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Proximate, Mineral and Phytochemical Analysis of Piliostigma Thonningii Stem Bark and Roots

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ABSTRACT

Proximate, mineral and phytochemical composition of Piliostigma thonningii stem bark and roots was investigated to determine the level of bioactive chemicals in the plant. The result revealed that proximate composition of Piliostigma thonningii stem bark (PSSB) contained moisture 7.11 %, dry matter (DM) 92.89 %, crude protein (CP) 4.22 %, crude fibre (CF) 52.81 %, ether extract (EE) 0.08 %, ash 8.11 %, carbohydrate 20.12 %, Nitrogen free extract (NFE) 26.67 % and energy 488.7 KJ/100g while Piliostigma thonningii root (PSSR) contained 8.34 % moisture, 91.66 % (DM), 7.40 % (CP), 41.60 % (CF), 2.10 % (EE), 12.80 % ash, 42.30 % carbohydrates, 27.76 % (NFE) and energy 922.6 KJ/100g. Mineral analysis of PSSB revealed the presence of Ca (55.19 mg/100g), P (29.93 mg/100g), Mg (40.10 mg/100g), K (18.73 mg/100g), Zn (12.09 mg/100g), Mn (3.11 mg/100g), Na (20.40 mg/100g), Cu (8.01 mg/100g), Fe (48.12 mg/100g), Cr (0.010 mg/100g), Cd (0.028 mg/100g), Co (0.021 mg/100g), Se (0.28 mg/100g) and Pb (0.010 mg/100g) while PSSR contained Ca (87.63 mg/100g), P (43.12 mg/100g), K (21.08 mg/100g), Mg (48.75 mg/100g), Zn (18.01 mg/100g), Mn (7.32 mg/100g), Fe (53.17 mg/100g), Na (22.72 mg/100g), Cu (12.54 mg/100g), Co (0.048 mg/100g), Cr (0.002 mg/100g), Se (1.56 mg/100g), Cd (0.040 mg/100g) and Pb (0.016 mg/100g). Phytochemical analysis of PSSR showed that it contained significant amount of bioactive chemicals of alkaloids (7.23 %), flavonoids (5.10 %), terpenoids (0.71 %), hydrolysable tannins (2.41 %), condensed tannins (0.10 %), phenols (5.02 %), saponins (0.17 %), steroids (2.00 %), phytates (0.77 %), glycosides (0.08 %) and cyanates (0.03 %) relative to alkaloids (5.11%), flavonoids (3.46 %), terpenoids (0.49 %), hydrolysable tannins (1.92 %), condensed tannins (0.19 %), phenols (4.00 %), saponins (0.22 %), steroids (1.18 %), phytates (0.51 %), glycosides (0.10 %) and cyanates (0.15 %) found in PSSB. It was concluded that PSSR contained appreciable amount of minerals and bioactive chemicals and could be considered as a potential alternative to antibiotics in livestock production.

1. Introduction

Due to the problems of antibiotics resistant bacteria and antibiotics residues in animal products and the dangers posed to human health, there is a renewed and growing interest in quest for alternatives to antibiotics for livestock medications. Recently, medicinal plants are been used as feed additives to improve livestock performance and ensure food safety (William and Losa, 2001; Oluwafemi et al., 2020). Medicinal plants or herbs have been reported to contain phytochemicals or bioactive chemicals (alkaloids, flavonoids, tannins, terpernoids, saponins, phenols etc.) which are found to be rich in minerals, vitamins, amino acids and other nutrients (Hyun et al, 2016). They have also been reported to be cheap, safe and effective without having any deleterious effect on the health of an animal (Alagbe, 2019). The bioactive chemicals in these herbs vary according to age of plants, species, method of processing/ storage, geographical location, soil type etc. (Hyun et al., 2016). Among the potential pharmacologically vital plants is *Piliostigma thonningii*.

