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Proximate, elemental and anti-nutritional composition of *Pachycarpus bisacculatus* (bitter butter) roots used locally as anti-snake venom

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Abstract
Snake bite remains a public health problem in many countries including Nigeria; hence search for snake antivenom has intensified. One plant that is potentially useful in this regard is *Pachycarpus bisacculatus* roots (bitter butter). This study determined; proximate, phytochemical, of the aqueous, *n*-hexane, methanol and ethyl acetate extracts of *Pachycarpus bisacculatus* roots. These extracts obtained by maceration were subjected to qualitative and quantitative phytochemical screening. Proximate, elemental, anti-nutritional compositions of this plant were carried out using standard methods. Results obtained were: moisture content (71.56±0.06, 7.53±0.37)%, crude protein (3.19±0.19, 1.92±0.05)%, crude fibre (11.33±0.31, 3.98±0.28)%, crude fat (1.05±0.07, 0.30±0.03)%, Ash (4.68±0.17, 2.32±0.32)%, carbohydrate (72.21±0.24, 19.92±0.48)% for the dried and fresh roots respectively. Elemental content showed that level of Ca was highest while that of Zn was the lowest. Al, Si, V, Pb were not detected. Anti-nutritional composition showed: oxalate (1.68±0.02 mg/100g), tannins (7.10±0.78 mg/100g), phytate (8.47±0.25 mg/100g) and cyanogenic glycosides (0.03±0.01 mg/100g). Extraction yields were 21.068% in aqueous solution, 1.6391% in ethyl acetate. The results indicate that root extracts of *Pachycarpus bisacculatus* contain bioactive chemicals and micronutrients which may be responsible for the medicinal properties reported for the plant.

Keywords: Proximate; Elemental; Nutritional; Antinutritional; *Pachycarpus bisacculatus*; Anti-snake venom

INTRODUCTION

Snakebite is a global medical problem especially in the rural areas of the tropics with about 40,000 deaths each year [1]. An estimated 5.4 million people worldwide are bitten by snakes each year with 1.8 to 2.7 million cases of envenoming. Snake bite is a neglected public health issue in many tropical and subtropical countries. Most of these occur in Africa, Asia and Latin America. In Africa, an estimated 435,000 to 580000 snake bite occur annually that need treatment. Most of these burdens takes place in countries where health systems are extremely weak and medical resources scarce like Nigeria [2]. The annual snakebite incidence in the savannah region of northern Nigeria has been estimated to be 497 per 100,000 populations, with 12.2% mortality rate due mainly to the carpet viper, *Echis ocellatus* [3] The commonest snakes of

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clinical importance in Nigeria comprise the cobras (*Naja species*) and the vipers (*Echis*). The carpet viper has been reported to be a very dangerous snake and its victims are mainly farmers, hunters and herdsmen; most of whom are of young productive ages. Nigerian carpet viper, *Echis ocellatus*, is commonly found in the Benue- Niger valley axis and the hilly north-eastern part of the country [4]. The supplies of antivenoms by the various governments are very unreliable; and where the products are available, they are very costly. The use of antivenoms for the treatment of snake venom poisoning is further restricted by their propensity to cause hypersensitivity reactions in sensitive patients. The inability of antivenoms to resolve the local effects of the venom is also a limiting factor [5]. The urgent need for the discovery of new anti-snake venom from local resources such as medicinal plants has therefore been recognized. According to these sources, the root bark of *Paullinia pinnata* (Bread and Cheese) is used by Okpameri people of Edo state, while the leaves of *Detarium microcarpum* (sweet dattock) are employed by traditional healers of Anaguta people of Jos, Plateau state, Nigeria to treat snakebite patients. However, this practice lacks scientific validation and therefore this type of treatment needs thorough scientific investigation.

