potent than *P. longifolia*. This study therefore sought to investigate the phytoconstituents and elemental composition of these two plant species in order to profer some scientific justification or otherwise to account for the postulated difference in medicinal properties.

1.1 Description

Guatteria longifolia (Sonn.) Wall. and *Uvaria longifolia* Sonn. are synonyms often used for *P. longifolia*[13]. *P. longifolia* is a columnar, pyramid-like, tree: main stem straight, undivided, growing up to 12 m or more. Branches are slender, short, about 1 or 2 m long, glabrous, and pendulous. Leaves alternate, distichous, exstipulate, mildly aromatic, 7.5 - 23 by 1.5 - 3.8 cm. Leaves are shining, glabrous, tapering to a fine acuminate apex, narrowly lanceolate, margin markedly undulate, pinnately veined, leathery or ubcoriaceous, shortly petiolate; with petiole about 6 mm long. Flowers arise from branches below the leaves, 2.5 to 3.5 cm across, yellowish to green, in fascicles or shortly pendunculate umbels; petals 6, 2 seriate, flat, from a broad base, lanceolate, long accumulate, spreading; and sepals 3, broad, short, triangular, the tips reflexed. Stamens are many, cuneate; connective truncately dilated beyond the cells. Ovaries indefinite; ovules 1 or 2; style oblong. Ripe fruits ovoid, 1.8 to 2 cm long, numerous, stalked, glabrous, 1 seeded; stalk 1.3 cm long, short, glabrous. Seeds smooth, shining. Flowering and fruiting: February-June[6].

2. Materials and Methods

2.1 Plant Sample Collection and Identification

P. longifolia cv *pendula* (Voucher number 032020-24), and *P. longifolia* (Voucher number 032020-24) samples leaves were harvested in March 2020 from the Kwabenya District (5.7021° N, -0.2446° W) and the University of Ghana campus (5.6539° N, -0.1859° W), both in the Greater Accra Region of Ghana. Plant materials were identified by Mrs. Gladys Schwinger, a Botanist at the Department of Plant and Environmental Sciences, University of Ghana, Legon. Two samples were harvested for each variety. The herbal material was thoroughly washed with distilled water and was air dried in a dust-free environment for 3 weeks (Figure 1).

Figure 1. Picture of *P. longifolia* cv. *pendula* and *P. longifolia*.

2.2 Extraction

Pulverized plant material of 2 g each were extracted with 20 mL of water, ethanol 50 % v/v and petroleum ether at room temperature. Extraction of the materials were performed by ultra-sonication for 30 minutes in each solvent, and subsequently centrifuged at 8000 × g for 5 min. The supernatant were pooled together and concentrated low *in vacuo* at 40 ºC and lyophilized.

2.3 High-performance Thin Layer Chromatographic (HPTLC) Analysis

2.3.1 Chemicals and Reagents

All the chemicals were purchased in the highest quality available and used as received unless otherwise stated. Highly purified deionized water was freshly obtained from Millipore® Simplicity (Billerica, U. S. A.).

2.3.2 Sample Preparation and Application

Ten microliters (10 µL) test solutions of 5 mg/L extracts dissolved in methanol were then applied to HPTLC plates of 20 × 10 cm dimensions of silica gel 60 F254 (Merck). The test solutions were applied as an 8 mm band with minimum of 11.4 mm distance between bands and 8 mm from lower edge of plate, and the bands dried.

2.3.3 Development

A 20 × 10 cm Twin Trough Chamber (CAMAG, Muttenz Switzerland), saturated for 15 minutes with about 10 mL mobile phase in each trough was used. The developing distance was about 70 mm from the lower edge of the plate. The plates were then allowed to air dry and documented before and after derivatization. For the aqueous extracts, acetic acid-water-butanol (10:40:50) was used as the mobile phase. For the 50% ethanol extract, ethyl acetate-water-formic acid 90:5:5 (v/v) was used as the mobile phase, and for the petroleum ether extract, toluene-ethyl acetate 90:10 (v/v) was the mobile phase.

2.3.4 Reagent Preparation

Reagents were prepared according to protocols described by Wagner and Bladt[14]. Anisaldehyde sulphuric acid, Natural Product Reagent (Naturstoff), and ferric chloride ($FeCl_3$) reagents were used to detect the presence of tannins, flavonoids, fatty acids and sterols respectively. Anisaldehyde reagent was prepared by adding 20 mL of acetic acid, 10 mL of sulfuric acid, and 1 mL of anisaldehyde to 170 mL of ice-cooled methanol and well mixed. Naturstoff reagent was prepared by dissolving diphenylboryloxyethylamine in methanol to produce 1 % w/v solution. Iron-III-Chloride reagent was a 10 % w/v aqueous solution of $FeCl_3$ in methanol.

2.3.5 Detection and Documentation

The HPTLC analysis was performed on CAMAG TLC Visualizer 2 equipped with visionCATS software (version: 3.0) (Muttenz, Switzerland). The plates were examined both when underivatized and derivatized under white light, short UV λ 254 nm and long UV 366 nm. For the petroleum ether extracts, the plates were derivatized with anisaldehyde reagent and then heated at 100 °C for five min.

