IAA Journal of Scientific Research 4(1):63-74, 2018. ISSN: 2736-7319

Proximate, Vitamin, Mineral and Phytochemical Analysis of Ethanol Root Extract and Fractions of *Sphenocentrum jollyanum*

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ABSTRACT

The proximate, vitamin composition, mineral and phytochemical analysis of ethanol root extract and fraction of *Sphenocentrum jollyanum* (SJ) were carried. The proximate, phytochemical, minerals, and vitamins of the ethanol root extract of SJ were analyzed using standard methods. The results revealed that the phytochemical constituents of the samples were in the order of phenols >terpenoids> flavonoids > tannins > glycosides > alkaloids > hydrogen cyanide >saponins>steroids in both the crude extract and fractions. However, phytochemicals were significantly ($P<0.05$) higher in extract than fractions.Proximate composition of the root indicated carbohydrates (40%) > proteins (20%) > moisture (15%) >fibre (8.9%) > fat (8.6%) > ash (7%). The minerals were found in the samples in the order of Ca (656 mg/100g)> Mg (384 mg/100g)> K (371 mg/100g)> Na (228 mg/100g) > Zn (2.78 mg/100g) > Fe (1.33 mg/100g) > P (0.64 mg/100g)> Cu (0.28 mg/100g). The levels of minerals were higher in the crude ethanol extract than in the fractions. The results of the vitamin composition revealed that the crude ethanol root extract and fractions of *S. jollyanum* contained vitamins A (2.46 mg/100g), B_1 (0.67 mg/100g), B_6 (0.47mg/100g), and C (0.56 mg/100g) in appreciable amounts. Vitamin A (2.46 mg/100g) was found to be significantly ($P<0.05$) higher than other vitamins analyzed. The vitamins were also found to be significantly ($P<0.05$) higher in the crude ethanol extract than the fractions. This study has shown that *Sphenocentrum jollyanum* root contain appreciable levels of nutrient components, useful minerals and phytochemicals.

Keywords: *Sphenocentrum jollyanum,* proximate, phytochemicals, minerals, vitamin

INTRODUCTION

Sphenocentrum jollyanum is an erect shrub that belongs to the family Menispermacea [1]. It is called “Ezeogwu” in Igbo, “AduroKoroo” or “Okramankote” in the Akan Language in Ghana [2]. *Sphenocentrum jollyanum* has been shown to have antihypertensive, antioxidant, antinociceptive, antiviral and anti-angiogenic effects in animals [3]; [4]. The plant is also documented for its use against chronic coughs, worms and other inflammatory conditions as well as tumors [5]; [6]. The plant is traditionally used as remedy for feverish conditions as well and as an aphrodisiac [7]; [8]. Studies have shown that the leaves possess significant antipyretic and analgesic activities [9]. The roots and leaves have been reported to be active against polio [10].

It is also believed to be emetic and purgative agents especially when poisoning is suspected; the sap is believed to relieve stomach ache and constipation and also boost appetite and sexual desire [11]. In Ivory Coast, pounded roots are taken against high blood pressure, while the boiled roots are given against epileptic fits [12]. In Ghana, the pulped root is used to treat breast tumors [13]. Different parts of the plant have been used extensively for the treatment of various ailments in Western African sub-regions [14]. Extracts from the root have been used for the relief of constipation, as stomachic, for sickle cell

disease, rheumatism and other inflammatory conditions [15]. The fruits are used as an anti-fatigue snack [16]. It has been also reported by [17] that the methanolic extract of the root of *Sphenocentrum jollyanum* increased the testosterone levels in a dose-dependent manner and also reduced the count, motility and viability of spermatozoa in albino rats.

Photochemicals are chemical constituents that are found in plants which protect the plants cells from insect attacks, disease causative agents, pollution, stress, drought and ultraviolet radiation. They are chemical compounds that are biologically active in plants. They contribute to plant colour, flavour and aroma and fight against disease [18]; [19]; [20]. Phytoconstituents of medicinal plants play an important role in the management of certain diseases such as diabetes mellitus, typhoid and malaria especially in developing countries where resources are meager. Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolities" of which there are several classes including alkaloids, flavonoids, glycosides, polysaccharides, phenols, tannins, saponins, terpenes and terpenoids [21]; [22]; [23]. Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables [24]. Phenolics are the largest group and most widely distributed of phytochemicals in plants. Phenolics include phenolic acids, polyphenols and flavonoids. Phenolics exhibit antioxidant property through free radical scavenging. Flavonoids are among the most studied groups of plant phenols [25]. Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides [26]; [27]. Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and haemolytic activity [28]; [29].