The antigen–antibody reaction is the basic mechanism for the neutralization of snake venom by antivenoms [6]. For small molecules, there are some hypotheses proposed on how those small compounds neutralize the toxic components of the venom. Several literature reports indicate the mechanism of inactivation; for example, through precipitation or inactivation of proteins, inactivation or enzyme inhibition, chelation, adjuvant action, antioxidant activity, or a combination of these activities. Among these, protein precipitation and enzyme inhibition are the most accepted. Several secondary metabolites with protein binding properties against snake envenomation include flavonoids, polyphenols, saponins, tannins, terpenoids, xanthenes, quinonoids, steroids, and alkaloids. These bind to the toxic proteins of the venom, thereby inactivating them. [7]. Compounds such as vitamins A, C and E, flavonoids, terpenoids, tannins, polyphenols, and some minerals (i.e., selenium) from plants have the ability to neutralize free radicals; hence, they are valuable natural antioxidants that can scavenge and remove oxygen free radicals, stabilize cell membranes, and act as immunomodulators. These classes of compounds are known to be powerful antioxidants both in hydrophilic and lipophilic environments. They can prevent, stop, or reduce oxidative damage due to PLA_2 activity by selectively binding to the active sites or modifying the conserved residues that are critical for the PLA_2 catalysis [8] The discovery of new antivenoms involves significant challenges in the assessment, design, and production of potential antivenoms, and the derivatization of identified compounds for improved activity. New and much improved antivenoms with high standards can be produced in adequate quantity when multidisciplinary and International collaborative efforts are undertaken. This would help serve not just a particular nation but the entire regions [9]

The study of the properties of medicinal plants used by indigenous people is an important approach towards the discovery and development of traditional herbal medicines. The concept of testing medicinal plants is underlined by the fact that some traditional herbal medicines have proved, on investigation, to be of value in orthodox medicine; and useful drugs have been developed from plants, some of which were originally used in traditional medicine [10]. The aim of this study is to determine the proximate, elemental and ant-nutritional composition of *Pachycarpus bisacculatus* roots.

EXPERIMENTAL METHODS

The plant samples of *Pachycarpus bisacculatus* root was collected from Langa-langa village of Nassarawa-Eggon local government area of Nassarawa State in July, 2019 based on ethno-botanical information. This plant was collected from their natural habitat and from identified herbalists. The plant was authenticated by a plant taxonomist in the Department of Plant Science and Biotechnology, University of Jos, Nigeria.

The root of *Pachycarpus bisacculatus* was cleaned using water until soil and other materials on it were removed. Thereafter, it was then air-dried under shade for a week. The plants materials were then ground into fine powder using the grinding mill (QBXO190-Q4 Euro Crank Arm) then wrapped in air-tight containers and placed in the laboratory at room temperature (25^{o}C) prior to further analysis.

Extraction of plant material. The plant materials were prepared by Soxhlet extraction method. About 20 grams of powdered plant materials were uniformly packed into a thimble and extracted with 250 cm^3 of different solvents using n-hexane, ethyl acetate, methanol and finally water to give n-hexane, ethyl acetate, methanol and aqueous extracts respectively. The process of extraction was continued for about 8 hours till the solvent in siphon tube of an extractor becomes colourless depending on the solvent used. The extracts were concentrated using rotary evaporator at 40-60^{o}C. The concentrated extracts were carefully removed and kept in refrigerator at 4^{o}C for phytochemical analysis [11]

Proximate analysis. The moisture, ash, crude fats, proteins and carbohydrates of all the fresh and air-dried samples of the root were carried out using standard AOAC method [12]. The moisture and ash were determined using weight difference method. Crude fat was extracted by means of the Soxhlet apparatus with petroleum ether (40 to 60°C) for 8 hours. Crude fibres were done by successive digestion of the defatted samples with 1.25% sulphuric acid and 1.25% sodium hydroxide solutions. The nitrogen value, which is the precursor for protein of a substance, were determined by micro Kjeldahl method involving digestions, distillation and finally titration of the sample. The nitrogen value was converted to protein by multiplying a factor of 6.25. Carbohydrate was determined by difference method. All the proximate values were reported in % [12]. The moisture content of each sample was calculated as loss in weight of the original sample and expressed as percentage [11].

The crude protein was determined by the Kjeldahl method with slight modification [11] by digestion, distillation and titration respectively. Estimation of fat content was performed using the Soxhlet extraction method. Ten grams (10 g) of the powdery form of each root sample were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser. Then 200 cm^3 of n–hexane was used to extract the lipid [11]. The ash content was also determined by method as described by [11]. The total ash content of a substance is the percentage of inorganic residue remaining after the organic matter has been ignited. Two grams of the pulverized plant samples was placed in a crucible and ignited in a muffle furnace at 550^{o}C for 6 hours. It was then cooled in a desiccator and weighed at room temperature to get the weight of the ash [11]. The carbohydrate content was determined by subtracting the summation of the percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100 [11].