Piliostigma thonningii (Schum) is a leguminous plant belonging to the family Caesalpiniacea. It is found in many countries including Nigeria, Niger, Cameroon, Togo, Botswana, Kenya, China, India, Indonesia, Cuba amongst others (Jimoh and Oladiji, 2005). The tree is perennial in nature and its petals are white to pinkish colour and the leaf characterized by green colour (Thagriki and Daniel, 2018). The plant is loaded with several phytochemicals which confers them ability to work as antimicrobial, anti-inflammatory, antifungal, antiviral, antioxidant etc. (Alfred, 2013). The leaves, roots and stem bark have been traditionally used for the treatment of chronic ulcers, diarrhea, toothache, gingivitis, cough, bronchitis, snake bites, hookworms and skin diseases (Daniyan et al., 2010). Alagbe et al. (2019) observed that the dried leaf of Piliostigma thonningii contains alkaloids, saponins, flavonoids, oxalate, tannins and phenols. Egharevba *et al*. (2010) reported that carbohydrates, glycosides, flavonoids, tannins, saponins, balsams, volatile oil and terpenes have been found in the leaves.

In view of these abundant potential in the plant, a research was carried out to further evaluate the proximate, mineral and phytochemical composition of *Piliostigma thonningii* stem bark and roots.

2. Materials and Methods

Plant collection and identification

Fresh and healthy roots of *Piliostigma thonningii* roots and stem bark were collected from different trees within Sumitra Teaching and Research Farm, Gujarat, India. It was identified and authenticated by a crop taxonomist (Dr. Stafford. F). The experiment was carried out between February to March, 2019.

Method of plant processing

The collected root and stem bark of *Piliostigma thonningii* were cut into pieces, washed with running tap water to remove all dirty particles and oven dried separately at 60^{o}C for 24 hours, it was later removed from the oven grinded into meal using a pulverizer and stored separately in a well labeled air tight container and kept for further analysis. *Piliostigma thonningii* stem bark was labeled as (PSSB) while *Piliostigma thonningii* root as (PSSR).

Laboratory analysis of PSSB and PSSR

Crude fibre, crude protein, moisture, ether extract and moisture content were determined according with the official methods of the association of official analytical chemist (AOAC, 2000) and all samples were evaluated in triplicates.

Dry matter (DM) = 100 – moisture content

Energy value (KJ/100g) was calculated using the equation below:

Energy = (37× Ether extract) + (17 × carbohydrate) + (17 × crude protein)

% NFE = % DM – (% EE + % CP + % ash + % CF)

Where NFE = nitrogen free extract; EE = ether extract; CP = crude protein; CF = crude fibre

Phytochemical evaluation of anthraquinones tannins, alkaloids, saponins, flavonoids, phenols, oxalate, glycosides, steroids and terpenoids were estimated using methods described by Harbone (1973), Odebiyi and Sofowora (1978), Boham and Kocipai (1974). Mineral analyses of calcium, phosphorus, potassium, sodium, magnesium, manganese, zinc, iron, cobalt, copper, chromium selenium, cadmium and lead were determined using Atomic Absorption Spectrophotometer (AAS – Model 156Y) based on (AOAC, 2000).

Statistical analysis

The analyses were done in triplicates and the data obtained were expressed as mean ± standard error of the means (mean ± S.E.M). The data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined Duncan multiple range test (Duncan, 1955). Significant was declared if $P \leq 0.05$.

3. Results and Discussion

Table 1 reveals the proximate composition of *Piliostigma thonningii stem* bark (PSSB) and *Piliostigma thonningii* root (PSSR). PSSB contained moisture, dry matter, crude protein, crude fibre, ether extract, ash, total carbohydrate, nitrogen free extract and energy at 7.11 %, 92.89 %, 4.22 %, 52.81 %, 0.08 %, 8.11 %, 20.12 %, 27.67 % and 488.7 (KJ/100g) respectively. PSSB contained moisture (8.34 %), dry matter (91.66 %), crude protein (7.40 %), crude fibre (41.60 %), ether extract (2.10 %), ash (2.10 %), carbohydrate (42.30 %), nitrogen free extract (27.76 %) and energy (922.6 KJ/100g). The moisture content reported

