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Journal of Pharmacognosy and Phytotherapy

*Full Length Research Paper*

# Phytochemical and zootechnical studies of *Physalis peruviana* L. leaves exposured to streptozotocin-induced diabetic rats

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**This is a phytochemical and zootechnical study on *Physalis peruviana* leaves in streptozotocin induced diabetic rats. This was part of a scientific development program of plant resources used in Congolese traditional medicine for the treatment of diabetes mellitus in which individual and community consequences are well established. Different fractions with hexane, ethyl acetate and the residue were obtained from the hydroalcoholic extract of *P. peruviana* leaves. Phytochemical screening was focused on the usual reactions of characterization based on precipitation and coloration with general reagents. The diabetic conditions were induced in rats by a single administration of streptozotocin (50 mg/kg body weight) intravenously. The positive control group received glibenclamide (6.5 mg/kg body weight) and each test group received 100 mg/kg of body weight. Those groups were compared with a control group which received only a Tween 20 solution (1 ml per 100 g body weight). Zootechnical profiles were evaluated by weight monitoring as well as food and water consumption in rats. Phytochemical screening showed the presence of polyphenols, flavonoids, alkaloids, saponins, tannins, anthocyanins, mucilages, cardiac glycosides, coumarins and betalains in the hydroalcoholic extract and its fractions. A highly significant difference ($P < 0.001$) of water consumption in opposition to the food intake and weight changes was observed. This study suggested the isolation and characterization of compounds from hydroalcoholic extract from the leaves of *P. peruviana* L. and its fractions for an extensive antidiabetic investigation.**

**Key words:** *Physalis peruviana*, phytochemical, antidiabetic activity, streptozotocin, zootechnical parameters.

## INTRODUCTION

Several pathophysiological processes are involved in the development of diabetes mellitus. These range from autoimmune destruction of the β-cells of the pancreas with a consequent insulin deficiency to abnormalities that result in resistance to insulin action (Armelle et al., 2008; Arika et al., 2016).

Diabetes mellitus is the most prevalent disease in the world, affecting 25% of the population and afflicts 150 million people and is predicted to rise to 300 million by 2025 (WHO, 2002; Babu, 2016). Conventional management of diabetes is expensive and therefore not affordable by many patients, especially in developing nations. More so, conventional drugs are not readily available and have been found to have side effects with long term use (Arika et al., 2015; Deeni and Sadiq, 2002). The distinctive traditional medical opinions and natural medicines have shown a bright future in the therapy of diabetes mellitus and its complications (Ekramul et al., 2002; Arika et al., 2016).

The World Health Organization (WHO, 2002) recommended the use of medicinal plants for the management of DM and further encouraged the expansion of the frontiers of scientific evaluation of hypoglycemic properties of diverse plant species (WHO, 2002; Kwete et al., 2002; Chikezie et al., 2015).

Plants have potential sources of hidden phyto-constituents which can be responsible for solving various potential health problems (Noumi and Yomi, 2001; Kwete et al., 2007). Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of plants (Li et al., 2007; Patil, 2016). They are also associated with reduced risks of cancer, cardiovascular disease, diabetes and lower mortality rates of several human diseases (Momeni et al., 2005; Ozkan et al, 2016).

*Physalis peruviana* (Solanaceae) is a medicinal plant widely used in folk medicine for treating diseases such as malaria, asthma, hepatitis, dermatitis, cancer, diuretic, rheumatism, antispasmodic, diuretic, antiseptic, sedative, analgesic diseases, and has antioxidant, antifungal, antibacterial, anti-inflammatory, cataract-cleaning, antidiabetic and anti-parasitic properties (Mariotte et al., 2005; Kasali et al., 2013a; Çakir et al., 2014; Joshi and Joshi, 2015; Lashin et al., 2016; Higaki et al., 2016; Chang et al., 2016).

In a survey conducted in the Eastern part of the Democratic Republic of the Congo (DRC), a number of traditional healers pointed out the use of *P. peruviana* L. leaves for this purpose (Kasali et al., 2013b). The objectif of this study was to analyze the phytochemical composition and evaluate zootechnical parameters of a hydroalcoholic extract and its fractions in diabetic rats.

## MATERIALS AND METHODS

### Study sites

The present study was undertaken at the laboratory of Pharmacognosy, Faculty of Medicine and Pharmacy, Official University of Bukavu/Republic Democratic of Congo) and chemical study of medicinal plants, bacteria, fungi and endophytes was done at Faculty of Sciences, University of Yaounde 1/Cameroon; Phytochemical laboratory of Higher Teachers' Training College (Faculty of Sciences, University of Yaounde 1) and laboratory of Toxicological and Pharmacological Studies (Faculty of Medicine and Biomedical Sciences/ University of Yaounde 1). This study was conducted between September 2015 and April 2016.

### Plant material

The leaves of *P. peruviana* L. (Solanaceae) were collected at Lwiro (Center for Research in Natural Sciences, Democratic Republic of Congo) situated at 50 km from Bukavu (South Kivu, Democratic Republic of Congo). They were identified a by Mr. Gentil IRAGI of Botany Department of this center and compared with voucher specimen No.2044. The leaves were air-dried and powdered for analysis.

### Preparation of hydroalcoholic extract and its fractions

800 g of the powdered leaves of *P. peruviana* were macerated with 6 L of 70% EtOH (Jothi et al., 2015) for 48 h and the combined filtrate (using the Whatman filter paper No. 1) was evaporated under reduced pressure using a rotary evaporator. A dried extract with a yield of 28.95% was obtained. One part of the filtered hydroalcoholic extract was stored in a refrigerator at 4°C. Another part of this extract was soaked in hexane and decanted into a funnel. The hexane fraction was concentrated in a rotary evaporator (BÜCHI 461 water Bath). This operation was repeated several times until total exhaustion (the solution has become colorless). The same operations were carried out with ethyl acetate. The residue from this fraction was concentrated under reduced pressure using a rotary evaporator. The following yields were obtained: 3.19 and 25.06%, respectively, for hexane and ethyl acetate.

