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Research Article

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Chemical and Proximate Contents of methanolic leaf extract of *Piliostigma Thonningii* schum (Camel foot)

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ABSTRACT

This study aims at investigating the phytochemical, elemental and proximate contents of the leaves of Piliostigma Thonningii schum. The air dried ground matter (500g) was macerated with 75% methanol for five days filtered and concentrates in vacuo using rotary evaporator. The extract concentrate yield was estimated in percentage $^{w}/_{w}$. The methanolic extracts were subjected to preliminary phytochemical analysis using standard procedures. Elemental contents of the leaf of P. Thonningi were determined using atomic absorption and flame emission spectroscopy. The proximate contents; ash, crude fibre, crude protein, carbohydrate, ether extract, dry matter and moisture content of the leaves of Piliostigma thonningii schum were also evaluated using standard protocols. The methanolic extract concentrate yield is 11.76$^{w}/_{w}$. Preliminary phytochemical analysis indicates the presence of tannin, saponins, terpeniods, flavonoids, carbohydrates and cardiac glycosides. Elemental content analysis P. thonningii leaves indicates that that the concentration of iron (Fe) in P. thonningii appeared to be within safety limits reported by World Health Organization (1996). However the concentration of Nickel (Ni) is slightly above the WHO recommended level. Mangnese (Mn), Copper (Cu), Chromium (Cr), Magnesium (Mg) Zinc (Zn) and Calcium (Ca) occur in low concentration. Na and As were not detected. Also Non-essential and toxic metals such as Pb and Cd were not detected The result of the proximate contents shows that the dry matter has the highest percentage of 95.4%, the moisture content is 6.12%, carbohydrate has 65.28% while crude fibre has a percentage of 20.00%, ash 4%, crude Protein 6.12% and ether 1%.

Keywords: Proximate, elemental, phytochemistry, *Piliostigma Thonningii*, extracts.

INTRODUCTION

Increased awareness of the significance of medicinal plants and nutrition to the health of individuals and communities has necessitated the need for knowledge of the food nutrients and Phytochemicals present in the various parts of different plants. The Phytochemicals contained in the plants are largely responsible for the definite physiological activity they exert on the human body[1] (Ighodaroro 2009) and their nutritional value is determined by the food nutrient they contain. Considerable information exists on the nutrient composition and medicinal value

of most well-known and easily cultivated plants. As a result of recent interest in the plant kingdom as a potential source of new drugs, strategies for the fractionation of plant extracts based on biological activity rather than a particular class of compound haven developed and the chemical examinations follow after isolation of the active fractions [2]. (Harborne, 1998). *Piliostigma thonningii* schum is an underexplored leguminous plant that belongs to the family of *caesalpiniacea* distributed in Africa and Asia in open woodlands and savannah regions that are moist as well as wooded grasslands in low to medium altitudes [3] and [4]. In the developing countries of the world, traditional herbal medicine is often used side by side western medicine with herbal medicine taking the upper hand when the cost of western medicine is beyond reach[5]. Moreover, different parts of *P. thonningii* have also been described as useful medicinally. Its root and twig have been used for the treatment of dysentery, fever, infections, respiratory ailments, snake bites, hookworm and skin diseases [3]. Although there are many reported folkloric claims on the medicinal usefulness of this plant on selected parts such as roots and stem bark. There is scanty research report on the phytochemsitry, elemental and proximate composition of the leaves of this plant in this part of Nigeria

EXPERIMENTAL SECTION

Plant Collection and Identification

The leaves of P. *thoniingi* were collected in Damaturu Local Government Area of Yobe State, Nigeria in March, 2012. The plant specimen was identified by a plant taxonomist, Prof. S. S. Sanusi, Department of Biological Science, while the voucher specimen No. 97883B was deposited at the Post-Graduate Research Laboratory, Department of Chemistry, and University of Maiduguri.