For the ethanol extracts, the plates were derivatized with Naturstoff reagent and examination was performed under 366 nm for the presence of flavonoids.

For the aqueous extracts, the plates were derivatized with ferric chloride reagent and examined under white light for the presence of tannins.

2.4 Ultra High Performance Liquid Chromatographic (UPLC) Analysis

The UPLC analysis of ethanolic extracts of *P. longifolia* was performed to develop the fingerprint chromatogram for the presence of rutin, quercetin,

myricetin, luteolin, and kaempferol. Instrumentation: Acquity™ UPLC, PDA eλ Detector, Quaternary Solvent Manager, Acquity UPLC H-Class manager, Sample Manager FTN Acquity UPLC, Milford, U.S.A. Stationary Phase: Acquity UPLC HSS T3, 1.8 µm, C_{18} 2.1 × 100 mm (Waters, Milford, U.S.A.). Mobile phase: A: HCOOH (0.1%) in water, B: HCOOH (0.1%) in Acetonitrile; Gradient: $t_{0\ min}$ A 95%, $t_{1\ min}$ A 95%, $t_{3\ min}$ A 90%, $t_{7\ min}$ A 0%, $t_{10\ min}$ A 0%; flow 0.5 mL/min; injection volume 2µL; column temperature 40^0C; detection λ 210–400 nm. Reference standards (97% HPLC) for external calibration: rutin (Sigma-Aldrich, Taufkirchen, Germany), and kaempferol (Roth, Karlsruhe, Germany) were dissolved in methanol at concentrations of 1, 0.5, 0.25, 0.125, and 0.0625 mg/mL.

2.5 Elemental Content Analysis by Inductively-coupled Plasma-optical Emission Spectrometric (ICP-OES)

2.5.1 Chemicals and Reagents

All the chemicals used were purchased in the highest quality available. Highly purified water was freshly obtained from an Aquatron A4000D system (Barloworld Scientific, Nemours Cedex, France).

2.5.2 Standard Solutions

The volumetric flasks for ICP-OES measurements were pre-treated with 2% v/v supra pure HNO_3 and purified water to minimize adsorption effects. The standard solutions of the metals were diluted between 1 and 5,000 µg/L with 2% *v/v* HNO_3 added from 1 g/L stock solutions of single-element standards.

2.5.3 Sample Preparation

A microwave digestion system (Multiwave Go, Anton Parr GmbH, Austria) was used to digest the plant samples. The samples (approximately 0.1 g each) were placed in Teflon vessels and 4 mL of HNO_3:HCl (3:1) was added to each. The vessels were heated at temperature programme as follows: 10 minutes heating to 100 °C and holding for 5 minutes, then heating from 100 °C to 150 °C for the next 10 minutes and holding for additional 5 minutes. Complete digestion was confirmed by decolourization of the sample solutions. The digests were cooled down to room temperature, transferred to 50 mL volumetric flasks, and made to volume with deionized water. A blank of HNO_3: HCl (3:1) was used for the analysis.

2.5.4 Elemental Analysis by ICP-OES

The elemental analysis was carried out using ArCos MV II (Spectro Ametek, Kleve, Germany) ICP-OES with axial plasma viewing. Gas flows were controlled by internal mass-flow controllers. A standard D-torch was employed. For sample introduction, the peristaltic pump of the system combined with a crossflow spray chamber was used under the following plasma conditions: 1200 W (RF-power), 13 L min^{-1} cooling gas, 0.8 L min^{-1}, and 0.8 L min^{-1} nebulizer gas.

3. Results

3.1 Phytochemistry Profiling by HPTLC

Upon HPTLC analysis of the extracts of both varieties of *P. longifolia* leaves, flavonoids were detected in the chromatograms (Figure 2). When underivatized, the spots quench fluorescence at λ 254 nm but a typical fluorescence at λ 366 nm was observed after derivatization with Naturstoff reagent. Phenol carboxylic acids (e.g. chlorogenic and caffeic acids) typically turn to blue bands while flavonols and flavones turn orange or yellow[14]. Two different profiles were detected for *P. longifolia* samples from different places. One sample of *P. longifolia* cv. *pendula* variety has a blue fluorescing spot at Rf ~ 0.30 and this is missing in both the other sample of similar variety and samples of the second variety. These samples originated from different places and could signal some sort of different chemotypes for the same species from different places.