in PSSB and PSSR were higher than values reported for Maerua angolensis stem bark (3.58 %), Moringa olifera stem bark (2.36 %), Moringa olifera seed husk (2.51 %), Urena lobata leaves (5.55 %), Daniellia oliveri stem bark (6.25 %) reported by Ezekiel et al. (2019); Andrew et al. (2018); Ogundele et al. (2017) and Alagbe et al. (2020) but much lower than that of Morinda lucida stem bark (9.00 %), Jatropha curcas root (9.77 %), Telfaria occidentalis stem (9.20 %) reported by Olanipekun et al. (2016) and Atamgba et al. (2015). According to Alagbe et al. (2020) low moisture content favours the shelf life of a sample. Crude protein (CP) in PSSR (7.40 %) is found higher than that of PSSB (4.22 %). However, both samples cannot be used as protein supplement in livestock feed because their CP level is less than 20 % (NRC, 1994). The CP values are also lower than values for *Eucalyptus camaldulensis* root (6.35 %) *and Hibiscus sabdariffa* stem bark (6.35 %) reported by Ojewumi and Dedeke (2020). Crude fibre, ether extract, ash, carbohydrate and energy in PSSB were significantly higher (*P <0.05*) than those of PSSR. Higher crude fibre is advantageous in efficient digestion of food, reduces the risk of cardiovascular diseases and lowers serum cholesterol level (Fasola *et al*., 2011). Ash content of a sample is a reflection of the amount of minerals it contains; therefore PSSB contains appreciable level of minerals which can contribute meaningfully to the nutrient needs of animals (Onwuka, 2005). According to Pamela et al. (2005); Alagbe et al. (2020) fats or ether extracts are important in diet for energy, play a key role in the transport of fat soluble vitamins and protection of internal tissues. PSSB is also rich in carbohydrate and energy which makes it useful energy source for livestock. The value reported for PSSR and PSSB are lower than values for Alstonia boonei stem bark (20.49 %), Alstonia boonei root (31.39 %) reported by Abu et al. (2006).

Table 1 Proximate composition of PSSB and PSSR

Parameters	PSSB	PSSR
Moisture (%)	7.11 ± 0.02	8.34 ± 0.01
Dry matter (%)	92.89 ± 0.01	91.66 ± 0.00
Crude protein (%)	4.22 ± 0.04^{b}	7.40 ± 0.05^{a}
Crude fibre (%)	52.81 ± 0.93^{b}	41.60 ± 0.15^{a}
Ether extract (%)	0.08 ± 0.01^{a}	2.10 ± 0.05^{b}
Ash (%)	8.11 ± 0.12^{b}	12.8 ± 0.10^{a}
Total carbohydrate	20.12 ± 0.40^{a}	42.30 ± 0.72^{b}
NFE	27.67 ± 0.01	27.76 ± 0.00
Energy (KJ/100g)	488.7 ± 10.91^{b}	922.6 ± 12.35^{a}

Values expressed as mean ± SEM (n=3)

Means in the same row with different superscripts differ significantly (*P*<0.05)

The mineral composition of PSSB and PSSR is presented in Table 2. PSSB contained calcium (55.19 mg/100g), phosphorus (29.93 mg/100g), potassium (18.73 mg/100g), magnesium (40.10 mg/100g), zinc (12.09 mg/100g), manganese (3.11 mg/100g), iron (48.12 mg/100g), sodium (20.40 mg/100g), copper (8.01 mg/100g), cobalt (0.021 mg/100g), chromium (0.010 mg/100g), selenium (0.28 mg/100g), cadmium (0.028 mg/100g) and lead (0.010 mg/100g) while those of PSSR contained calcium, phosphorus, potassium, magnesium, zinc, manganese, iron, sodium, copper, cobalt, chromium, selenium, cadmium and lead at 87.63, 43.12, 21.08, 48.95, 18.01, 7.32, 53.17, 27.12, 12.54, 0.048, 0.002, 1.56, 0.040 and 0.016 (mg/100g). The mineral composition of PSSB and PSSR revealed a significant difference (P<0.05) indicating that PSSR leave is rich in minerals when compared to PSSB. Calcium, phosphorus, potassium, magnesium and sodium values obtained in PSSB and PSSR were lower than the values reported for Waltheria indica root and stem bark (110, 120, 110, 140 and 90 mg/100g) and (120, 140, 120, 150 and 90 mg/100g) respectively by Afisu et al. (2016). However, all values were within the range recommended by WHO (1991). Calcium provides rigidity to the skeleton and acts as an activator for several key enzymes, including pancreatic lipase, acid phosphatase, cholinesterase, ATPases, and succinic dehydrogenase (Arinola et al., 2008). Phosphorus performs a key role in energy, cell metabolism and regulates the normal acid base balance (Ibrahim et al., 2001). Magnesium stimulates muscle and nerve irritability (contraction), is involved in the regulation of intracellular acid-base balance, and plays an important role in carbohydrate, protein and lipid metabolism (NHWC, 2002). Potassium is the major cation of intracellular fluid, and regulates intracellular osmotic pressure and acid-base balance (Asagba et al., 2004). Iron is a key component of the respiratory pigments haemoglobin, myoglobin and various enzyme systems including the cytochromes, catalases, peroxidases, and the enzymes xanthine and aldehyde oxidase, and succinic dehydrogenase (Malhotra, 1998; Alagbe 2020). Zinc serves as a cofactor in many enzyme systems and also plays a vital role in lipid, protein, and carbohydrate metabolism; being particularly active in the synthesis and metabolism of nucleic acids (RNA) and proteins (NHWC, 2002). Manganese is essential for bone formation, the regeneration of red blood cells, carbohydrate metabolism, and the reproductive cycle