Vitamins are group of complex organic compounds that is required by the body for its normal metabolism. It is present in small amounts in food. They are needed for the maintenance of optimal health, growth and reproduction [30]. Deficiency symptoms (avitaminosis) occur when a single vitamin in the diet is omitted especially when it is needed for the proper functioning of the body [31]. Vitamins are classified into two broad classes - the water soluble vitamins and the fat-soluble vitamins [32]. Vitamin A (retinol) is involved in growth and development, boosting of immune system and good eye sight (vision) [33]. Vitamin D improves magnesium, phosphate, calcium, iron and zinc absorption by the intestines. Research has clearly shown that vitamin D deficiency is part of the seasonal nature of cold and flu outbreaks, low levels of vitamin D leads to lower immunity [34], [35]. The major role of vitamin E is in scavenging reactive oxygen species (peroxyl radical) and prevents polyunsaturated fatty acids oxidation thereby disabling the released free radicals that would have caused damage to tissues [36]; [37]. Vitamin C functions as a cofactor in many enzymatic activities, some of which are important in healing of injuries and in prevention of bleeding. Ascorbate also functions as an antioxidant [38]. The reduced state of ascorbate is enhanced by the presence of glutathione in body cells and extracellular fluids [39].Vitamin B complex are a group of water-soluble vitamins that play important roles in cell metabolism [40].

Proximate analysis is determination of a group of closely related components together (total protein and fat). It conventionally includes determinations of the amount of water, protein, fat, ash and fiber, with nitrogen-free extract (sometimes termed Nifext) being estimated by subtracting the sum of these five percentages from 100 [41]. Proximate analysis is the partitioning of compounds in a feed into six categories (moisture, ash, crude protein or (Kjeldahl protein), crude lipid, crude fibre and nitrogen-free extracts (digestible carbohydrates) [44]. This is done by decomposition of consumable goods into their major components. Proximate approximation of the contents of packaged goods serve as a cheap and easy way for verification of

nutritional panels. Since many herbal products are used orally, to know the proximate and nutrient analysis of these products and raw material used therein plays a crucial role in assessing nutritional significance and health effects [45]; [46]; [47].

Minerals are organic substances present in all body fluids and tissues. Minerals are needed as important nutrients by the body to stay healthy. Their presence is necessary for the maintenance of certain physicochemical processes that are essential to life. Although they yield no energy, they have important roles to play in many body activities [48]. Minearals are classified into macrominerals and trace minearals. Macro minerals are needed in large amounts by the body and they include magnesium, sodium, calcium, chloride, sulphur, phosphorus and potassium. Trace minerals are needed in small amounts by the body. They include iron, iodine, cobalt, fluoride, zinc, manganese, copper and selenium. Minerals can be sourced from different food varieties [49]; [50]; [51]; [52]. The micronutrient deficiencies which are of greatest public health significance are iron deficiency, causing varying degrees of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, and pregnancy complications e.g. reduced psychomotor skills [53]. Minerals are involved in the maintenance of acid-base balance and in the regulation of body fluids. Some are cofactors in enzymatic reaction. Minerals are used in hemoglobin and thyroxin formation. Some minerals play roles in antioxidant functions. They transport gases and also help in muscle contractions [54]. Concentration on which Zn affect human health ranges from 100 to 500 mg/l [55]. Calcium plays significant roles in the contraction of muscle and in many enzyme functions. Calcium functions as a constituent of bones and teeth and in regulation of nerve and muscle functions. It is involved in teeth and bone formation, in clotting of blood and enhances proper heart rhythm [56]. Iron is involved in the formation of haemoglobin which aids in the transportation of oxygen in cellular respiration [7]. It functions as essential component of enzymes involved in biological oxidation such as cytochromes C, C_1 and A_1 [11]; [12]; [13]. Zinc functions as a cofactor and is a constituent of many enzymes such as lactate dehydrogenase, glutamic dehydrogenase, DNA and RNA polymerases, alkaline phosphatase, carbonic anhydrase and alcohol dehydrogenase. Zinc dependent enzymes are involved in macronutrient metabolism and cell replication [18]. Magnesium is important in teeth and bone formation, proper nerve and muscle activity and helps combat stress [21]. Sodium ions are the major cations in the extracellular fluid (ECF) and as such constitute the major contributor to the ECF osmotic pressure. Fluids are retained when sodium is available thereby counteracting dehydration. It also induces thirst for more fluid intake [25]. Potassium is a very significant body mineral, important to both cellular and electrical function [26]. It is one of the main blood minerals called electrolytes which means it carries a tiny electrical charge (potential) [30].