The corresponding video-densitometric profiles for the two types of profiles observed are shown in Figure 3. Comparing the densitometric profiles of the different channels for *P. longifolia* samples, only red channel profiles were similar for the samples. The green, blue and grayscale channel profiles all show the differences at the same Rf ~ 0.30. These observations are outlined in Figure 3. For samples belonging to the same variety of *P. longifolia*, similar profiles were seen irrespective of the origin, and this is evident in the densitometric profiles in Figure 3. With the exception of the profile with the distinct fluorescing band at Rf ≃ 0.3, the profiles developed with the use of Naturstoff reagent for

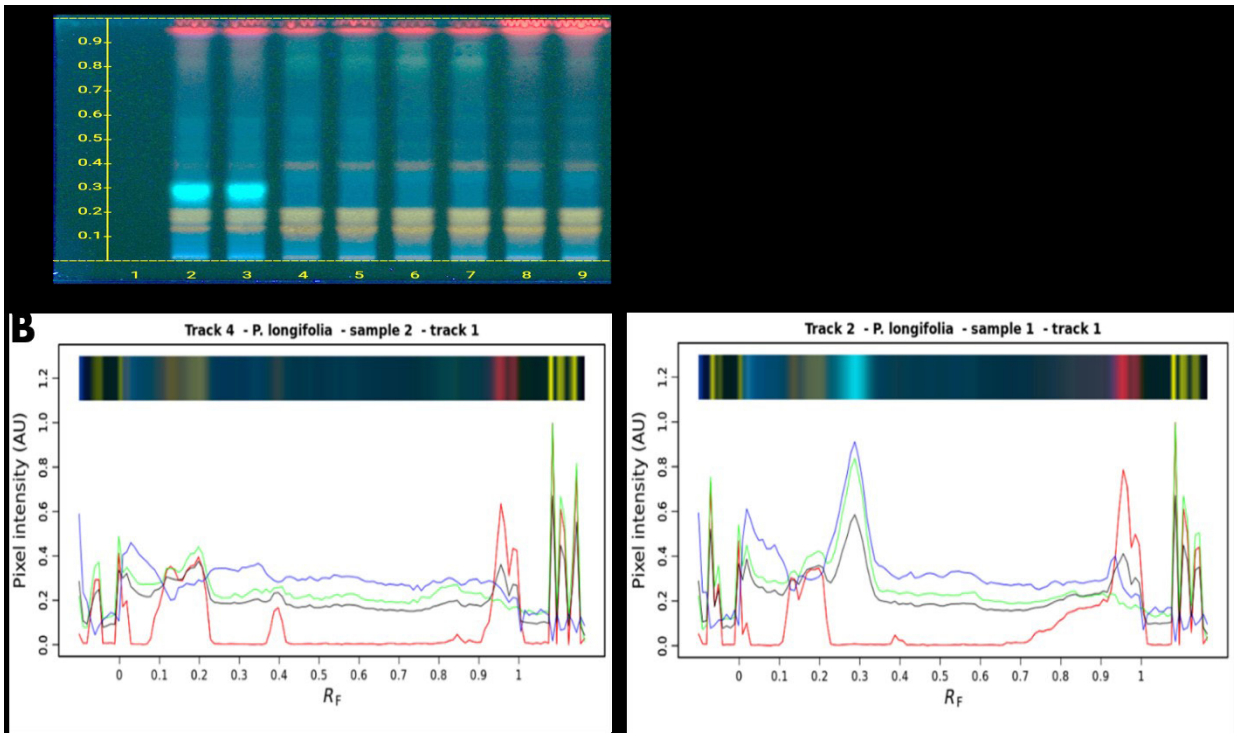

Figure 2. HPTLC profiles of the *P. longifolia* samples obtained after derivatization with Naturstoff reagent and detection at λ 366 nm. **(A).** *P. longifolia* cv. *pendula* (Tracks 2-5) and *P. longifolia* samples (Tracks 6-9). **(B).** Summary of video-densitometric profiles of the corresponding two different HPTLC profiles of the *P. longifolia* samples analyzed.

all the samples (*P. longifolia* cv. *pendula* and *P. longifolia varieties)* were similar.

Analysis of the petroleum ether extracts of both varieties of *P. longifolia* upon detection with anisaldehyde-sulphuric acid reagent, showed the presence of phenols, terpenes, sugars, and steroids by forming violet, blue, red, grey or green bands at day light (Figure 4D)[14]. Visual inspection of the HPTLC profiles showed they were similar, and this could be used to confirm the high level of similarity in phytochemical composition among the two different varieties of the plants.

Iron-III-Chloride reagent revealed dark green and brown spots in the aqueous extract, as seen in Figure 5, indicating the presence of tannins[14]. Similarly, the pattern of bands observed were the same for the samples from the different varieties. This could imply that the tannins profiles in the two varieties were similar.

3.2 HPLC Fingerprint Chromatogram

HPLC analysis also confirmed similar fingerprint chromatograms (Figure 6) of all samples of *P. longifolia* cv *pendula* and *P. longifolia* samples irrespective of the origin.

The typical fingerprint chromatogram (Figure 7) of the two *P. longifolia* varieties showed 4 main peaks at retention times: 4.364, 4.478, 4.481 and 7.194 min.

3.3 Flavonoid Content

Spiking samples of *P. longifolia* ethanol 50% extracts with reference flavonoids showed the possible presence of rutin and the absence of quercetin, myricetin, luteolin and kaempferol (Figure 8).