Elemental content evaluation

Ashing, digestion and analysis of samples

The air-dried plant samples were pulverized manually in wooden mortar and pestle into coarse powder. 5.0g of each sample was independently packed into an acid-wash porcelain crucible and then placed in a muffle furnace for three (3) hours at 550^0c. The crucible were removed from the furnace and cooled. 10ml of 6 M HCl were added and covered; this content was heated on a steam bath for 15minutes. 1ml of HNO_3 was later added for an hour so as to dehydrate Silica and completely digest organic substances. Lastly 5ml of 6M HCl and 10ml of water were added and the mixture was heated on a steam bath to complete dissolution. The mixture was cooled and filtered through a Whatman No.1 filter paper into a 100ml volumetric flask and then made up to the mark with distilled water [6].

Elemental contents analysis

The air dried sample (10g) was dried to a constant weigh in an oven at 80^oC . The sample (5g) was then ashed in a hotspot furnace at 500^oC for 3 hours. The ashed material was then digested using standard procedures (Radojevie and Baskin 1999) and analysed for the determination of trace and heavy elements present in *Vitex doniana* leaves using a combination of flame emission spectrometry (FES) (GallenKamp) and Atomic Absorption Spectrometry (AAS) (SPG Unicam Model No.1) at appropriate wavelength, temperature and lamp current for each element under study [6]. Flame emission spectrometry (FES) (Gallen Kamp FGA 330) was use to determine sodium and potassium. Elements such as magnesium, calcium, iron, lead zinc, manganese, cadmium and copper was determined by atomic absorption spectrometry (AAS).

Extraction and Phytochemical analysis

The weighed powdered air-dried sample (200g) of P. *thoniingi* was macerated with 95% ethanol for five days filtered and evaporated in vacuo at 40°C using a rotary evaporator. The extract concentrate was labeled and the percentage yield was calculated in $^w/_w$. The methanolic extract was subjected to qualitative chemical screening for identification of the secondary metabolites such as flavonoids, alkaloids, sterols, triterpenes, saponins, anthraquinones, tannins, polyuronides, emodol, etc as described by Ioan, 1982 [7]; Sofowora,1993a&b [8,9]; Trease and Evans 2002 [10].

Proximate Composition determination

The air-dried leaves were and ground into fine powder. About 10.0g of the grounded leaves was exhaustively processed for various parameters according to the Association of Official Analytical Chemists methods ;[11,12]. The proximate analysis (carbohydrates, fats, crude protein, moisture, dry matter, crude fiber, nitrogen free extract and ash) of the leaves were determined using AOAC methods. Using weight difference, moisture and ash were obtained. The fiber content was estimated from the loss in weight of crucible and its content on ignition.

Carbohydrate was determined when the sum of the percentage of moisture, ash, crude protein and fats were subtracted from 100. The nitrogen value, which is the precursor for protein of a substance, was determined by micro kjeldahi method, involving digestion, distillation and finally titration of the sample [12]. The nitrogen value was converted to protein by multiplying with a factor of 6.25. The determination of crude lipids content of the samples was done using soxhlet type of direct solvent extraction method. The solvent used was petroleum ether (boiling range 40-60^{0}c). While the nitrogen free extract was calculated indirectly by difference as the sum of crude protein, fibre, fats and ash subtracted from 100. The result of proximate value was all estimated as percentage [11,12].