MATERIALS AND METHODS

Collection of Biological Material

The present study was carried out using the roots of *Sphenocentrum jollyanum*. Fresh roots of *Sphenocentrum jollyanum* were collected from Ovoko in Igbo-Eze South Local Government Area of Enugu State, Nigeria and was authenticated in the *Herbarium* Unit of Department of Botany, University of Nigeria, Nsukka by Mr O. Onyeukwu. Part of the authenticated plant was deposited in the *herbarium* for reference purposes.

Preparation of the Plant Extract

The roots of *Sphenocentrum jollyanum* were harvested and washed under tap water to remove contaminants and air dried under shade. They were pulverized using laboratory milling machine and sifted using 0.25 mm sieve. One thousand five hundred gram (1,500g) of the powdered root sample of *Sphenocentrum jollyanum* was soaked in 7500 ml of ethanol for 48 hours with agitation. The resulting ethanol root extract was filtered using muslin cloth and evaporated to dryness using rotary evaporator at a temperature of 45^{0}C. The concentrated ethanol root extract of *Sphenocentrum*

jollyanum was used for subsequent analyses.

Fractionation of the Crude Extract of *Sphenocentrum jollyanum* Roots

The ethanol root extract of *Sphenocentrum jollyanum* (20 g) was fractionated in a glass column (150 cm x 1.5 cm) packed with 200 g of a slurry of silica gel G. (70-230 mesh). The column was eluted in succession with 500 ml ethyl acetate and 500 ml methanol to obtain ethyl acetate (EAF) and methanol (MF) fractions respectively. The resulting fractions were evaporated to dryness using rotary evaporator at a temperature of 45^{0}C. The concentrated ethyl acetate (EAF) and methanol root fractions of *Sphenocentrum jollyanum* were used for subsequent analyses.

Quantitative Phytochemical Analysis of Ethanol Root Extract of *Sphenocentrum jollyanum*

Flavonoids and Tannins were determined according to the method of Trease and Evans, (2002). While the determination of Alkaloids, Phenols, Terpenoids and Saponins were done using the method of [33]. Glycosides and Hydrogen Cyanide were done according to the method of [37], while Steroids was determined by the method described by [42].

Determination of Proximate Composition of *Sphenocentrum jollyanum* Coarse Root Sample

The Determination of Moisture Content, Crude Fibre, Total Ash, Crude Fat, Crude Protein, and Carbohydrate were done by the method of [7].

Determination of Mineral Contents of Ethanol Root Extract of *Sphenocentrum jollyanum*

Phosphorus, Iron, Calcium, Magnesium, Copper, Zinc, Sodium and Potassium were determined by the method of [7].

Determination of Vitamin Contents of Ethanol Root Extract of *Sphenocentrum jollyanum*

Vitamin A, Vitamin D and Vitamin E content were determined by the [7] method, while Vitamin $B_{1,}$ Vitamin $B_{2,}$ Vitamin B_3 content were determined by the method of [44]. Vitamin $B_{6,}$ Vitamin $B_{7,}$ Vitamin $B_{9,}$ Vitamin B_{12} were determined by the method of [8], and Vitamin Cwas determined using the method of [49].

