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Qualitative and Quantitative Phytochemical Analysis of Methanol Extracts of *Phragmanthera incana* (schum) Leaves Parasitized on South-West-Nigeria Host Trees

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Abstract

Phragmanthera incana (Schum), a specie of mistletoe, belonging to the family *Loranthaceae*, is a hemi-parasitic plant growing on trees in South-Western part of Nigeria. The phytochemical constituents of *P. incana* from four host trees; *Psidium guajava* (guava), C*ola acuminata* (kolanut), *Anacardium occidentale* (cashew) and *Mangifera indica* (mango) were analysed qualitatively and quantitatively following standard protocols. The Qualitative screening showed the presence of tannins, anthraquinone, flavonoids, phenols, reducing sugar, cardiac glycosides, terpernoids, saponin and steroids in all the four selected host trees. Quantitative evaluation showed a significantly high ($p < 0.05$) Phenol and Steroid contents in *P. incana* from cashew tree when compared with *P. incana* from guava, kolanut and mango trees. A significant increase ($p < 0.05$) in Saponin, Terpernoids and Cardiac glycosides was recorded in *P. incana* from mango tree when compared with *P. incana* from cashew, guava and kolanut trees while a significantly high ($p < 0.05$) reducing sugar was in *P. incana* from kolanut tree when compared with *P. incana* leaves from cashew, guava and mango trees. It can be concluded that the plant has immense potential to be used in the area of pharmacology and as a prospective target for drug formulation.

Key Words: Phytochemicals, *Phragmanthera incana*, Mistletoes, Hemi-parasitic plants

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Introduction

Phytochemicals are natural constituents present in plants, which work in conjunction with nutrients and dietary fibers to act as defense against diseases and to slow the aging process (Ekwueme *et al.,* 2015; Igwenyi *et al*., 2011). The medicinal values of plants lie in these chemical constituents which produce definite physiological action on the human body (Akinmoladun *et al.*, 2007). The chemical constituents are divided into two groups; primary and secondary constituents, based on their functions in plant metabolism. The primary constituents are common sugars, amino acids, proteins and chlorophyll, while the secondary constituents are alkaloids, terpenoids, flavonoids, tannins, phenolic compounds, saponins, cardiac glycosides and many more (Krishnaiah *et al*., 2009).

Phragmanthera incana (Schum), a specie of mistletoe belonging to the family *Loranthaceae*, is a hemi-parasitic plant growing on trees in South-Western parts of Nigeria. It is locally referred to as “Afomo Onishana” in Yoruba, “Kauchin” in Hausa and “Awuruse” in Igbo language. *P. incana* is a woody plant, with stems up to 2 m long; its young parts are densely covered with brown hairs and the berries are red in color (Figure 1). It is majorly found in secondary jungle and bush savanna area; from Sierra Leone to West Cameroon and Fernando Po Island (Gulf of Guinea that forms part of Equatorial Guinea), and extending across the Congo basin to Zaire, Angola and Nigeria (Ogunmefun et al., 2013). Its taxonomy kingdom is Plantae, *division*: Tracheophyta, *subdivision*: Spermatophytina, *class*: *Magnoliopsida, order: Santalales, family: Loranthaceae, genus: Phragmanthera, species: incana* and *botanical name: Phragmanthera incana.* Ogunmefun et al., (2013) had previously reported the phytochemical analysis of *P. incana* leaves on cocoa and kolanut trees however this study aimed to investigate the qualitative and quantitative phytochemical constituents of *P. incana* leaves on four host trees; *P. guajava* (Guava), C. *acuminata* (Kolanut), *A. occidentale* (Cashew) and *M. indica* (Mango) and compare them.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh leaves of *P. incana* from four host trees were collected from their natural habitat in a forest at Imota, Ikorodu Local Government Area of Lagos State Nigeria. It was identified and authenticated at Forest herbarium of Forest Research Institute (FRIN) Ibadan.