3.4 Elemental Content

As seen in Table 1, the presence or absence of a total of 22 elements were detected in the samples of the two varieties of the plant. Qualitatively, the elements present in both varieties were similar. Out of the 22 elements investigated, only Iridium (Ir) was not detected among all samples. Regarding toxic heavy metals whose presence and contents are usually regulated, most of them (including As, Pb, Cd, and Hg) had their levels above the recommended limits. The exceptions were for chromium (Cr) and copper (Cu) where their median levels recorded for each variety were less than the recommended limits of 2 mg/kg and

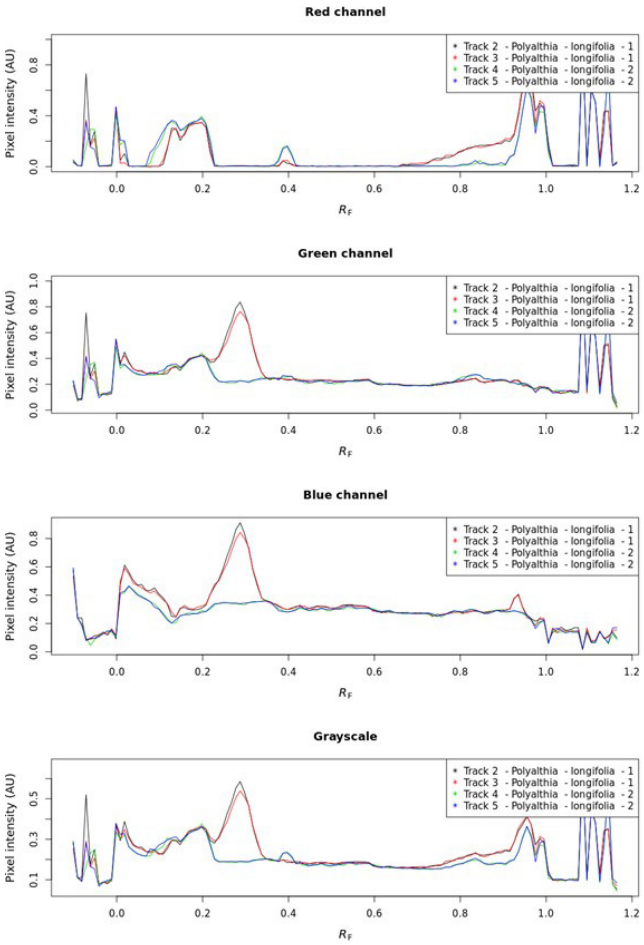

Figure 3. Densitograms of *P. longifolia* samples at the different channels.

150 mg/kg respectively. Comparatively, the levels of each of the elements in both varieties were comparable ($p>0.05$). The presence of the other elements detected demonstrate the additional benefits the plants possess with respective to their nutritional and medicinal values. Similar to previous results, the contents of all elements were similar ($p>0.05$).

IQR – Interquartile range showing the lower quartile (Q1) and upper quartile (Q3); LOD – Limit of detection; Levels of elements in the two varieties were compared using the non-parametric Mann-Whitney test. Level of significance were determined at 95 % confidence level. NS – Not Significant.

4. Discussion

The qualitative comparative study of the two varieties of *P. longifolia* by HPTLC revealed no distinguishable differences. In comparing the ethanol extracts of *P. longifolia* from various places, there was a high level of similarity. One sample which was different due to the presence of one spot could be said to be different from the other *Polyalthia* samples or a chemotype of *P. longifolia*. As reported in other literature, this study confirmed the presence of flavonoids and phenols (tannins)[10,12] in both varieties of *P. longifolia*. *P. longifolia* is rich in flavonoids and based on the HPLC retention time, rutin which is suspected to be a constituent, has been reported to be an active constituent of this plant[15]. The high level of similarity from the chromatographic profiles is due to the similarity in the phytochemical compositions of the two plant varieties. Contrary to the accession that one of the varieties has more medicinal value than the other which is considered more for ornamental purposes. The observations from this study, shows that both varieties could be considered for medicinal uses. This assertion is further supported by the similarities of the elemental compositions. The physiological importance of these elements as outlined in several literature could also be thought to augment the medicinal importance of the plant[16,17]. Additionally, the phytochemical constituents also account for the medicinal properties. Phytochemical constituents such as flavonoid derivatives have shown to have antimalarial activity[18]. The flavonoids antioxidants also act to provide protection against free radicals that damage cells and tissues. In the treatment of high blood pressure, flavonoids have also been shown to have a potential to inhibit angiotensin converting enzyme *in vitro*[19]. Tannins promote healing of wounds, and are effective in diarrhea, colitis and peptic ulcers[20]. Phenolic such as catechins have an anti-hyperglycemic action, lowering both blood-glucose and normalizing insulin release[21].