Anti-nutritional content. The cyanogenic glycoside and oxalate were determined by the alkaline picrate method of Opeyemi, et al [12]. Trypsin inhibitor, phytic acid and tannins were

determined by the method outlined by Harinder et al [13].

Mineral content. The ash obtained was used for the determination of mineral content. The resulting ash was dissolved in 10 cm^3 of 10% HNO_3 and heated slowly for 20 minutes. After heating, it was filtered and the filtrate was used for Atomic Absorption spectrophotometer (AAS) to determine Cu, Fe, Zn, Mn and toxicity level of Pb and Cd [14].

Qualitative tests on the phytochemicals. This was performed according to the standard methods described by Yadav and Agarwala, [15].

Test for phenolic compounds. About 5 cm^3 of aqueous extract was mixed with 2 cm^3 of 2% solution of $FeCl_3$. Appearance of a blue-green or black colour indicated the presence of phenols and tannins [15].

Test for flavonoids. The water extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicates the presence of flavonoids [11]. Aqueous extract was mixed with 2 cm^3 of 2% solution of NaOH gave an intense yellow colour which turned colourless on the addition of few drops of dilute HCl acid indicating the presence of flavonoids [15].

Test for saponins. Equal volume of aqueous extract mixed with distilled water in a test tube and shaken vigorously gave the formation of stable foam which was taken as an indication for the presence of saponins [15].

Test for steroidal glycosides. About 5 cm^3 of aqueous extract was mixed with 2 cm^3 of chloroform, then 2 cm^3 of concentrated H_2SO_4 was added carefully and shaken gently. Appearance of a reddish-brown colour indicated the presence of steroidal ring, i.e., aglycone portion of the glycoside [15].

Test for cardiac glycosides. The aqueous extract was mixed with 2 cm^3 of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 cm^3concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides [15].

Test for steroids and terpenoids. aqueous extract was mixed with 2 cm^3 of chloroform and concentrated H_2SO_4 was added sidewise, a red colour produced in the lower chloroform layer which indicated the presence of steroids. About 5 cm^3 of aqueous extract was dissolved in 2 cm^3 of chloroform and evaporated to dryness. To this was added 2 cm^3 of concentrated H_2SO_4 was added and heated for about 2 minutes. Appearance of a greyish colour indicated the presence of terpenoids [15].

Test for alkaloids. The aqueous extract (5 cm^3) was mixed with 2 cm^3 of 1% HCl and heated gently. Two drops of Mayer's reagent (prepared by dissolving a mixture of 1.36 g of mercuric chloride and 5 g of potassium iodide in 100 cm^3 distilled water) and Dragendorff's reagent was then added to the mixture. Turbidity resulting from the formation of precipitate was taken as evidence for the presence of alkaloids [15].

Quantitative analysis of the phytochemicals. The quantity of flavonoids was determined by taking 5 g of plant sample weighed in a 250 cm^3 titration flask, and adding 100 cm^3 of the 80% aqueous methanol at room temperature and shaken for 4 hours in an electric shaker. The entire solution was filtered through Whatman filter paper number 42 (125 mm). The filtrate was later transferred into a weighed crucible and evaporated to dryness over a water bath and weighed again [16]. The difference in weight gave the weight of flavonoids which was expressed as a percentage of the weight of sample analysed.

For alkaloids determination, 5 g of each sample was weighed into a 250 cm^3 beaker, and 200 cm^3 of 20% acetic acid in ethanol was added and allowed to stand for 4 hours. This was filtered and the extract was concentrated using a water bath to evaporate about one-quarter of the original volume. The concentrated ammonium solution was added dropwise to the extract until the precipitation was completed. The entire solution was allowed to settle and washed using distilled water then filtered. The weight of the precipitate was recorded [17].