Preparation and Extraction of Plant Material

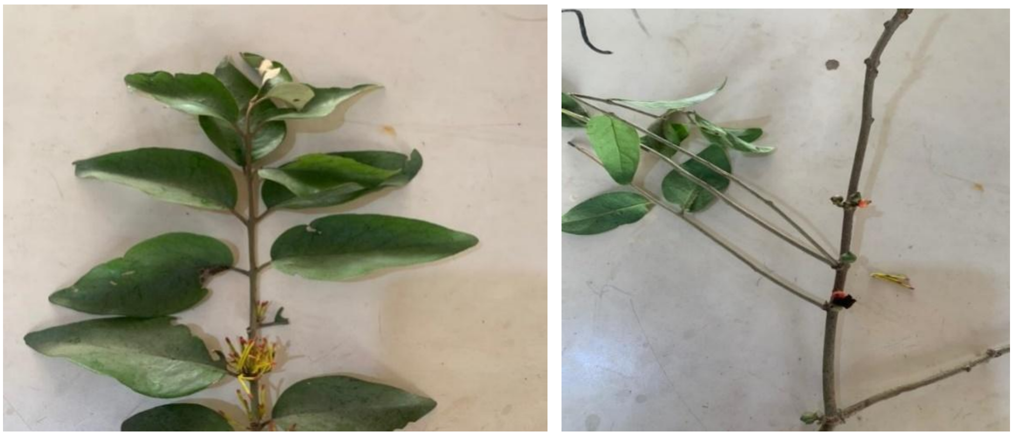
The leaves were washed under running water to remove debris and contaminants and air dried separately for one week. The dried leaves were pulverized using mechanical grinder and stored in an air tight container until further use. The pulverized samples were extracted for 48 h by cold maceration using 70% methanol at 1:6 w/v. The mixture was filtered using Whatman filter paper no. 1, and then concentrated in a rotary evaporator at 40 °C. The extracts obtained were stored at below 4 ^{o}C until further use.

Qualitative Phytochemical Screening of Extracts

The detection of phytochemical composition of the four methanol extracts of *P. incana* leaves were carried out using procedures reported by Anyasor *et al.,* (2010); Evans (1996) and Harborne (1973). The samples were qualitatively assayed for tannins, flavonoids, alkaloids, phenol, saponin, terpenoid, anthraquinones, cardiac glycosides, reducing sugar and steroids.

Quantitative Phytochemical Analysis

All the phytochemicals were determined using colorimetric method as follows. Tannin content was assayed according to Noha et al., (2011). Flavonoids were measured against quercetin standard (Ordonez et al., 2006). Alkaloids were determined according to Harborne (1973), while total phenolic content was estimated using Folin-Ciocalteau method of Singleton et al., (1999). Terpenoid and Steroids were also determined according to Trease and Evans (1989). Saponins were measured according to the method of Okwu and Josiah (2006). Cardiac glycosides were assayed using Baljet’s reagent according to Solich et al., (1992) as modified by Tofighi et al., (2016). Reducing sugars were determined using the method described by Trease and Evans 1989 as modified by Krivorotova & Sereikaite, (2014).



a) b)

Figure 1: *P. incana* leaves, flower and berries

RESULTS AND DISCUSSION

The qualitative phytochemical content as represented in Table 1 indicates the presence of tannin, flavonoids, phenol, saponin, terpernoid, cardiac glycosides, anthraquinone, reducing sugar and steroids in *Phragmanthera incana* leaves analysed. Anthraquinone was present in *P. incana* leaves from cashew and kolanut but not detected in the other two host trees. Alkaloid is present in *P. incana* leaves from guava, cashew and kolanut but not detected in *P. incana* leaves from mango. This study further confirms the presence of the identified chemical constituents in *P. incana* as previously reported by Ogunmefun et al., 2013.