RESULTS AND DISCUSSION

The methanolic extract concentrate percentage yield is 11.76 $^w/_w$. The Phytochemical analysis indicates the presence of phytochemicals such as flavonoid, saponins, tannins and cardiac glycosides in moderate concentrations as shown in table 1. Alkaloids and Anthraquinones were not found in the methanolic extracts. This phytochemicals have been implicated in having diverse medicinal and pharmacological properties. Terpenes are very important group of organic compounds that have been reported as potent drugs used in treatment of wide range of ailments. They can be simple essential oils to the more complex triterpenes and teraterpenes. The most rapidly acting anti-malarial, artemisinin and its derivatives are Terpenes [10]. The presence of terpenes will encourage further research for possible new drugs leads. Cardiac glycosides are known to work by inhibiting the Na^+/K^+ pump. This caused an increase in the level of sodium ions in the myocytes and then led to a rise in the level of Ca^{2+}. This inhibition increase the amount of Ca^{2+} ions available for contraction of the heart muscle which improves cardiac output and reduces distention of heart; thus are used in the treatment of congestive heart failure and cardiac arrhythmia[13]. Saponins from plants have long been employed for their detergent properties. It is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and weight loss etc [13]. (Ngbede, . It is also been reported to have anti-fungal properties [14]. Some saponins glycosides are cardiotonics while others are contraceptives and precursors for other sex hormones[10]. Tannins sacs are known to be common in *Caesalpinoidea*e and known to exhibit antiviral, antibacterial and anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectively and is also used as diuretic. Plant tannins are also source of commercial tannic acids and tanning agents [10]. Flavonoid has been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. Some flavonoids have also reported to behave like the some coumarins in the inhibition of giant cell formation in HIV infected cell cultures [10]. This study may provides support to the previous folkloric reports that indicates that the leaves are being used in treatment of ailments such as arthritis, Headache, malaria fever, antiseptic (healing ability) and backache etc.

Table 1. Phytochemical analysis of the leaf of P.*thonningii* methanolic extract

Constituents	Test	Inference
Tannins		++
Alkaloids		-
Terpenes		++
Saponins		++
Anthraquinone		-
Cardiac glycosides	1.Salkowski's 2. Liebermann	++ ++
Flavonoids	1. Shinoda's test 2. Ferric Chloride 3. Lead acetate 4. Sodium hydroxide	+ ++ ++ ++
Tannins		+
Terpenes		++
Alkaloids		-
Carbohydrate	1. General test (Molish) 2. Test for free reducing sugar (fehling's 3. Test for combined reducing sugars 4. Test for pentoses 5. Test for ketoses	++ ++ ++ - -

Note: (+++) Highly present, (++) Moderate present, (+) Present and (–) Absence

Elemental content analysis results in the Table **2**, shows that the concentration of iron (Fe) in P. *thonningii* appeared to be within safety limits reported by World Health Organization (1996) [15]. However the concentration of Nickel (Ni) is slightly above the WHO recommended level. Mangnese (Mn), Copper (Cu), Chromium (Cr), Magnesium (Mg) Zinc (Zn) and Calcium (Ca) occur in low concentration. Na and As were not detected. Also Non-essential and toxic metals such as Pb and Cd were not detected Animal needs Arsenic in very small amount to metabolise protein, amino acids and taurine.. Mineral elements are essential in many vital processes in both plants and animals. Trace elements play an important role in health and diseases. Macro nutrients such as sodium and calcium regulate the fluid balance of the body and thereby influence the cardiac output and changes in their level result in hypertention[16,17]. . Mn is essential for normal functioning of central nervous system and is good anti-oxidant [18]. . Ca is needed in the development of bone teeth, regulates heart rhythm, helps in blood clothing, maintain proper nerve and muscle functions and lowers the blood pressure. Copper is a common environmental metal and is essential in cellular metabolism but at high concentrations, it can be highly toxic to fish [19]. Zinc is an essential element in the human diet and deficiency in the diet may be more detrimental to human health than too much of it in the diet [20]. Although Zn is not carcinogenic, ingestion of large doses can cause death [20]. From the research, Zn and Fe concentration is within safety limit described by WHO, (1996) [15].