Irrespective of the above-mentioned benefits, the presence of some heavy toxic heavy metals were also documented in high quantities. As, Cd, Hg and Pb exceeded their recommended limits. Many studies have demonstrated that reactive oxygen species production and oxidative stress play a key role in the toxicity and carcinogenicity of these four metals. These metals have a high degree of toxicity, and are ranked among the priority metals that are of great public health significance. They are all systemic toxicants with known activities to induce multiple organ damage, even at lower doses. These metals are classified as "known" or "probable"

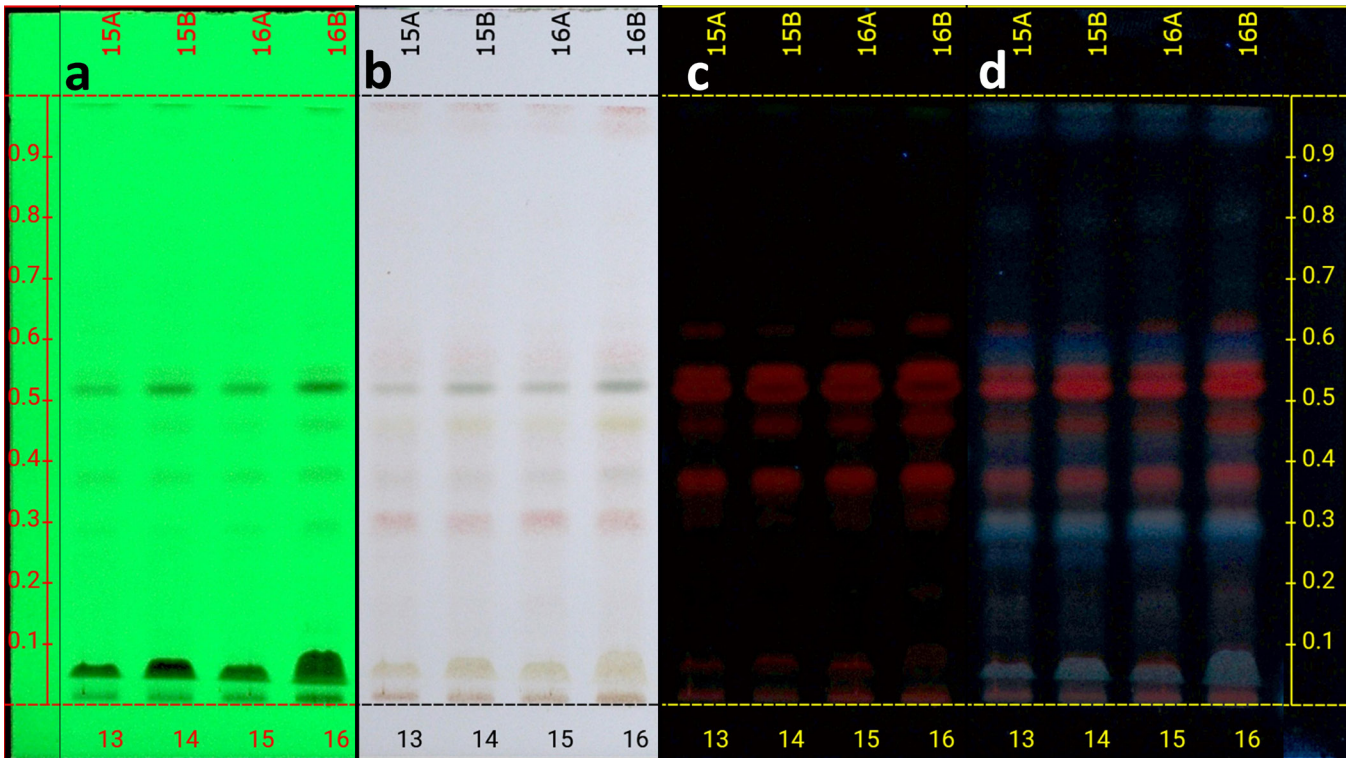

Figure 4. HPTLC profile of petroleum ether extract of *P. longifolia* var *pendula* (tracks 13, 14) and *P. longifolia* (tracks 15, 16). Samples were derivatized with anisaldehyde sulphuric acid and documented: **a.** underivatized under λ 244 nm, and derivatized; **b.** white light c. λ 244 nm d. λ 366 nm.