About 20 g of plant samples were dispersed in 200 cm^3 of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55^{o}C. The mixture was filtered and the residue re-extracted with another 200 cm^3 of 20% ethanol. The combined extracts were reduced to 40 cm^3 over water bath at about 90^{o}C. The concentrate was transferred into a 250 cm^3 separating funnel and 20 cm^3 of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. Then, 60 cm^3 of n-butanol was added to the aqueous layer. The solution of n-butanol extract was washed twice with 10 cm^3 of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage [17].

To determine the total phenols, 5 g of the plant sample was weighed into a 250 cm^3 titration flask and 100 cm^3 n-hexane was added twice for 4 hours interval each; the filtrates were discarded for fat free sample preparation. Then, 100 cm^3 diethyl ether was added, heated for 15 min cooled up to room temperature and was filtered into a separating funnel. About 100 cm^3 of the 10% NaOH solution was added and swirled to separate the aqueous layer from the organic layer. It was washed three times with 25 cm^3 de-ionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10% HCl solution and 50 cm^3 dichloromethane (CH_2Cl_2) to acidify the aqueous layer in the separating flask. Consequently, the organic layer was collected, dried and then weighed (18).

RESULTS AND DISCUSSION

Table 1 shows the proximate composition of dried and fresh samples of *Pachycarpus bisacculatus* roots. Crude protein content of the dried sample was 3.19±0.19% slightly differs with the value obtained for the fresh sample which is 1.92±0.05% crude protein. On the other hand, these two values are different from the values obtained for the protein content of *Crassocephalum crepdioides* (27.13±0.01%) reported by Adjatin et al [19]. However, the results shows that *Pachycarpus bisacculatus* is not a good source of protein and it can not be used as a substitute to improve the protein content of low-quality protein diet in both human and animal population in the developing countries [20].

Fat content was 5.64±0.01%. The relatively low fat in the dried and fresh *Pachycarpus bisacculatus* roots shows that the roots can be recommended as weight reducing diet and can therefore be consumed in large quantities with safety without risk of cardiovascular disease, obesity and other related diseases [21, 22].

The ash content of the plant part is a reflection of its mineral elements. The value of 4.68±0.17% in the dried sample and 2.32±0.32% in the fresh samples of *Pachycarpus bisacculatus* roots shows that it is not very rich in nutritionally important minerals, such as potassium, calcium, sodium, magnesium, phosphorus, zinc, copper, iron and selenium. Minerals are utilized by the body in many ways and they play vital roles in the nutritional development of humans and animals [23]. The Crude fibre in *Pachycarpus bisacculatus* roots had the value of 11.33±0.31% in the dried sample and

3.98±0.28% in the fresh sample. Dietary fibre promotes growth and protects beneficial intestinal flora. Furthermore, high intake of fibre is said to promote digestion and reduce the risk of colon cancer [24, 25]. Plants with high fibre content are used for the treatment of obesity, diabetes, cancer, and gastrointestinal disorders [26]. Though the percentage of fibre is moderate, it is nonetheless a dietary advantage to its consumers knowing that it assists digestion and limits cholesterol absorption as observed by Ngaha et al [27]. Moisture content is an integral part of proximate composition analysis of food [25]. It is an index of its water activity and is used as a measure of stability and susceptibility to microbial contamination [28]. This study recorded moisture content as 7.53±0.37% for the dried and 71.56±0.06% for the fresh samples respectively. This implies that dried samples of *Pachycarpus bisacculatus* roots can be stored easily and the moisture content can contribute in slowing down growth and development of microorganisms and in hindering hydrolysis of components present in plant material [27]. While the fresh samples have high moisture content which will promote the growth and development of microorganisms; also, it will reduce the shelf life of the sample. The Nitrogen free extracts (NFE) content in the dried sample (72.21±0.24)% is higher than in the fresh sample (19.92±0.48)%. The nitrogen free extracts could be a good source of energy and thus, a useful supplement in animal feed formulation and human diet.

Table 2 shows the percentage yields and the respective weight of the Hexane, Ethyl-acetate, methanol and aqueous extracts of the roots. From the results, water extracted more efficiently than methanol, hexane and ethyl acetate extracted the least.