Figure 1. Proximate contents of P. *thonningii* methanolic leaves extract

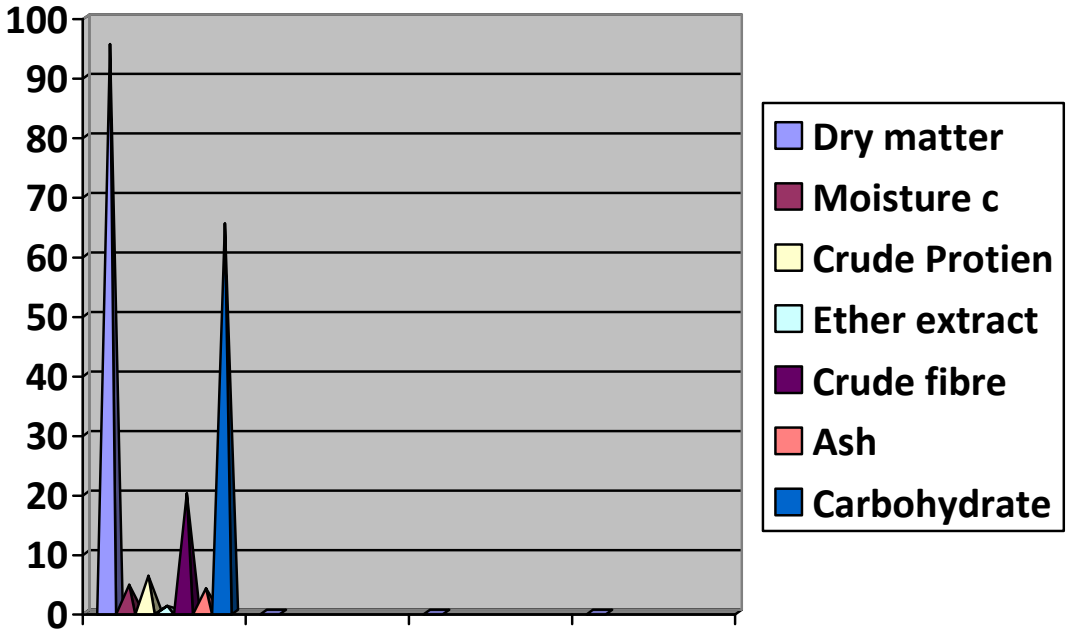

Table 2. Concentrations in ug/gof some Elemental contents in the leaves of P. *Thonningii*

Elements	**Concentration (ug/g)**	**WHO Standard** in ug/g **(1999)**
Calcium (Ca	0.8	360-800
Chromium(Cr)	**0.4**	12.8
Copper (Cu)	0.12	100-300
Iron (Fe)	69.2	50-5000
Lead (Pb)	**ND**	5-30
Manganese (Mn)	0.07	100-20000
Zinc (Zn)	32	150-20000
Nickel (Ni)	8.2	≥ 5
Sodium (Na)	ND	400-500
Arsenic (As)	ND	≥ 0.9
Lead (Pb)	ND	5-30

ND= Not detected

Table 3 and figure 1 below shows the percentages of moisture, ash, carbohydrate, fibre, fat, crude protein and dry matter contents of the leaves of P.*thoningii.* The result of the proximate contents as shown in table one (1) indicate that the Dry matter has higher percentage of 95.4%, the moisture content is 6.12%, Carbohydrate has 65.28%.While crude fibre has a percentage of 20.00%,Ash 4% Crude Protein 6.12% and Ether 1%.

Table 3. Proximate contents result of P.*thoningii* leaves

Serial Number	Sample Codes	% Dry Matter	%Moisture Content	%Crude Protein	% fats	% Crude fiber	% Ash	%Carbohydrate
1	B.	95.4	4.6	6.12	1.0	20.0	4.0	65.28

CONCLUSION

Pre-liminary phytochemical analysis revealed the presence of secondary metabolites such as tannins, flavonoid, saponins and cardiac glycosides in the methanolic extract of P.*thoiningi* leaves.However alkaloids and anthraquinone were not found in the extracts. Proximate evaluation of the leaves of P. *thoningi* indicates that dry matter has higher percentage of 95.4%, followed by Carbohydrate which is 65.28% and crude fibre is 20.00%, moisture content is 6.12%, Crude Protein is 6.12%, Ash has a percentage of 4 and Ether has the lowest percentage of 1.

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