(Olafadehan et al., 2020). Cobalt is an integral component of cyanocobalamin (vitamin B12), and as such is essential for red blood cell formation and the maintenance of nerve tissue (Abdennour et al., 2014). Copper plays a key role iron metabolism, skin pigmentation, formation of bone and connective tissue (Beldi et al., 2006). Selenium is an essential component of the enzyme glutathione peroxidase and it influences the absorption and retention of vitamin E (Chia et al., 1992). Chromium is a cofactor for the hormone insulin and is pivotal in cholesterol and amino acid metabolism (Asagba and Obi, 2000). Sodium is an important intracellular cation involved in the regulation of acid base balance as well as muscle contraction (Akpanyung, 2005). Lead and cadmium are heavy metals when in excess can cause a deleterious effect in the general performance of an animal (Alagbe, 2016).

Table 2 Mineral composition of PSSB and PSSR

Parameters	PSSB (mg/100g)	PSSR (mg/100g)	WHO recommendation in mg/100g (1991)
Calcium	55.19 ± 0.01^{a}	87.63 ± 0.02^{b}	36.0 – 80.00
Phosphorus	29.93 ± 0.02^{a}	43.12 ± 0.01^{b}	20.0 – 45.00
Potassium	18.73 ± 0.01^{b}	21.08 ± 0.02^{a}	10.0 – 25.00
Magnesium	40.10 ± 0.00	48.95 ± 0.17	-
Zinc	12.09 ± 0.02	18.01 ± 0.00	15.0 – 50.00
Manganese	3.11 ± 0.03^{b}	7.32 ± 0.01^{a}	10.0 – 20.00
Iron	48.12 ± 0.15^{b}	53.17 ± 0.04^{a}	5.00 – 50.00
Sodium	20.40 ± 0.09	22.72 ± 0.56	4.00 - 50.00
Copper	8.01 ± 0.02^{b}	12.54 ± 0.01^{a}	10.0 – 30.00
Cobalt	0.021 ± 0.000^{b}	0.048 ± 0.000^{a}	-
Chromium	0.010 ± 0.000	0.002 ± 0.000	0.12 - 0.80
Selenium	0.28 ± 0.03	1.56 ± 0.02	-
Cadmium	0.028 ± 0.000	0.040 ± 0.000	-
Lead	0.010 ± 0.000^{b}	0.016 ± 0.000^{a}	0.50 – 0.300

Values expressed as mean ± SEM (n=3)

Means in the same row with different superscripts differ significantly (P<0.05)

The phytochemical analysis of PSSB and PSSR is presented in Table 3. PSSB contained alkaloids (5.11 %), flavonoids (3.46 %), terpenoids (0.49 %), hydrolysable tannins (1.92 %), condensed tannins (0.19 %), phenols (4.00 %), saponins (0.22 %), steroids (1.18 %), phytates (0.51 %), glycosides (0.10 %) and cyanates (0.15 %) while PSSR contained alkaloids, flavonoids, terpenoids, hydrolysable tannins, condensed tannins, phenols, saponins, steroids, phytates, glycosides and cyanates at 7.23 %, 5.10 %, 0.71 %, 2.41 %, 0.10 %, 5.02 %, 0.17 %, 2.00 %, 0.77 %, 0.08 % and 0.03 % respectively. Both samples follow similar pattern as alkaloids > phenols > flavonoids > hydrolysable tannins > steroids > terpenoids > phytates > saponins > condensed tannins > cyanates > glycosides. The phytochemical analysis of the *Piliostigma thonningii* plant showed that the root is abundant in bioactive chemicals or secondary metabolites when compared to the stem bark ($P < 0.05$). According to Oluwafemi et al. (2020) variations in phytochemicals or bioactive chemicals in plants could be attributed to geographical locations, age or stage of maturity, extraction or processing method, soil type etc. The presence of these chemicals confers plants the ability to performs multiple biological activities such as inflammatory, antimicrobial, antifungal, antihelminthic, antiviral, antioxidants etc. (Akintayo and Alagbe, 2020).