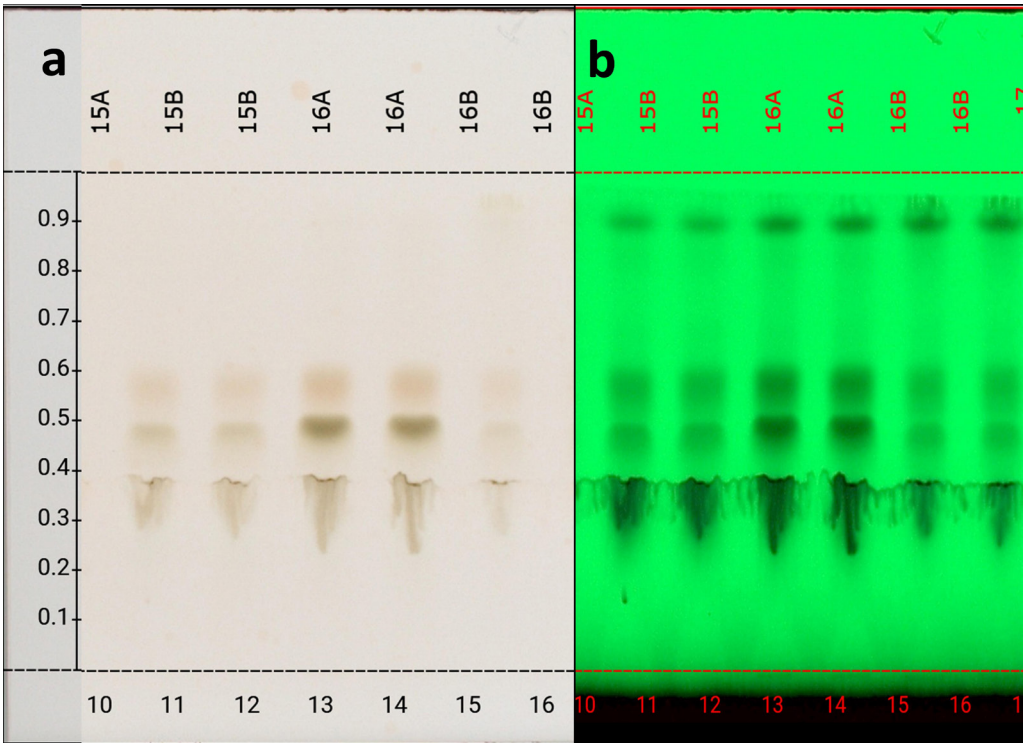

Figure 5. HPTLC profile of aqueous extract of *P. longifolia* cv. *pendula* (tracks 10, 11, 12) and *P. longifolia* (tracks 13, 14, and 15). Samples were derivatized with $FeCl_3$ chloride and documented: **a.** white light, and **b.** λ 244 nm.

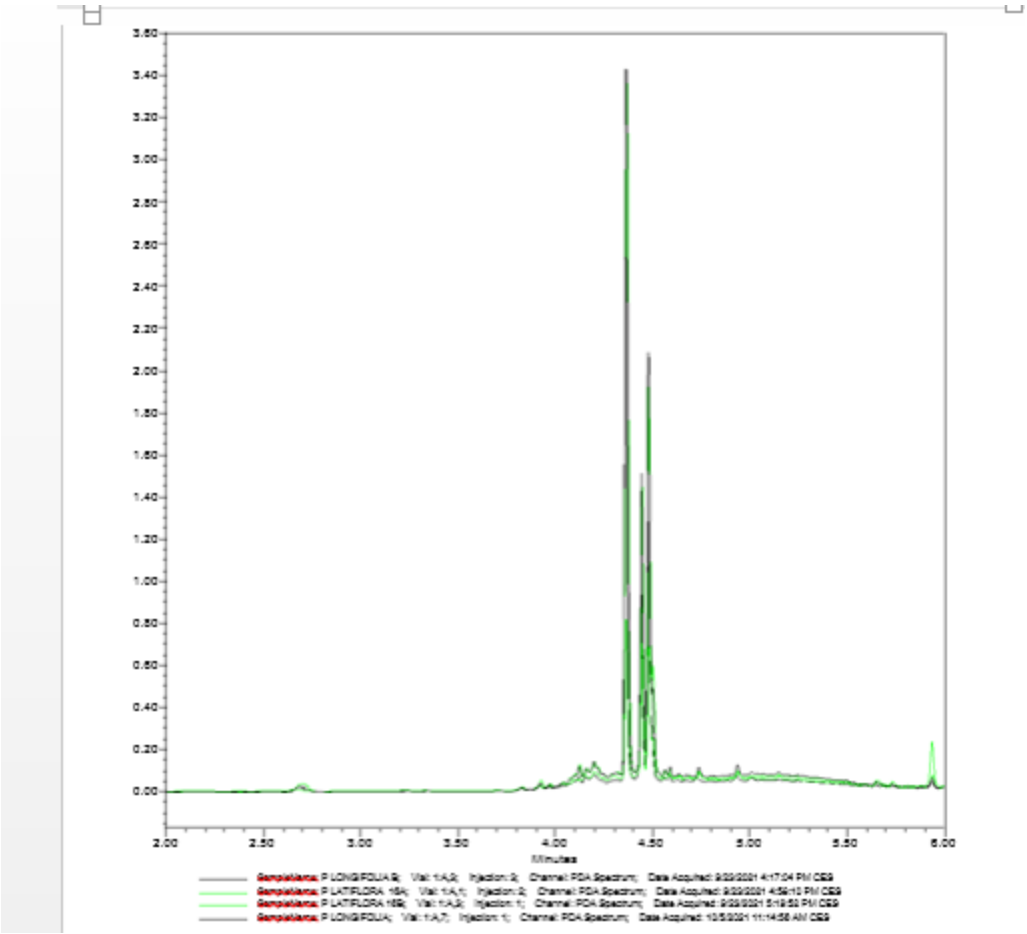

Figure 6. HPLC overlaid chromatograms of *P. longifolia* cv *pendula* and *P. longifolia* samples.