STATISTICAL ANALYSIS

Results were expressed as mean± standard deviations where applicable. The data were subjected to one-way analysis of variance (ANOVA), followed by Post hoc Duncan multiple comparison test using SPSS software version 21 and $p < 0.05$ was regarded as significant

RESULTS AND DISCUSSION

Chemical Composition of *Sphenocentrum jollyanum*.Quantitative phytochemical composition of crude ethanol root extract, ethylacetate and methanol fractions of *Sphenocentrum jollyanum*.

The result of quantitative phytochemical analyses of crude ethanol root extract, ethylacetate and methanol fractions of *S. jollyanum* is shown in Table 1. The results revealed that the extract and fractions contain phytochemicals in varying amounts and occurred in the order of phenols>terpenoids> flavonoids> tannins > glycosides> alkaloids > hydrogen cyanide>saponins>steroids in both the crude extract and fractions. However, phytochemicals were significantly ($P<0.05$) higher in extract than fractions.This result agrees with the earlier work of Mbaka*et al.* (2009) which showed that the root extract of *Sphenocentrum jollyanum* contain alkaloids, terpenoids, flavonoids, tannins and glycosides. [13] also showed that methanol stem bark extract of *Sphenocentrum jollyanum* contain tannins, alkaloids and terpenoids. The presence of these biologically active compounds suggest that *Sphenocentrum jollyanum* could serve as a potential source of antidiabetic drug because there secondary metabolites could exert some biological activities when taken by animals [9].

Phytochemicals identified from medicinal plants present an exciting opportunity for the development of new types of therapeutics for diabetes mellitus. Most prevalent among phytochemical groups are the alkaloids, glycosides, flavonoids, terpenoids and steroids [2].

Alkaloids has been shown to exert a wide range of antidiabetic activities. Different

alkaloids have been isolated from several medicinal plants and investigated for their possible antidiabetic activity in different animal models [1].

The alkaloid trigonelline isolated from the seeds of *Abrusprecatorius* decreased blood glucose levels in alloxan-induced diabetic rats as well as reduced the activity of glucose-6-phosphatase and glycogen phosphorylase, two enzymes important for glucose production [49]. Berberine, an isoquinoline alkaloid is obtained from the roots and stem bark of *Berberis L.* (Berberidaceae) [50], acts as antihyperglycemic agent by inhibiting the activity of disaccharidases in Caco-2 cells [54]; [55].

Jamboline, a glycoside present in the seeds of *Syzygiumcumini* (*Eugenia jambolana*) has been shown to possess antidiabetic properties [16]. Jamboline exerts hypoglycemic action by preventing conversion of starch into sugar and also diminishes quantity of sugar in urine and reduces thirst [25]; [26].

In vitro study of [4] suggested that anthocyanins, a class of flavonoids decreased the intestinal absorption of glucose by retarding the release of glucose during digestion.

Proximate Composition of *Sphenocentrum jollyanum* Root

Proximate composition of the root of *Sphenocentrum jollyanum*is shown in Figure 1. The results revealed that the root of *S. jollyanum* have high levels of carbohydrates (40%) and protein (20%). The results indicated that carbohydrates (40%) > proteins (20%) > moisture (15%) >fibre (8.9%) > fat (8.6%) > ash (7%). The results of this research are in agreement with the earlier findings of [31] who also reported similar contents of carbohydrates, proteins, moisture, fibre, fat and ash in some selected medicinal seeds. Proteins, fats and carbohydrates are essential for life and studies have indicated that life is sustained by nutrient mixtures of foods containing them [11]. The carbohydrate value of 40 % obtained in the root of *S. jollyanum* is moderate and within the range reported by [19], [20]. Carbohydrates are known to be important components in many foods, and the digestible carbohydrates are considered as an important source of energy [16]. The protein value of 20 % falls within the range of findings by [22]. Availability of such high contents of protein is helpful in maintaining proper growth and development in adults, children, and pregnant women that require good quantity of protein daily [26]. As a nutritive value of food, fibers in the diet are necessary for digestion and for effective elimination of wastes, and can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer [29]. Thus *S. jollyanum* can be considered as a valuable source of dietary fiber in human nutrition with a fibre value of 8.9% (figure 1). [38] suggested a strong correlation between moisture contents and fiber, which could be of interest to human health as the fibre are easily digested and disintegrated.