Quantitative phytochemical studies revealed that in *P. incana* leaves, there are varying amount of phytochemicals present in the selected host trees similar to the report by Fasanu and Oyedapo, (2008) and Ishiwu et al., (2013) on mistletoe. The quantitative analysis corroborate the identified phytochemical content variation of *P. incana* leaves as reported by Ogunmefun et al., (2013). Phytochemicals such as phenols, tannin, flavonoids, saponin, terpernoids, cardiac glycosides and reducing sugar have been reported for their values in health care management. Previous studies of plant extracts and fractions have linked plant bioactive compounds to be involved in the prevention of gastrointestinal, cardiovascular and neurodegenerative diseases (Fernandes *et al.*, 2017; Sharman & Kanwar, 2018). Quantitative phytochemical analysis on *P. incana* leaves from the four host trees as shown in Table 2 indicates that tannin composition in the samples ranged from 50.34 ± 0.14 mg/100 g in *P. incana* leaves from mango to 52.33 ± 0.20 mg/100 g in *P. incana* leaves from guava. Highest flavonoids was in *P. incana* leaves from cashew. Flavonoids such as quecertin shows anti-lipase activity, prevents adipogenesis and induces cell death in mouse pre-adipocytes (Zheng *et al.*, 2010; Fang *et al.*, 2008). Kaempferol extracted from *Bauhinia fortificate* leaves reduces hyperglycemia (Jorge *et al.*, 2004). Isorhamnetin found in medicinal plants such as *Ginkgo bilobia, Hippophae rhamnoides, Oenanthe javanica and Opuntia ficus-indica* possess anti-obesity activities (Rodriguez-Rodriguez *et al.*, 2015). Flavonoids have be shown to be hypolipidermic by preventing the oxidation of low density lipoprotein. (Scalbert *et al.*, 2005).

Alkaloid was present in *P. incana* leaves from guava, cashew and kolanut with *P. incana* leaves fron guava having the highest amount (56.24 ± 0.13 mg/100 g). Alkaloids such as isoliensinine, liensinine, synephrine, piperine, piperlongumine neferine and nuciferine have exhibited potential effect on obesity (Acharya & Shrivastava, 2008). Caffeine and chlorogenic acid causes reduction in body mass and body fat by decreasing glucose absorption. Decrease in glucose absorption

Table 1: Qualitative Phytochemical Contents of P. incana Leaves from Four Host Trees

	Tannin	Flavonoids	Alkaloid	Phenol	Saponin	Terpernoid	Anthra-quinone	Cardiac glycoside	Reducing sugar	Steroid
PIPG	+	+	+	+	+	+	-	+	+	+
PICA	+	+	+	+	+	+	+	+	+	+
PIAC	+	+	+	+	+	+	+	+	+	+
PIMI	+	+	-	+	+	+	-	+	+	+

+ indicates present, - indicates not present
PIPG (P. incana from *P. guajava), PICA (P. incana from C. acuminata) PIAO (P. incana* from *A. occidentale) PIMI (P. incana from M. indica)*

Table 2: Quantitative chemical constituents

Phytochemical (mg/100g)	**PIPG**	**PICA**	**PIAO**	**PIMI**
Tannin	52.33 ± 0.20	51.05 ± 0.17	51.82 ± 0.85	50.34 ± 0.14
Flavonoids	29.97 ± 0.18	28.48 ± 0.22	32.66 ± 0.14	30.93 ± 0.14
Alkaloid	56.24 ± 0.13	46.51 ± 0.23	48.63 ± 0.22	-
Phenol	46.60 ± 0.39	48.44 ± 0.12	50.00 ±0.20[**]	46.02 ± 0.20
Saponin	43.11 ± 0.40	44.29 ± 0.14	42.91 ±0.33	48.95 ± 0.20[**]
Terpernoid	25.00 ± 0.13	24.15 ± 0.13	30.94 ± 0.21	32.09 ± 0.26[**]
Cardiac glycosides	36.36 ± 0.26	33.58 ± 0.16	33.75 ± 0.13	39.63 ± 0.25[**]
Reducing sugar	29.99 ± 0.38	36.90 ± 0.19[a**]	30.29 ± 0.23	33.92 ± 0.15
Steroid	24.18 ± 0.25	25.30 ± 0.16	26.43± 0.16[**]	23.25 ± 0.20

- Indicates not detected

[**] Significantly increased ($p < 0.05$) amount when compared with other methanol extracts

PIPG (P. incana from *P. guajava), PICA (P. incana* from *C. acuminata) PIAO (P. incana* from *A. occidentale) PIMI (P. incana* from *M. indica)* eventually causes an increase in the utilization of fats reserves, due to reduced availability of glucose as an energy source (Hom, 2007; Tajik *et al.,* 2017).