### Animals

Healthy male albino Wistar rats (body weight 175 ± 10.6 g) aged 2-3 months, were used in the study. The rats were maintained under standard laboratory conditions at 27.75 ± 1°C, and normal photo period (12 h dark/12 h light) was used for the experiment. The rats were acclimatized to the laboratory conditions a week prior to the experiment.

The experimental protocol and the maintenance of the experimental animals was done in accordance with the regulations of the Organization for Economic Co-operation and Development (OECD) guide since in Cameroon, the ethics committee focuses only clinical studies. The animal experiment protocols were carried out in accordance with the guidelines of the ICH on preclinical pharmaceutical testing in mouse (OECD, 2001; Tsague et al., 2016).

### Animal ethical regulatory consideration

Healthy male albino Wistar rats (body weight 150 to 250 g) were ethically required for use for the experiment according to

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the ICH guidelines.

The experimental protocol and the maintenance of the experimental animals was done in accordance with the regulations of the OEDD guide, the EU parliament directives on the protection of animals used for scientific purposes, since in Cameroon, the ethics committee focuses only on clinical studies. The animal experiment protocols was carried out in accordance with the guidelines of the ICH on preclinical pharmaceutical testing in mouse (OECD, 2001; Akbarzadeh et al., 2007).

### Phytochemical screening

Qualitative phytochemical tests of R. heudelotti methanolic extract were carried out according to Odebiyi and Sofowora (1978) methods to identify some components such as alkaloids, saponins, tannins, flavonoids, polyphenols and anthraquinones.

**Test for alkaloids:** 0.5 g of the sample was stirred with 5 ml of 1% aqueous HCl on a steam bath and then filtered. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1 ml portion was treated similarly with Dragendroff reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids in the extract.

**Test for saponins:** The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used for the screening test. 0.5 g of plant extract was shaken with water in a test tube. Frothing which persisted on warming was taken as evidence for the presence of saponins.

**Test for tannins:** 0.5 g of dried extract was stirred with 5.0 ml of distilled water. This was filtered and ferric chloric reagent was added to the filtrate. A blue-black precipitate was taken as evidence for the presence of tannins.

**Test for phenol and polyphenols:** 0.5 g of plant extract was heated for 30 min in a water bath. 3 ml of 5% $FeCl_2$ was added to the mixture, then followed by the addition of 1 ml of 1.00% potassium ferrocyanide. The mixture was filtered and green (phenol) and blue (polyphenol) colours were observed.

**Test for anthraquinones:** 0.5 g of plant extract was shaken with 5 ml of benzene, filtered and 2 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink or violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxy anthraquinones.

**Test for flavonoids:** 0.5 g of plant extract was dissolved in 5 ml of NaOH at 1 N. The change of the yellow colour obtained after adding HCl 1 N indicated the presence of flavonoïds.

### Zootechnical study in Streptozotocin-induced diabetic rats

#### *Induction of diabetes*

Diabetes was induced in fasted rats injecting 50 mg/kg streptozotocin (Sigma, France) in the tail vein. STZ was dissolved in 0.1 M citrate buffer (pH 4.5) (Tadjeddine et al., 2013). Streptozotocin induces diabetes within 3 days by destroying the beta cells (Ziane et al., 2015). After 48 h of STZ administration, blood glucose level of each rat was determined (Anthikat et al., 2016). Before induction, all rats were fasted 12 h (Ngueguim et al., 2016). Rats with serum glucose level above 300 mg/dl were considered as diabetic (Khathi et al., 2013).

### Experimental protocol

The rats were divided into six groups of five rats in each group. Group 1: Untreated rats (control), received vehicle alone (1% tween 20, 1 ml per orally); Group 2: Rats treated with 6.5 mg/kg of glibenclamide, positive control; Group 3: Rats treated with 100 mg/kg of hydroalcoholic extract of *P. peruviana*; Group 4: Rats treated with 100 mg/kg of the hexane fraction of *P. peruviana*; Group 5: Rats treated with 100 mg/kg of the ethyl acetate fraction of *P. peruviana*; Group 6: Rats treated with 100 mg/kg of the residue fraction of *P. peruviana*.

All rats were administered single dose of drug (orally) daily for 28 days. Daily administration was through a gastric gavage by inducing a gastric tube (Gatierrez et al., 2014). The day of administration of first dose was considered the zero day of treatment.

At the end of the experimental period, all animals were deprived of food overnight and then sacrificed by cervical decapitation after anesthetizing by either inhalation (Saini and Sharma, 2013). One touch electronic Glucometer (One Touch Ultra®) was used for glucose measurement.

### Water consumption and food intake

The body weight of each rat was measured once each week and the total amount of food consumed was recorded 3 times per week (Gutierrez et al., 2005).

### Body weight monitoring

Body weights of all animals in each group were monitored using a top loader weighing balance throughout the experimental period (Ofusori et al., 2012).

### Statistical analysis

All results were expressed as mean ± standard error (SE) for each sample. Statistical analysis was performed using GraphPad Prism 5.02 statistical package (GraphPad Software, USA). The data were analyzed by one way analysis of variance (ANOVA) followed by Turkey's multiple comparison post test. Differences between groups were considered to be significant at $P < 0.05$.

## RESULTS

## Phytochemical analysis

The results in the Table 1 represent the phytochemical analysis of some fractions from the hydroalcoholic extract of *P. peruviana* leaves. According to these results, the phytochemical analysis showed the presence of tannins and saponins in all fractions except in the hexane fraction. However, the method used did not show resins and oxalates. In addition, the highest percentage of positive tests were obtained from the hydroalcoholic extract (37.5%) respectively, followed by fractions with ethyl acetate residue (25%) and ethyl acetate (25 %), and finally the hexane fraction (12.5%).