The mineral composition of crude ethanol root extract, ethylacetate and methanol fractions of *Sphenocentrum jollyanum*.

The results ofthe mineral composition of ethanol root extract, ethylacetate and methanol fractions of *S. jollyanum* are shown in Table 2. Analysis of the result revealed the presence of the following minerals: Na (228 mg/100g), K (371 mg/100g), Ca (656 mg/100g), Mg (384 mg/100g), (Cu 0.28 mg/100g), Fe (1.33 mg/100g), Zn (2.78 mg/100g) and P (0.64 mg/100g) with Ca (656 mg/100g) having the highest value in both the crude extract and fractions. The magnitude of occurrence of the minerals was in the following order: Ca (656 mg/100g)> Mg (384 mg/100g) > K (371 mg/100g) > Na (228 mg/100g) > Zn (2.78 mg/100g) > Fe (1.33 mg/100g) > P (0.64 mg/100g)> Cu (0.28 mg/100g). The levels of minerals were higher in the crude ethanol extract than in the fractions while the magnitude of occurrence of Na, K, Ca, Mg and Fe were in the following order: crude ethanol extract > methanol fraction >ethylacetate fraction. These results agree with the earlier reported works of [7]; [8], [9] which showed the presence of minerals in medicinal plants in appreciable amounts.Minerals are required for normal growth, activities of muscles and skeletal

development, cellular activity and oxygen transport (copper and iron), chemical reaction in the body and intestinal absorption (magnesium), fluid balance and nerve transmission (sodium and potassium) [14]. Iron is useful in prevention of anemia and other related diseases. Iron is required in mammalian nutrition to prevent anemia and is part of hemoglobin and myoglobulin molecules involved in oxygen transport to and within cells. [17]. Manganese plays a role in energy production and in supporting the immune system [43]. Deficiency of these nutrients and minerals are known to affect the performance and health in both humans and other animals [8]. Zinc forms metalloproteinase and enzymes complexes which cannot be dissociated without loss of activity. Calcium is an important constituent of body fluids and is used in bone formation in conjunction with phosphorus. Magnesium is an activator in enzyme systems which maintains electrical potential in nerves, while sodium and potassium influence osmotic pressure and contributes to normal pH equilibrium [15]. Nutrients rich foods are vital for proper growth both in adults and children. Since *S. jollyanum* can be taken in combination with other dietary components, some of which may be better sources of the minerals under consideration, this plant could be of value in supplementing the minerals available from these other sources [10]. Recent promotion of herbs and medicinal plants as health foods commonly includes reference to their mineral contents. Unfortunately, little consideration is generally given to the fact that only five mineral elements are considered essential for metabolism in substantial amounts (calcium, magnesium, potassium, phosphorus and sodium), while ten others (chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenium, selenium and zinc) are important in trace amounts only; of these, probably only selenium, molybdenuim, manganese, chromium and fluorine are essential [55]. Controversy exists over metabolism need versus optimal intake. Toxic levels are often very near the required dosages for "normal diets" and minerals like lead (Pb), arsenic (As), mercury (Hg), silver (Ag) and cyanide (CN^-) are toxic and of no significant use in the human body [51]. In case of the Pb concentration, the suggested concentration in plant species that is "safe" is 0.2 to 0.6 mg/100ml, however, WHO recommendations for Pb level in humans, is that it should not exceed 10 ppm (WHO, 2007). It is noteworthy that lead (Pb) was not detected in *S. jollyanum* root used in this study and so, there is no danger of Pb toxicity. There has been some speculation that mild deficiencies of minerals may be beneficial (WHO, 2007). Dietary deficiencies are common with iron, calcium, iodine and fluorine [4].

The vitamin composition of crude ethanol root extract, ethylacetate and methanol fractions of *Sphenocentrum jollyanum*.