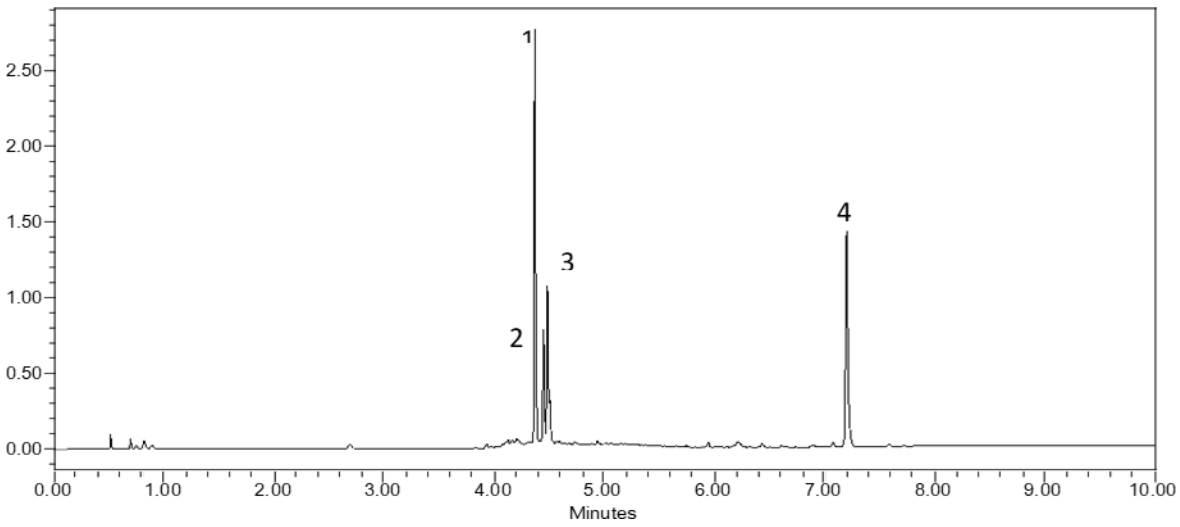

Figure 7. Typical UPLC fingerprint chromatogram of *P. longifolia* at λ 250 nm.