**Table 1.** Phytochemical screening of *Physalis peruviana* leaves.

| Metabolite | Reagent methods | HydAE | HexF | EthAF | ResEAF |
|---|---|---|---|---|---|
| Polyphenols | Ferric chloride<br>Lead acetate | + | - | + | + |
| Flavonoids | Iso amyl alcohol/Mg<br>+ hydrochloric acid | + | - | + | + |
| Alkaloids | Hodger | - | - | - | - |
| | Wagner | + | - | - | + |
| | Mayer | + | + | - | + |
| Cardiac glycosides | Glacial acetic acid/ $FeCl_3$<br>+ Sulfuric acid | + | - | + | + |
| Saponosides | Frothing test | + | - | + | + |
| Tannins | Ferric chloride | + | - | + | + |
| Anthocyanins | Sulfuric acid/Ammonia | + | + | + | - |
| Quinones | Sodium hydroxyde | - | - | - | - |
| Mucilages | Ethanol 95° | + | + | + | - |
| Resins | Glacial acetic acid/ $H_2SO_4$ | - | - | - | - |
| Betalains | Sodium hydroxyde | + | + | - | - |
| Terpenoids and steroids | Liebermann-Burchard | + | - | - | - |
| Coumarins | Ferric chloride/$NHO_3$ | + | - | + | + |
| Oxalates | Glacial acetic acid | - | - | - | - |

+: Positive test, -: negative test, HydAE: hydroalcoholic extract, HexF: hexane fraction, EthAF: ethyl acetate fraction, ResEAF: residue ethyl acetate fraction.

## Zootechnical evaluations

### *Water consumption and food intake*

The water consumption showed a highly significant increase (*** = P > 0.001) in all animals treated against healthy animals (control), however intake food variation showed a highly significant decrease (P > 0.001) in all animals treated as compared to the healthy animals (Figure 1).

### *Body weight monitoring*

There was a highly significant (p< 0.001) body weight changes in all treated rat groups as compared to the control the group (Figure 2). Between groups treated (with glibenclamide and plant extract/fractions), there was no significant difference (P<0.05). There was a significant difference (p< 0.05) between the control group and those treated with the aqueous alcoholic extract.

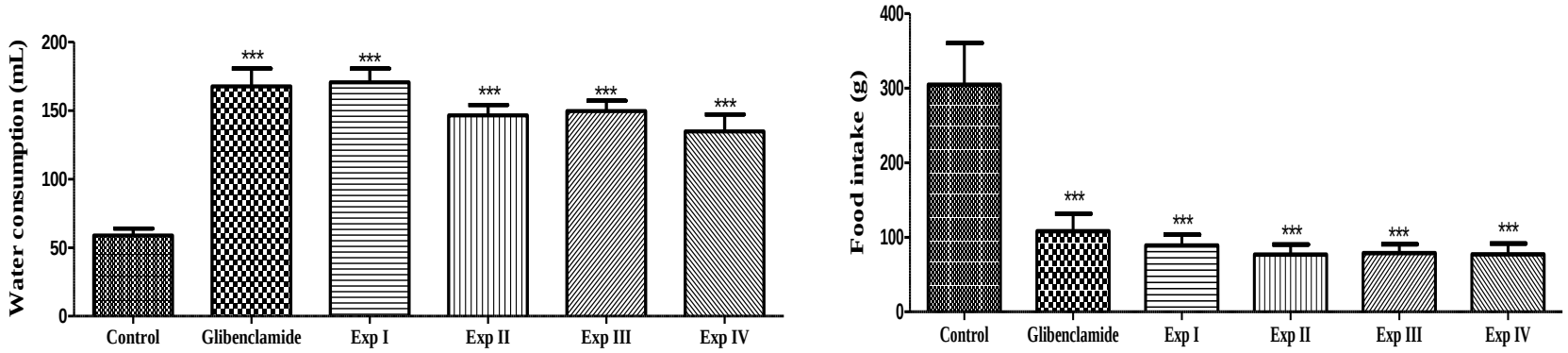
### *Assessment of weight variation of organs*

From the study as shown in Table 2, there was no significant difference (p< 0.05) in the change of organ weight in all animals treated for heart, liver, brain, spleen and the two kidneys. However, a significant difference (p< 0.05) in weight variation of pancreas, liver, brain, lungs and testicles was noticed (Table 2).

## DISCUSSION

There was an uneven distribution in this study of secondary metabolites in the hydroalcoholic extract and its fractions. Saponins and tannins tests were positive in the extract and in two of its fractions (ethyl acetate and its residue fractions), and having 25% of positive tests. Polyphenols compounds, flavonoids, anthocyanins, mucilage, cardiac glycosides and coumarin represented 9.4% in each category. This category is followed by grouping betalains (6.2%). Alkaloids represented 15.6% of positive tests for three different reagents used, which gave an average of 5.2% per reagent. Steroids and terpenes represented 3.1%. Quinones, resins and oxalates are not found (0%).

Many preceding studies reported the presence of some secondary metabolites in the fruit or the leaves of *P. peruviana*. Some studies indicated that cardiac glycoside, alkaloid, saponins, tannins, steroids and terpenoids and flavonoids were present while anthraquinones were absent