The results of the vitamin composition of crude ethanol root extract, ethylacetate and methanol fractions of *S. jollyanum* are shown in Figure 2. The results revealed that the crude ethanol root extract and fractions of *S. jollyanum* contained vitamins A (2.46 mg/100g), B_1 (0.67 mg/100g), B_6 (0.47mg/100g), and C (0.56 mg/100g) in appreciable amounts but vitamin A (2.46 mg/100g) was found to be significantly ($P<0.05$) higher than other vitamins analyzed. The vitamins were also found to be significantly ($P<0.05$) higher in the crude ethanol extract than the fractions. Our result are in agreement with the work of [12] who reported the presence of vitamins A, C, E, B_1, B_2 and B_3 in the leaves of some anti-diabetic medicinal plants: *Azadirachtaindica, Vernoniaamygdalina* and *Gongronemalatifolium*. It has been shown that overproduction of free radicals are associated with A, C and E avitaminosis [37]. Vitamins A, C and E are antioxidants that function to scavenge reactive oxygen species released in the pathogenesis of diabetes mellitus due to abnormalities in glucose metabolism [40]. Our result from this research showed that crude ethanol root extract and fractions of *Sphenocentrum jollyanum*are rich in these antioxidant vitamins A, C and E which could be responsible for the

possible anti-diabetic effect of *Sphenocentrum jollyanum* [9]

Table 1: Quantitative phytochemical composition of *Sphenocentrum jollyanum* crude ethanol root extract, ethylacetate and methanol fractions

Phytochemicals (mg/100g)	Ethanol Extract	Ethylacetate Fraction	Methanol Fraction
Terpenoids	1904.72 ± 0.45[a]	935.80± 0.24[c]	968.92± 0.02[b]
Tannins	614.34 ± 0.01[a]	278.82 ± 0.01[c]	335.35± 0.01[b]
Alkaloids	332.78 ± 0.01[a]	179.41 ±0.01[b]	153.17±0.01[c]
Flavonoids	1646.92 ± 0.01[a]	970.03± 0.01[b]	676.13± 0.01[c]
Phenols	2217.19± 0.01[a]	917.07 ±0.01[c]	1300.12±0.01[b]
Glycosides	613.63± 0.02[a]	238.87± 0.01[c]	375.23± 0.01[b]
Saponins	1.89±0.01[a]	0.82±0.09[c]	1.07 ±0.04[b]
Steroids	1.53± 0.01[a]	0.74 ±0.06 [b]	0.79±0.07[b]
Hydrogen cyanide	2.12 ±0.02[a]	0.77 ±0.05 [c]	1.35±0.01[b]

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts within the same row are significantly different at P<0.05.

Table 2: Mineral composition of crude ethanol leaf extract, ethylacetate and methanol fractions of *Sphenocentrum jollyanum*.

Minerals (mg/100g)	Ethanol Extract	Ethylacetate Fraction	Methanol Fraction
Sodium	228.19 ± 1.16[a]	112.55±0.02[b]	116.18±0.01[b]
Potassium	371.42± 0.012[a]	173.08±0.01[c]	198.34± 0.01[b]
Calcium	656.88 ± 0.01[a]	276.61 ±0.01[c]	380.27±0.01[b]
Magnesium	384.05 ± 0.01[a]	118.24± 0.01[c]	266.29± 0.01[b]
Copper	0.28± 0.01[a]	0.14 ±0.01[b]	0.14±0.01[b]
Iron	1.33± 0.013[a]	0.68± 0.01[b]	0.65± 0.03[b]
Zinc	2.78±0.04[a]	1.59±0.01[b]	1.19 ±0.01[c]
Phosphorus	0.64± 0.01[a]	0.35 ±0.01[b]	0.29±0.01[b]

Values are the mean ± standard deviation of 3 replicate values.
Values with different superscripts within the same row are significantly different at P<0.05.