Phenol concentration varies from 46.02 ± 0.20 mg/g in *P. incana* leaves from mango to 46.60 ± 0.39 mg/100 g in *P. incana* leaves from guava. Phenols such as p-coumaric, cafferic acid, ferulic acid, cinnamic acid, ellagic and p-hydroxybenzonic acid are abundant class of naturally occurring phytochemicals which have been shown to modulate physiological and molecular pathways that are involved in energy metabolism, adiposity and obesity (Nobili *et al.,* 2009; de Melo *et al.,* 2017).

Saponin ranged from 42.91 ±0.33 in PIAO to 48.95 ± 0.20 mg/100 g in *P. incana* leaves from mango. Saponins are steroid or triterpenoid glycosides characterized by their astringent taste and foaming properties. They possesses antidiarrheal, anti-inflammatory and cholesterol lowering property (Mandeau *et al.,* 2005). The non-sugar part of saponins has a direct antioxidant activity which may result in reduced risk of cancer and heart diseases (Manjunatha, 2006). Dietary teasaponin, saponins from Platycodi Radix, and onion skin extract were reported to decrease postprandial plasma triglyceride response after lipid load and showed anti-obesity effects in animals fed high-fat diets (Kim, 2007; Sharman & Karwar, 2018).

Terpenoids have the lowest amount in *P. incana* leaves from guava and the highest in *P. incana* from Mango tree. Cardiac glycosides are more abundant in *P. incana* leaves from mango (39.63 ± 0.25 mg/100 g) when compared with *P. incana* from the other host trees analysed. Reducing sugar amount was highest (36.90 ± 0.19 mg/100 g) in *P. incana* from kolanut. Steroid estimation from the four host trees shows that PIAO possess the highest amount 26.43 ± 0.16 mg/100 g while PIMI has the lowest 23.25 ± 0.20 mg/100 g. There was a significantly high (p < 0.05) Phenol and Steroid contents in *P. incana* from cashew tree when compared with *P. incana* from guava, mango and kolanut trees. A significantly high (p < 0.05) Saponin, Terpernoids and Cardiac glycosides was in *P. incana* from mango tree when compared *P. incana* from cashew, guava and kolanut trees while a significantly high (p < 0.05) reducing sugar in *P. incana* from kolanut tree when compared with *P. incana* from cashew, mango and guava trees. Phytosterols such as diosgenin, campesterol, brassicasterol, sitosterol, stigmaterol and guggulsterone appear to reduce obesity. High intakes of these compounds decrease LDL-cholesterol levels Terpenoids such as Gymnemic acid, oleanolic acid and corosolic acid have potential therapeutic activity on obesity (Kanetkar *et al.,* 2007; Osman *et al.,* 2010). Geranyl geraniol, farnesol and geraniols terpenoids are ligands with potential to activate PPAR, dietary lipid sensors that control energy homeostasis as well as lipid and carbohydrate disorders (Takahashi *et al.,* 2002).

CONCLUSION

The qualitative and quantitative phytochemical analysis showed that *P. incana* leaves from the four selected host trees contains flavonoids, tannins, terpenoid, phenols, reducing sugar, cardiac glycosides, terpenoids, saponin and steroids. The plant contains chemical constituents of therapeutic potentials. However, composition varies from one host tree to the other. The leaves from the host trees should be further explored for their pharmacologic properties.

Conflict of Interest

The authors declares that there is no conflict of interest.

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