**Figures 1.** Water consumption and food Intake.

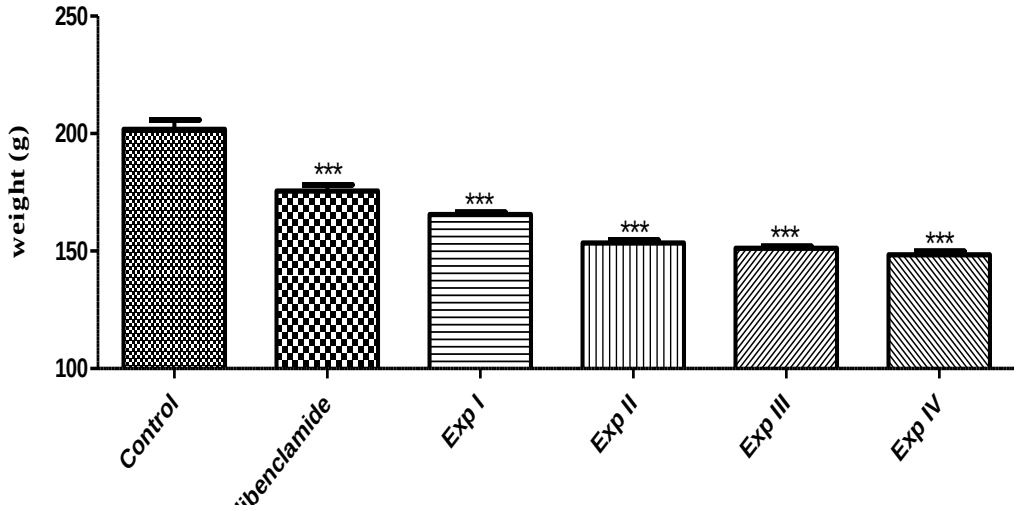



**Figure 2.** Body weight monitoring of test rats when compared with the control.

in the leaves (Moabe et al., 2013; Magambu et al., 2014). A phytochemical screening in regeneration plant, callus from seed, leaf and fruits from mother plants of *P. peruviana*, showed the present of alkaloids, glycosides, cardiac glycosides, saponins, phenol, sterol, tannins, flavonoids and diterpene (Lashin and Elhaw, 2016). A phytochemical investigation of the crude ethanolic extract of *P. peruviana* L. revealed the presence of phenols, flavonoids, phytosterols, glycosides, sterols, saponins, tannins and alkaloids (Ahmed, 2014). Previous phytochemical studies have isolated a number of compounds from *P. peruviana*, such as ticloidine, withanolides, phenolics and phytosterols (Gautam et al., 2015).

**Table 2.** Weight variation of organs.

| Organs | Group 1 | Reference | Exp. I | Exp. II | Exp. III | Exp. IV |
|---|---|---|---|---|---|---|
| Heart | 0.66±0.09 | 0.73±0.04 | 0.57±0.10 | 0.59±0.06 | 0.48±0.06 | 0.55±0.14 |
| Lung | 2.20±0.27 | 2.57±0.08 | 1.06±0.19** | 1.78±0.58 | 1.05±0.1** | 1.90±0.14 |
| Liver | 5.33±0.5 | 7.40±0.56** | 5.72±0.58 | 5.68±0.62 | 5.58±0.39 | 5.69±0.64 |
| Brain | 1.62±0.05 | 1,52±0.07 | 1.52±0.12 | 1.4±0,13* | 1.55±0.08 | 1.55±0.08 |
| Pancreas | 0.77±0.09 | 0.48±0.04** | 0.57±0.05* | 0.45±0.1** | 0.55±0.01** | 0.52±0.1* |
| Spleen | 0.76±0.14 | 0.71±0.15 | 0.69±0.15 | 0.65±0.09 | 0.50±0.10 | 0.96±0.07 |
| Right kidney | 0.67±0.06 | 0.85±0.01 | 0.79±0.11 | 0.67±0.11 | 0.62±0.06 | 0.63±0.02 |
| Left kidney | 0.73±0.05 | 0.84±0.03 | 0.77±0.11 | 0.69±0.14 | 0.62±0.09 | 0.63±0.05 |
| Right testicle | 1.52±0.41 | 1.39±0.03 | 1.03±0.46 | 0.72±0.2** | 0.62±0.03** | 0.62±0.01* |
| Left testicle | 1.54±0.33 | 1.42±0.01 | 1.08±0.41* | 0.71±0.1** | 0.56±0.01** | 0.61±0.02* |

The values are expressed as mean ± SEM of the respective groups (n=5). The weight values of groups are compared with normal control animals, value *p< 0.05 and **p< 0.01. Exp. I: Group treated with 100 mg/kg of hydroalcoholic extract of the plant; Exp. II: Group treated with 100 mg/kg of the hexane fraction of plant; Exp. III: Group treated with 100 mg/kg of the ethyl acetate fraction of plant, Exp. IV: Group treated with 100 mg/kg of the residue fraction of the plant.

*P. peruviana* contain the pseudo-steroids (physalines) and glycosides which show the anticancer activity. From the aerial parts of *P. peruviana*, various withanolide glycosides have been isolated. From the whole plant material, there is isolation of two withanolides (Sharma et al., 2015). Three new physalin steroids, physalinIII, physalin IV, 3-O-methylphysalin X, together with five known physalins were isolated from the 80% EtOH extract of calyces of *P. alkekengi* var. franchetii (Yu et al., 2013).

Withanolides are natural steroidal lactones produced mainly by plants in the Solanaceae that often have many health benefits such as anti-inflammatory activity (Ahmed, 2014). *Physalis* (L.) species contains various carbohydrates, lipids, minerals, vitamins and phytosteroles (Sharma et al., 2015). This genus contains calystegins, alkaloids from nortropane, and steroidal glycoalcalkaloids from spirosolane (Jouzier et al., 2005; Xu et al., 2013).

A number of phytochemical are known, some of which include: alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids. They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more. Phytochemicals are responsible for the medicinal activity of plants and they have protected human from various diseases (Savithramma et al., 2011). Many classes of plants secondary metabolites, such as alkaloids, terpenoids, polyphenols, flavonoids and many others show promising antidiabetic potentials. These natural constituents may act as a promising source of delivering oral hypoglycemic effect with minimal side effects (Singab et al., 2014).

According to the results, the administration of a single dose of Streptozotocin (50 mg/kg weight body) increased water consumption. Other studies have shown that diabetes mellitus is characterized by classical symptoms such as polyphagia, and polydipsia which are exhibited in HFD-STZ diabetic rats and this may be attributed to the impaired glucose homeostasis as a result of insulin inefficiency (Akbarzadeh et al., 2007). Water consumption was inversely related to food intake, this was an indication that the decrease in food intake in diabetic animals was linked to the significant amount of sugars in the blood that had an impact on the index of satiety. High levels of sugars were associated with decreased appetite and short-term food intake as has been reported also (Anderson and Woodend, 2003). Oral treatment with the fruit extract of *P. peruviana* to diabetic group of rats decreased food and fluid consumptions which could be due to improved glycemic status (Sathyadevi et al., 2014).