Table 1: Proximate composition of *Pachycarpus bisacculatus* roots

	Dried samples	Fresh samples
Mmoisture	7.53±0.37	71.56±0.06
Crude Protein	3.19±0.19	1.92±0.05
Crude Fibre	11.33±0.31	3.98±0.28
Crude Fat	1.05±0.07	0.30±0.03
Ash	4.68±0.17	2.32±0.32
Nitrogen Free Extract	72.21±0.24	19.92±0.48

Values are percentages for triplicate analysis

Table 2: Percentage yield of *Pachycarpus bisacculatus* in n-hexane, ethyl-acetate, methanol and aqueous extracts

Extracts	Percentage yield (%)	Weight (g)
Hexane	1.877	1.502
Ethyl acetate	1.6391	1.313
Methanol	10.342	3.275
Water	21.068	16.860

Table 3: Phytochemical screening of *Parchycarpus bisacculatus* root extracts

	n-Hexane	Ethyl acetate	Methanol	Aqueous
Alkaloid	-	+	+	+
Saponin	-	-	+	+
Tannins	-	+	+	+
Flavonoids	-	-	+	+
Carbohydrate	+	+	+	+
Steroids	+	+	-	-
Triterpenoids	+	+	-	-
Anthraquinones	-	-	-	-
Cardiac glycoside.	+	+	+	+

+ = Present; absent - = Absent; nd = Not detected

Table 4: Anti-nutritional composition of *Pachycarpus bisacculatus* root extracts

Parameters	Values (mg/100g)
Oxalate	1.68±0.02
Tannins	7.10±0.78
Phytate	8.47±0.25
Cyanogenic glycosides	0.03±0.01

Table 5: Elemental composition of *Pachycarpus bisacculatus* root extracts

Elements	Values	Reference Values (WHO, 2017)
Magnesium (Mg)	0.68±0.06	615.00
Aluminium (Al)	ND	0.90
Silicon (Si)	ND	4.50
Phosphorus (P)	0.75±0.03	950.00
Sulphur (S)	0.42±0.07	7.50
Potassium (K)	14.76±0.45	422.00
Calcium (Ca)	22.67±1.51	1200.00
Titanium (Ti)	0.18±0.03	0.50
Vanadium (V)	ND	0.05
Chromium (Cr)	0.13±0.01	0.50
Manganese (Mn)	0.16±0.01	2.00
Iron (Fe)	2.14±0.29	5.00
Nickel (Ni)	0.14±0.02	0.70
Copper (Cu)	0.43±0.07	40.00
Zinc (Zn)	0.07±0.01	50.00
Rubidium (Rb)	0.22±0.01	1.50
Barium (Ba)	0.83±0.07	0.70
Strontium (Sr)	0.66±0.04	1.50
Lead (Pb)	ND	0.30

This may be due to the polarity of water and low-polar nature of ethyl acetate and hexane. This implies that *Pachycarpus bisacculatus* roots contains more polar constituents than non-polar constituents.

Table 3 presents the results of the qualitative phytochemical screening which revealed that saponins, tannins, terpenoids, flavonoids, cardiac glycosides, alkaloids, steroids and carbohydrate were present and the result agrees with the report by Sadat et al [29], where they reported also the presence of cardiac glycosides in the work. Phytochemicals found in fruits and vegetables are generally known for being responsible for protective health benefits in man and animals [30]. Phytochemicals found in this analysis justifies the use of the plant in traditional medical practice for the cure or prevention of diseases such as its use as anti-snake venom, in wound healing, and as antidiabetic agent and for lowering of cholesterol level [31]. Saponins, from recent evidence seem to possess hypo-cholesterolaemic, immune-stimulatory, anti-carcinogenic as well as anti-snake venom properties. In addition, they reduce the risk of heart diseases in humans [32]. Terpenoids are said to help in preventing metabolic disorders, fight cancer and exert anti-aging benefits. As phytochemicals, terpenoids are responsible for a wide variety of flavours and aromas, and have been found to possess anti venom, analgesic, anti-inflammatory, anti-fungal, anti-microbial, anti-viral and anti-parasitic properties [33]. Alkaloids have a wide range of pharmacological activities including anti-malarial, anti-asthma, anticancer, vasodilatory, anti-arrhythmic, analgesic antibacterial, anti-hyperglycaemic and anti-snake venom activities. Many have found use in traditional or modern medicine, or as starting points for

drug discovery [34]. The occurrence of these phytochemicals in *Pachycarpus bisacculatus* roots extracts gives the plant a nutritional advantage. Alkaloids, terpenes, flavonoids and saponins in various plants have portray good anti venom properties in different extract solutions [35]