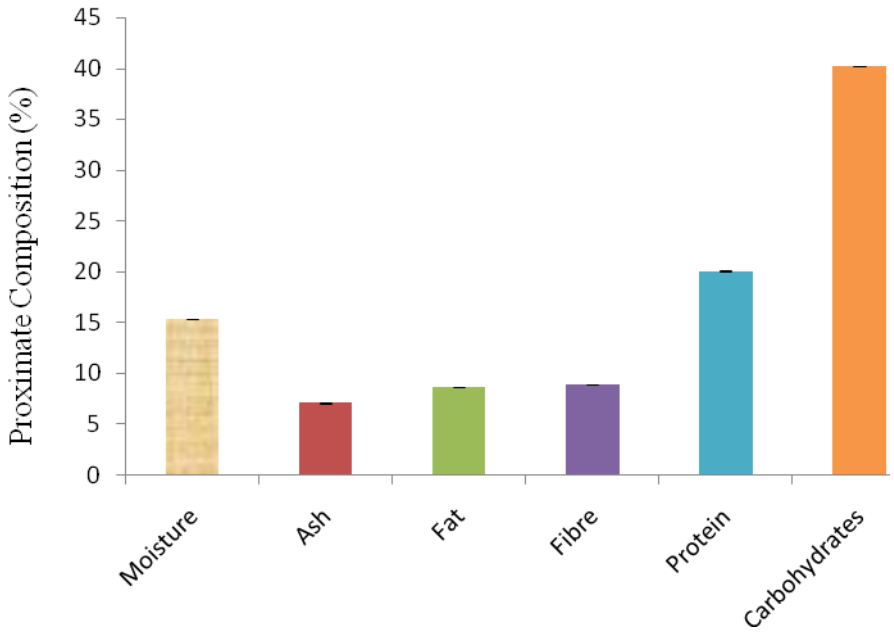

Figure 1: Proximate composition of *Sphenocentrumjollyanum* root.

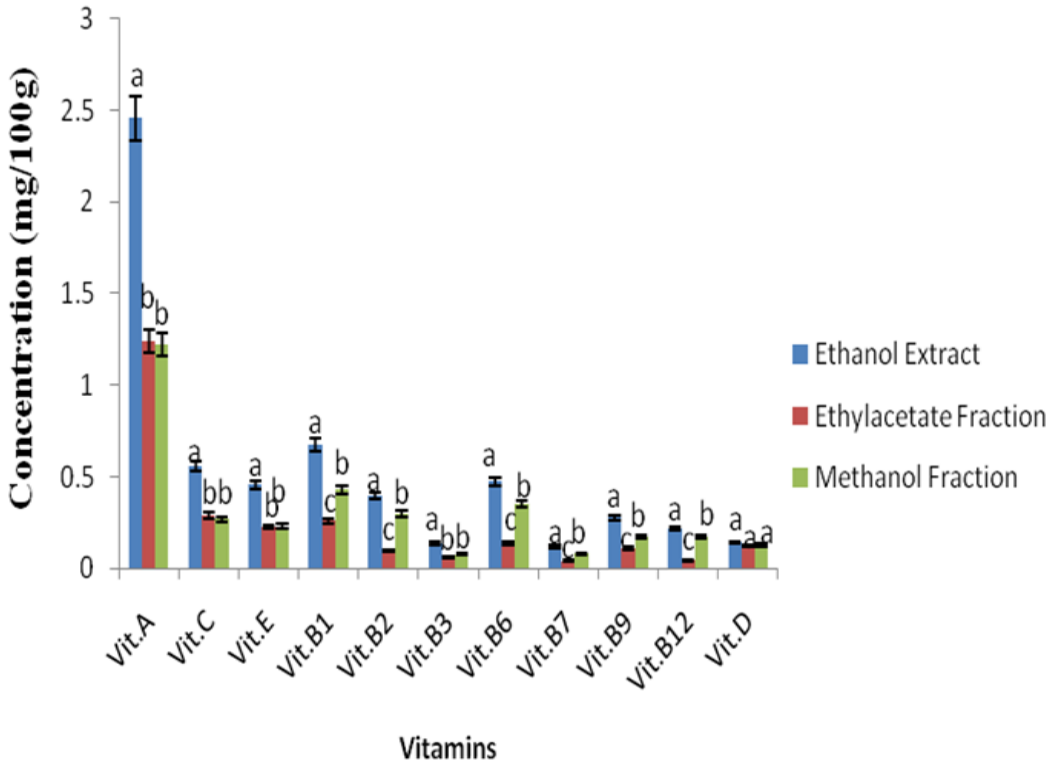

Figure 2: Vitamin composition of crude ethanol root extract, ethylacetate and methanol fractions of *S. jollyanum*.
Bars in a group with the same letter are not significantly different at $p<0.05$.

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