This study showed a significant decrease in final weight, weight gain at p< 0.001 and also food intake at p< 0.05 as compared to control group. Both *Physalis* powder and juice treated groups showed a significant decrease in final weight (p< 0.01 and 0.05, respectively), weight gain and FER

at p< 0.05 as compared to the control group. Aqua *Physalis* extract and methanol *Physalis* extract treated groups showed non-significant difference in these parameters as compared to the control group. *Physalis* powder, juice, aqua *Physalis* and methanol extract treated groups showed a significant increase in final weight, weight gain, food intake as compared to reference group (Hafez et al., 2011). This study also showed differences in some changes in organ weight and body weight Diabetic condition has been known to be associated with weight loss as reported by Anderson and Woodend (2003). The weight loss recorded in untreated diabetic animals could be a symptom of ill health, which may have been caused by the release of free radicals (Abdelmoaty et al., 2010).

The streptozotocin-induced diabetes rats showed significant loss of body weight with respect to the extract treated and controlled groups. Kumar et al. (2011) reported that antidiabetic and antihyperlipidemic effects is best induced in rat models using streptozotocin-induced rat models for better comparison with test plants, and give a better result profile of the test battery. With respect to the reference group, the inability of the plant to improve the animals' weight, at the end of treatment was observed, although a stabilization of weight was recorded at the end of treatment.

## Conclusion

The result of this present study showed that the hydroalcoholic extract of *P. peruviana* and its fractions contains many secondary metabolites which will be used against diabetes. It is interesting to isolate and characterize some compounds of this plant and to extend their antidiabetic potential investigations.

## CONFLICT OF INTERESTS

The author(s) have not declared any conflict of interests.

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# Pharmacognostic studies and elemental analysis of *Cassytha filiformis* Linn

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***Cassytha filiformis,*** **a leafless and perennial vine with small scales as a replacement of the leaves is currently being used in the treatment of various disease conditions such as jaundice. Macroscopic/ organoleptic characters, microscopic, chemo-microscopic characters, numerical standards, and elemental analysis were determined from the whole plant of *C. filiformis.* Findings from this study revealed the presence of some diagnostic microscopical features such as paracytic stomata, unicellular covering trichomes with cystoliths, prismatic calcium oxalate crystals and annular xylem vessels. Quantitative physical constants include moisture contents (5.5 %), ash value (17 %), acid insoluble ash value (1 %), total tannins (27.3 %), swelling index (165 %), water, ethanol and oil extractive indices (20.6, 13.6 and 1.6%, respectively). Trace metals such as Fe (165.4279 ppm), Mn (14.4093 ppm) and Ni (2.7933 ppm) detected in *C. filiformis* were higher than FAO/WHO (1984) permissible limit for edible plants, While others: Pb (0.0568 ppm), Zn (0.1094 ppm), Cd (0.0103 ppm) and Cu (0.0535 ppm) were found to be within the safety limit. The aim of this work was to study the pharmacognostic, characters, elemental analysis and numerical standard of *C. filiformis*.**

**Key words:** Atomic absorption spectrophotometer (AAS), Rumfar gada, Lauraceae, pharmacognosy.

## INTRODUCTION

Plants of the Lauraceae are nearly all woody trees and shrubs comprising 32 genera and about 2000 to 2500 species. An exception is the vining, leafless, parasitic genus, *Cassytha* (Watson and Dallwitz, 1993). This genus is considered to be unique in the family of Lauraceae as it is a parasite. The genus derived its name, *Cassytha*, from the Greek name, *Cuscuta* (meaning dodder). The vine has several common names in the regions of the tropics. For example, South Sea Islanders called this vine *"tentanini"* which has the meaning "to go round and round," and this seems to be a true descriptive adjective for the plants entwining habit (Mythili et al., 2011). Hausas in northern Nigeria call the plant “Rumfar Gada”.

*Cassytha filiformis* is a plant used for its various ethnomedical purposes in Nigeria. The plant is used in

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traditional treatment of many diseases e.g vermifuge and also in the suppression of lactation after still birth by several tribes in Nigeria (Burkill, 1995). The plant (stem and leaves) is boiled in water and administered for varying lengths of time to treat jaundice (Personal communications). Men were also reported to use it in the love magic while women used the extracts of the vine as a colouring agent or as a dye to provide a black color for the fabrics (Schroeder, 1967). In the traditional Ayurveda, *C. filiformis* is used as the major substitute for *Cuscuta* (Sakshy et al*.,* 2010). The brown colour of the stem is used as the colouring agent and hence have a major application in the dyeing industries (Sharma et al., 2009). Several aporphinoid alkaloids were isolated from the samples originating from Taiwan, Brazil, Australia and New Guinea but compositions were found to be quite variable among the different origins. Six aporphines from *C. filiformis* were shown to have *in vitro* cytotoxic properties out of which actinodaphnine, cassythine and dicentrine, also show *in vitro* antitrypanosomal properties against *Trypanosoma brucei brucei* (Quetin-Leclercq et al., 2004). Aqueous and alcoholic extracts of *C. filiformis* were tested for their diuretic activity in Wister rats. Total urine output volume and the concentration of $Na^+$, $K^+$ and $Cl^-$ ions excretion in the urine were finally estimated. Aqueous and alcoholic extracts of *C. filiformis* were found to exhibit significant diuretic activity by causing a marked increase in the $Na^+$ and $K^+$ excretion (Sharma et al*.,* 2009).

The design of this study was to determine some of the pharmacognostic standards of diagnostic importance, elemental content and numerical standarda for smooth and easy identification of *C. filiformis.*

## MATERIALS AND METHODS

All chemicals and reagents used during the study were of analytical grade purchased from Sigma Aldrich chemical company and Merck (From Distributors, Lagos, Nigeria). The instruments were well calibrated before use.