The alkaloids value in PSSB and PSSR is higher than the values for Adansonia digitata stem bark (0.42 %), Adansonia digitata root (0.097 %), Alstonia boonei stem (0.33 %), Alstonia boonei root (0.07%), Anacardium occidentalis root (0.20 %), Anacardium occidentalis stem (0.40 %), Azadirachta indica stem bark (0.47 %), Azadirachta indica root (0.22 %), Carica papaya stem bark (0.11 %), Carica papaya root (0.32 %), Chromolaen aodorata root (0.17 %), Chromolaen aodorata stem bark (0.27 %), Chrysophylla malbidum root (0.23 %) and Citrus aurantifolia stem bark (0.11 %) reported by Taiye and Pass (2014).

Tannins are a very complex group of plant secondary metabolites, which are soluble in polar solution and are distinguished from other polyphenolic compounds by their ability to precipitate proteins (Silanikove *et al.*, 2001). Tannins are secondary compounds present in plants and comprise polyphenols of great diversity (Hoste *et al.*, 2006). Condensed tannins are more widely distributed in higher plant species than the hydrolysable variety and are thought to be more active in precipitating proteins (Dykes *et al.,* 2005). Tannins are also known to posses' antibacterial and antiviral activities and type of tannins

synthesized by plants vary considerably depending on plant species, stage of development and environmental condition (Cornell, 2005; Enzo, 2007). Phytic acid and/or phytates compete with essential dietary minerals such as calcium, zinc, iron and magnesium to make them biologically unavailable for absorption (Alagbe, 2020). High oxalate diet can increase the risk of renal calcium absorption and has been implicated as a source of kidney stones (Chai and Liebman, 2004). Saponins have been suggested to be involved in antibacterial and anti-inflammatory activities (Cheeke, 2000). Phenolic acids are derivatives of benzoic or cinnamic acids derivatives to form hydroxybenzoic and hydroxycinnamic acids, respectively (Dykes and Rooney, 2006). They are antioxidants which are capable of scavenging free radicals thus preventing diseases (Olafadehan et al., 2020). Flavonoids perform multiple activities of antifungal, antibacterial and antioxidant properties (Saleem et al., 2005). Alkaloids have been shown to perform analgelsics, antibacterial and antiplasmodic properties (Kasolo et al, 2010).

Table 3 Phytochemical analysis of PSSB and PSSR

Parameters (%)	PSSB	PSSR
Alkaloids	5.11 ± 0.01^{a}	7.23 ± 0.03^{b}
Flavonoids	3.46 ± 0.03^{b}	5.10 ± 0.00^{a}
Terpenoids	0.49 ± 0.00^{a}	0.71 ± 0.02^{b}
Hydrolysable tannins	1.92 ± 0.02^{b}	2.41 ± 0.00^{a}
Condensed tannins	0.19 ± 0.01^{a}	0.10 ± 0.02^{b}
Phenols	4.00 ± 0.04^{b}	5.02 ± 0.04^{a}
Saponins	0.22 ± 0.00^{a}	0.17 ± 0.00^{b}
Steroids	1.18 ± 0.02^{a}	2.00 ± 0.04^{b}
Phytates	0.51 ± 0.01^{a}	0.77 ± 0.02^{b}
Glycosides	0.10 ± 0.03^{a}	0.08 ± 0.00^{b}
Cyanates	0.15 ± 0.02^{a}	0.03 ± 0.01^{b}

Values expressed as mean ± SEM (n=3)

Means in the same row with different superscripts differ significantly ($P<0.05$)

4. Conclusion

The increasing concern about the extensive and indiscriminate use of antibiotics in livestock production has encouraged several researches on the use of medicinal plants as potential alternatives because of the presence of phytochemicals which have been identified to perform multiple biological activities. To ensure food safety natural products needs to be prioritized; it was concluded from the experiment that PSSR contains higher concentrations of minerals and bioactive chemicals which when explored in livestock production will improve performance and its general health status.

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