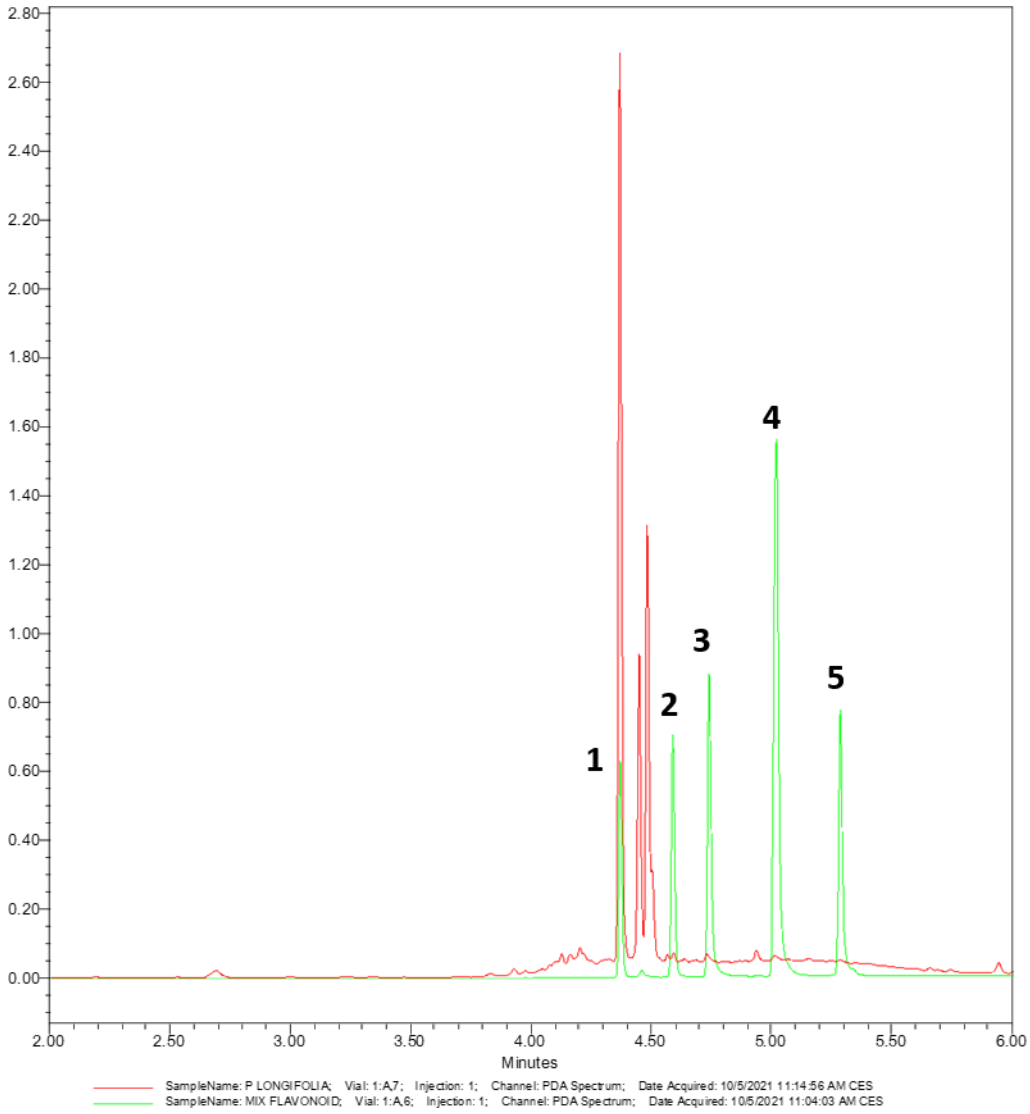

Figure 8. Overlaid UPLC chromatogram of 50% ethanol extract of *P. longifolia* with reference flavonoids at λ 250 nm; 1. Rutin, 2. Quercetin, 3. Myricetin, 4. Luteolin, 5. Kaempferol.

Table 1. Elemental content of *P. longifolia* leaf samples

Element	Median (IQR) amount in *P. longifolia cv pendula* (mg/kg)	Median (IQR) amount in *P. longifolia* (mg/kg)	Limit (mg/kg)
Al	217.2 (180.2–286.2)	216.6 (183.4–274.9) ^{ns}	
As	11.40 (7.881–16.59)	9.251 (7.220–23.54) ^{ns}	5
Ba	20.58 (11.81–31.00)	30.94 (1.875–31.72) ^{ns}	
Br	2320 (2244–2396)	2261 (2069–2452) ^{ns}	
Ca	10234 (7877–13072)	9541 (8193–11429) ^{ns}	
Cd	4.199 (0.000–36.46)	7.697 (0.000–57.06) ^{ns}	0.3
Cr	0.1836 (0.000–1.753)	0.000 (0.000 – 1.340) ^{ns}	2
Cu	6.070 (0.000–14.11)	9.739 (0.000–11.82) ^{ns}	150
Fe	434.4 (275.5–600.9)	302.7 (281.4–340.6) ^{ns}	
Hg	5.208 (4.666–5.751)	4.432 (2.257–6.608) ^{ns}	0.5

Table 1. Continued...

Element	Median (IQR) amount in *P. longifolia cv pendula* (mg/kg)	Median (IQR) amount in *P. longifolia* (mg/kg)	Limit (mg/kg)
Ir	< LOD	< LOD	
K	13878 (8479–18978)	15351 (10239–21786) ^{ns}	
Mg	1573 (1252–1886)	1452 (1118–1782) ^{ns}	
Mn	34.92 (26.40–41.96)	18.91 (6.783–31.78) ^{ns}	
Ni	18.69 (14.12–23.26)	11.76 (1.701–21.82) ^{ns}	
P	1474 (1305–1748)	1529 (1358–1725) ^{ns}	
Pb	26.77 (21.21 – 32.33)	35.49 (27.76 – 43.21) ^{ns}	10
S	2147 (1586 – 2832)	2188 (1923 – 2570) ^{ns}	
Sn	3.368 (0.000 – 6.736)	2.765 (0.209 – 5.321) ^{ns}	
Sr	56.21 (36.18 – 76.23)	44.52 (14.54 – 74.49) ^{ns}	
Ti	5.643 (4.748 – 5.918)	5.178 (1.513 – 7.288) ^{ns}	
Zn	15.12 (13.92 – 16.32)	14.50 (13.87 – 15.12) ^{ns}	

human carcinogens[22]. Thus, and with such high levels reported, the safe use of this plant becomes questionable. However, this phenomenon is more reflective of the kind of anthropological and or industrial activities that occur around the collection sites of the plants. The main factor that affects the content of elements in medicinal plants are from the soil, effects of biological, geographical, and agrochemical activities, climatic conditions and he ability of some plants to specifically accumulate elements[16]. It is therefore necessary to strengthen the control measures applicable to medicinal plants, especially for those which are prepared to be orally administrable. Measures like Good Agricultural and Collection Practices should be encouraged to help control the presence and levels of some of these impurities.

5. Conclusion

Irrespective of the minor morphological difference observed in the two varieties of *P. longifolia*, the HPTLC and UPLC analysis demonstrated similar phytochemical compositions in terms of flavonoids. As such, both varieties could be considered for medicinal uses. Similarly, they also possess nutritional values because of the high levels of essential elements. However, there are also high level of heavy metals in the analysed plant samples. For this reason, regulatory measures regarding the use of medicinal plants should be enhanced. There may also be the need to further investigate these plant varieties in terms of differences in other phytochemical components.

6. Funding

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