The plants, *C. filiformis* were collected from Ahmadu Bello University (ABU) Dam area in the month of August, 2016. The plant was identified and authenticated as *C. filiformis* (Family: Lauraceae) by U. S. Gallah, a Taxonomist at the Department of Biological Sciences, Ahmadu Bello University, Zaria, the voucher specimens (No. 2314) were preserved at the Department herbarium library.

### Macroscopy

The following macroscopic characters of the fresh aerial parts were noted: color, odor, taste, size and shape, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina and texture (Evans, 2009).

### Microscopy

The free hand thin transverse and longitudinal sections of the fresh aerial stem of the plant material were treated with different staining reagents and observed for the general and specific microscopic characteristic. Furthermore, small quantity of the powdered plant material was cleared, mounted and observed for diagnostic powder characteristics (WHO, 2011).

### Physicochemical investigations

The fresh and dried aerial parts powdered plant material was used for the determination of numerical standards e.g ash values, extractive values, swellimg index, bitterness value, crude fibre, etc. The chemomicroscopic examination powder with chemical reagents were also studied (WHO, 2011).

### Analysis of metals of the powdered *C. filiformis* using atomic absorption spectrophotometry

Macro and micronutrients of *C. filiformis* were obtained using atomic absorption spectrophotometer (AAS, Shimadzu 2010, Japan) available at National Research Institute for Chemical Technology (NARICT), Zaria.

## RESULTS

### Macroscopic and organoleptic properties of *C. filiformis*

Stem of *C. filiformis is* green to yellow, filiform and glabrous. Leaves are reduced to minute Scale 1 mm long, near the tips of stem. Flowers are sessile and borne in small panicles (Plate 1). The organoleptic characters include, dark greenish colour of the dried powdered plant material, it has a distinct odour, a slightly bitter taste. The fracture of the bark is fibrous and the texture is smooth and hairy.

### Microscopic examination of *C. filiformis*

Diagnostic features identified from the aerial stem parts of *C. filiformis* include: unicellular covering trichome that is cornical in shape, with thick walls. Scattered paracytic stomata, appearing on the aerial stem. Some parenchyma cells which are oval in shape, the trichomes which contain cystoliths, conducting elements e.g annular xylem vessel were observed (Plates 2, 3 and 4).

### Chemomicroscopical examination of powdered *C. filiformis*

Some of the chemo-microscopical features identified were starch, and calcium oxalate crystals, (cell inclusions), tannins and calcium carbonate (cell constituents), and cellulose, suberin and cuticles (cell wall materials) (Table 1).

### Numerical standards of *C. filiformis*

Some of the numerical standards of powdered *C. filiformis*

**Plate 1.** The plant, *Cassytha filiformis.*

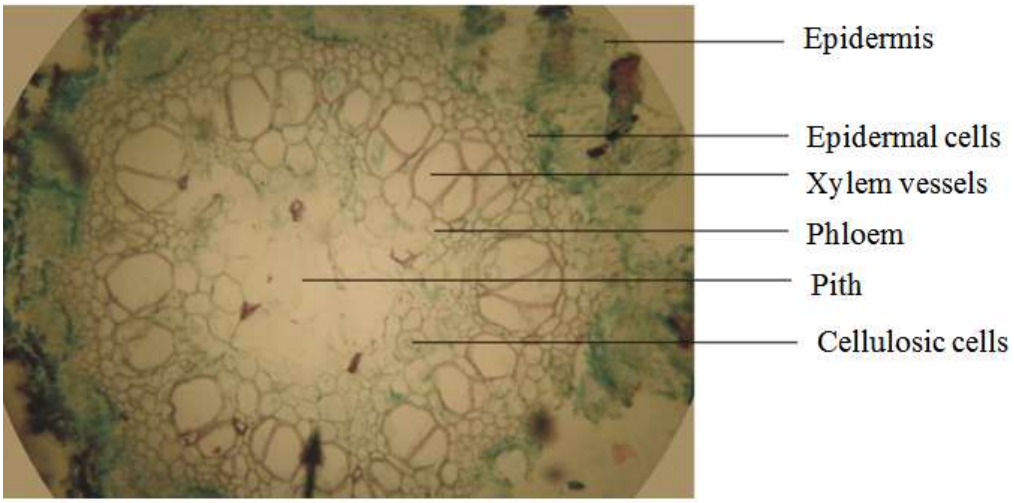


**Plate 2.** Transverse section of *C. filifomis* stem (Safranin and Fast green Stain. X 200).

determined under this work include: moisture content, total ash value, acid insoluble ash value, total tannins, swelling index, bitterness value, alcohol and water soluble extractive values, oil content and crude fibre (Table 2).

### Analysis of metals detected in powdered *C. filiformis*

Elemental analysis was carried out on the powdered *C. filiformis.* Some of the analysed metals include: copper, chromium, iron, manganese, potassium, calcium, sodium, nickel, cadmium, zinc and lead (Table 3).

## DISCUSSION

*C. filiformis* is used in many cultures for the treatment of various disease conditions including jaundice without standardization. In this study, crude form of *C. filiformis* has been evaluated with the view to provide useful and diagnostic parameters for the standardization of the drug.