Table 4 presents the result of the anti-nutritional content of the plant. The presence of oxalate in foods or vegetables above acceptable levels causes irritation in the mouth and the lining of the gut [32] and also hinders the absorption of divalent minerals, particularly calcium [36]. This in effect makes calcium inaccessible to the body, especially for the maintenance of strong bones, teeth, co-factor in enzymic reactions, nerve impulse transmission and blood clothing [37]. However, the concentration of oxalate in this work (1.68±0.02 mg/100g) is within the acceptable level of below 25 mg/100g fresh sample [38]. The consumption of *Pachycarpus bisacculatus* roots in effect may not be harmful or toxic. The tannins level of 7.10±0.78 mg/100g is much less than the lethal dose of tannins (30 mg/kg) reported by Inuwa et al [39], and is higher than the concentration of tannins in *Corchorus olitorius* reported by Ifemeje et al [40] in which they recorded the concentration of tannins as 1.45±0.03%. The dietary use of the vegetable should therefore be encouraged as it would not cause any harm, going by the lethal dose of tannins. Tannins are known to be heat stable and they interfere with the digestion of protein in humans and animals, probably by making protein partially unavailable or by inhibiting digestive enzymes and increasing faecal nitrogen. Tannins present in food products have been found to inhibit the activities of trypsin, chemotrypsin, amylase and lipase. This suggests that the leaves and roots are safe for consumption with regard to cyanogenic glycosides value of 0.03±0.01mg/100g. The phytate level/value recorded in this work is 8.47±0.25 mg/100g. The value is higher than the phytate in *Garcinia kola* (0.634%) reported by Dike and Nnamdi [41]. All the same, the value obtained is below toxic levels and so does not pose any danger to consumers, with respect to medicinal plants. Phytic acids are found in abundance in fibre-rich foods and are recommended because they protect human from cardiovascular diseases and some forms of cancer [42, 43]. With this advantage, yet phytic acid reduces the bioavailability of minerals because of its strong binding affinity to them. They chelate metal ions such as calcium, copper, iron, zinc, magnesium and molybdenum forming insoluble complexes that are poorly absorbed from gastrointestinal tract [43, 45].

Table 5 presents the mineral content of this plant. The values indicate high amount of minerals required by the body, Calcium having highest value (22.67±1.51 mg/kg). This is also in agreement with the elemental analysis by Olaofe and Sanni [46], who reported potassium to be the most abundant mineral in Nigeria agricultural products. Though, potassium is the second most abundant element after calcium (14.76±0.45 mg/kg). High amount of potassium, calcium and magnesium (as macro element) could help to lower the blood pressure [47]. Several clinical studies have shown potassium, calcium and magnesium to be effective blood pressure lowering agents [48]. Hence, consumption of this root may help achieve this purpose. The iron (Fe) content of the seed was 2.14±0.29 mg/kg which shows that it is rich in iron. The mineral contents are within the range when compared with the WHO permissible limits for foods as shown in Table 5. Barium slightly exceeds the permissible level as compared to world health organization permissible level for edible foods [49].

Conclusion. The high content of carbohydrate in the roots of *Pachycarpus bisacculatus* implies that it can be used as energy booster. The high percentage yield of *Pachycarpus bisacculatus* extract in water and methanol greater than in hexane and ethyl acetate implies

that *Pachycarpus bisacculatus* roots contain more polar constituents than non-polar constituents. Further study on the effects of this plant extract on the snake venom is recommended. The high presence of cardiac glycoside in the four solvents shows that it is heart friendly. Cardiac glycosides are a class of organic compounds that increase the output force of the heart and increase its rate of contractions by acting on the cellular sodium-potassium ATPase pump. The anti-nutritional composition shows that the consumption of *Pachycarpus bisacculatus* roots in effect may not be harmful or toxic.

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