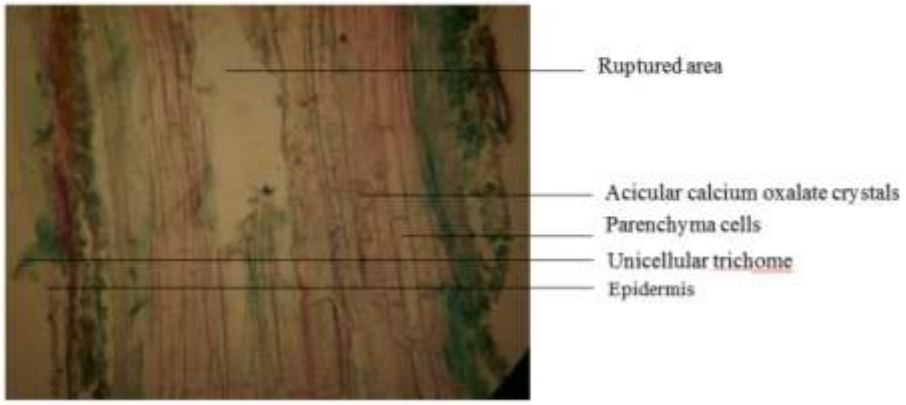


**Plate 3.** Longitudinal section of *C. filifomis* stem (Safranin and Fast green Stain. X 200).

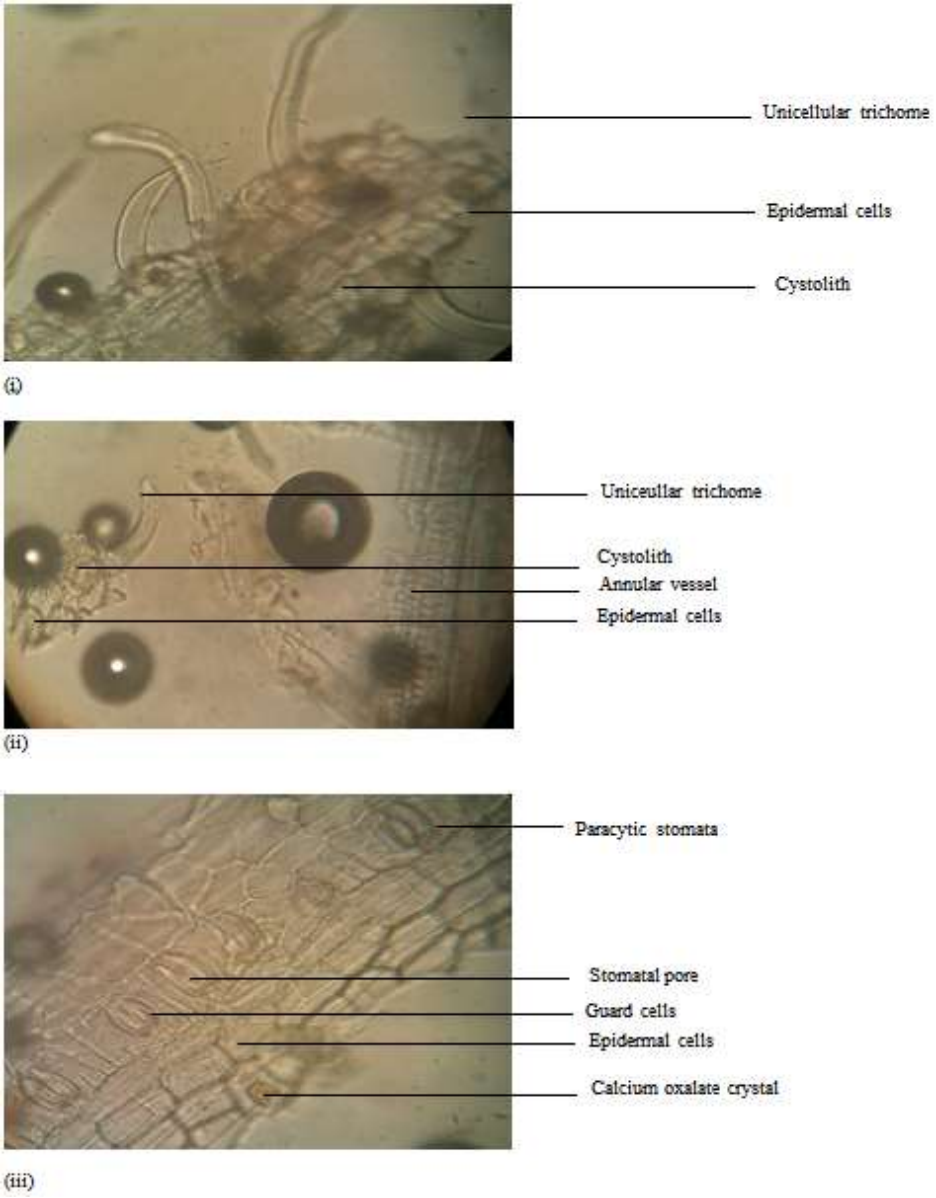


**Plate 4.** Microscopical features of powdered whole plant of C. *filifomis* (200X).

**Table 1.** Chemomicroscopical features from the powdered *C. filiformis.*

| Reagents | Constituents | Observation | Inference |
|---|---|---|---|
| **CELL WALL MATERIALS** | | | |
| Chlor-zinc-iodine + Conc. HCl | Cellulose | Blue-violet | Cellulose |
| Sudan iv | Suberin/Cuticle | Orange red | Suberin/Cuticle |
| Phloroglucinol + Conc. HCl | Lignin | Pink | Lignin |
| **CELL CONSTITUENTS** | | | |
| 5 % Ferric chloride | Tannins | Greenish black | Tannins |
| N/50 Iodine | Starch | Blue | Starch |
| Million's reagent | Protein | Red colouration | Protein |
| 80% $H_2SO_4$ | Calcium oxalate crystals | Shiny crystals dissolves | Calcium oxalate crystals |
| 5% acetic acid | Calcium carbonate | Crystals dissolves with effervescence | $CaCO_3$ |

**Table 2.** Numerical standards of *C. filiformis.*

| Numerical standards | *C. filiformis* |
|---|---|
| Moisture content | 5.50±0.82 |
| Ash value | 17.00±1.08 |
| Acid insoluble ash | 1.00±0.41 |
| Water soluble extractive value | 20.60±0.77 |
| Ethanol soluble extractive value | 13.60±0.69 |
| Total tannins | 27.30±6.81 |
| Bitterness value | 0.23±0.01 |
| Swelling index | 165.00±10.00 |
| Crude fibre | 22.40±0.10 |
| Fixed oil | 1.60±0.16 |

n =5.

**Table 3.** Elemental analysis of powdered *C. filiformis.*

| Elements | Concentration (ppm) | FAO/WHO (1984) limit* (ppm) |
|---|---|---|
| Na | 5.1735 | - |
| Mg | 9.3911 | - |
| Ca | 84.3993 | - |
| Cr | 7.7940 | - |
| Cu | 0.0535 | 3.0 |
| Fe | 165.4279 | 20 |
| K | 0.8313 | - |
| Mn | 14.4093 | 2.0 |
| Zn | 0.1094 | 27.4 |
| Pb | 0.0568 | 0.43 |
| Cd | 0.0103 | 0.21 |
| Ni | 2.7933 | 1.63 |
| Co | 0.4621 | - |

*For edible plants; ppm: Parts per million.

The parameters obtained include microscopical features (Plates 2, 3 and 4), chemomicroscopical features (Table 1) and numerical standards (Table 2).

The leafless plant, *C. filiformis* was found to have paracytic stomata located in between thin and thick walled parenchymatous cells in the epidermis. This result

is in conformity with the previous work by Sharma et al. (2009). The presence of stomata promotes heat dissipation by water loss, maximizing the control of water loss by leaf and increases photosynthetic potential (Woodward, 1998), these features are essential for the plant as the whole of its aerial part is involved in photosynthesis. Acicular calcium oxalate (Plate 3) found scattered in ground parenchymatous cells are important parameters for identification and standardization of *C. filiformis* and it is a clear indication that the plant is rich in oxalic acid with which higher plants syntheses the crystals and deposit them in specialized in any organ or tissue (Nakata, 2003; Webb, 1999). Presence of unicellular covering trichomes with cystoliths (calcium carbonate deposit) is an excellent diagnostic feature for *C. filiformis*. The presence of cystoliths in the base of the trichomes found by this study is reported for the first time. Trichomes are epidermal outgrowths of considerable value for taxonomic purposes for some plants. These outgrowths play a role in plant defense especially with regard to phytophagous insects (Metcalfe and Chalk, 1988). They may also be involved in the regulation of temperature and water repellency as well (Neinhuis and Barthlott, 1997).

Taking into consideration, the diversity in chemical nature and properties of contents of drugs, various solvents are used for extractives values. This study found the extractive value of water (20.60%) to be the highest followed by alcohol (13.60%) then lipid (oil content) or diethyl ether extract (1.6%). This is expected as water extracts of most polar compounds such as carbohydrates which are the commonest in most plants. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired (Kokate et al., 2009).

Studies of numerical standards can serve as a valuable source of information and are usually used in judging the purity and quality of the drug (Nisharaj and Radhammany, 2012). The moisture content of *C. filiformis* is exceptionally low as compared to the pharmacopoeia (EP, 2011) limit (10 to 12%). This may not be unconnected to the absence of the leaves and could be essential in preventing decomposition of the crude drug either due to chemical change or microbial contamination during drying and storing. The ash value and acid insoluble ash value of *C. filiformis* were found to be 17 and 1% w/w, respectively. The acceptable (WHO) limits for total ash and acid insoluble ash vary according to the vegetable drug. Some typical examples include the total ash and acid insoluble ash values of *Centella asiatica* which should not be more than 19% and not less than 6%, respectively (WHO, 1999), similarly, in *Pericarpium granati*, the total ash should not be more than 4% and the acid insoluble acid should not be less than 1% (WHO, 2009). The ash value is a measure of the earthy matter or inorganic composition and/or other impurities present along with the drug such as carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium (Wallis, 2005). The low values of ash in *C. filiformis* are indications that these minerals occur only in trace quantities. The bitterness value of *C. filiformis* was found to be 0.23. The acceptable limit varies according to the vegetable drug. Plant materials that have a strong bitter taste (“bitters”) are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice. The total tannins of *C. filiformis* were found to be 27.30%. Other pharmacognostic parameters found by this study include swelling index, crude fibre and bitterness value. These parameters are diagnostic characteristic of *C. filiformis* and are been reported for the first time in this plant. The parameters can be considered as additional indices for the authenticity of the drug. The swelling index of *C. filiformis* found was 165% of the original volume of the plant material. Many plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties- especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose. Therefore, swelling index gives an idea on the mucilaginous and pectin content of crude drug. The crude fibre content of *C. filiformis* was found to be 22.40%. Determination of crude fibre is useful in distinguishing between similar drugs or in the detection of adulteration (Thomas et al., 2008). It also helps to remove the more resistant parts of plant organs which can be used for microscopic examination.

Concentrations of minerals in *C. filiformis* determined by this study include iron (165.4279 ppm), manganese (14.4093 ppm) and nickel (2.7933 ppm) as against the permissible limit set by FAO/WHO (1984) for edible plants (Table 3). However, for medicinal plants, the WHO (2005) limits has not yet been established for Fe, Mn and Ni. Sheded et al. (2006) showed similarity in Fe content (between 261 and 1239 ppm), and wide differences in Mn (44.6 and 339 ppm) content in selective medicinal plants of Egypt. Trace elements with lower concentration in *C. filiformis* include Zn (0.1094 ppm), Cu (0.0535 ppm), lead (0.0568 ppm), and cadmium (0.0103 ppm) which are below the permissible limit, Zn (27.4 ppm), Cu (3.00 ppm), Cd (0.21 ppm) as set by FAO/WHO (1984) for edible plants. However, these results are within the permissible limits for Cu set by China and Singapore as 20 and 150 ppm, respectively and the limit for lead (10 ppm) as set by China, Malaysia, Thailand (WHO, 2005). The overall results indicated clearly, the contents of the essential metals such as iron, manganese and nickel were within acceptable limits of the toxic metals such as lead which are within safe limit (Table 3). Therefore, *C. filiformis* can also be beneficial sources of appropriate and essential trace elements.

## Conclusion

The pharmacognostic studies of C*. filiformis* yielded a set of qualitative and quantitative parameters that are useful in ascertaining the identity of the plant and in determining

the quality and purity of the drug materials for future studies. The parameters which are reported here especially the bitterness value, total tannins, swelling index and crude fibre which are reported for the first time in this study can be considered distinctive enough to identify and decide the authenticity of the drug.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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