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DETERMINATION OF PROXIMATE AND MINERAL ELEMENT COMPOSITION OF THE CRUDE ETHANOL EXTRACT OF THE ROOTS OF *SPHENOCENTRUM JOLLYANUM*

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Abstract

The present study is aimed at investigating the proximate composition and mineral element composition of ethanol extract of *S. jollyanum* in Wistar albino rats. In proximate analysis, moisture. Ash, crude fibre, carbohydrates, crude fats and crude protein were determined. And for mineral analysis, potassium, sodium was determined using Flame Photometer, while calcium, magnesium, iron, zinc, calcium and chromium were determined using Atomic Absorption Spectrophotometer. Proximate composition of *S. jollyanum* indicated the presence of crude protein (1.05%), moisture (4.93%), crude lipid (2.78%), ash (1.25%) with high content of fiber (6.59%) and carbohydrates (83.4%). The results also showed low levels of minerals in Na(1,72mg/kg), Cr(4.87mg/kg), Mg(5.1mg/kg), Zn(3.42mg/kg), and Ca(8.98mg/kg) with high levels of minerals in K(62.62mg/kg), and Fe (31.06mg/kg). The results of this study concluded that the root extract of *S. jollyanum* contained high amount of nutritional, energy values and minerals which suggest its folkloric uses as an ergogenic aid and could be beneficial to enhance the performance of athletes in sporting events.

Keywords: Proximate Composition, Mineral element Composition, Sphenocentrum Jollyanum, Ergogenic aids

1.0 INTRODUCTION

Sports is a very lucrative venture all over the world. Countries, organizations and individuals invest heavily in different sports and sporting event with the sole aim of winning and pride. Every athlete desire to win laurels and break records. This drive and desire to win at all cost most often pushes the athlete to the limits of top performance. Athletes are usually sought after by organizations and even countries based on their quality and tendency to win and bring home trophies. Several researches have been conducted towards peak performance in athletes and this has led to the discovery of various substances (natural and synthetic) which can aid athletes achieve higher levels of muscle response towards achieving the goals of winning and being on top form. An ergogenic substance is a physical, mechanical, nutritional, psychological, or pharmacological substance or treatment that either directly improves physiological variables associated with exercise performance or removes subjective restraints which may limit physiological capacity" (Robergs, 2010). Ergogenic aids are also substances, devices, or practices that enhance sports performance. These include mechanical aids (such as special clothing and equipment), nutritional aids (such as sports drinks), physiological aids (such as blood transfusions), pharmacological aids (such as steroids), and psychological aids (such as meditation) (Millard, 2013). Due to the high rate of illegal substance use as ergogenic aids and the threat of being banned from sporting activities and stripping off of laurels from affected athletes, focus has turned from pharmacological ergogenic aids to nutritional and herbal aids. Nutritional ergogenic aids includes metabolic fuels e.g. carbohydrate, protein, pyruvate, lactate, fat, caffeine, branched chain amino acids, etc.; Limiting cellular components creatine, carnitine, vitamins, phosphate, $NaHCO_3^-$, etc., anabolic or stimulatory substances e.g. protein, chromium, vanadium, dichloroacetate, ephedrine, β-hydroxy- β -methylbutarate (HMB),Androstenedione, caffeine, etc.; Anti-Catabolic anti-oxidants, β-hydroxy- β -methylbutarate (HMB), etc.; Substances that

may enhance thermoregulation and/or prevent dehydration e.g. fluid, electrolytes, glycerol, sports drinks, etc. pharmacological ergogenic aids include erythropoietin, β-blockers, antihistamines, growth hormone, anabolic-androgenic steroids, caffeine, amphetamines, ephedrine, β -hydroxy- β -methylbutarate (HMB), androstenedione, dehydroepiandrosterone etc, Physiological ergogenic aids include blood doping, saline infusion, warm-up, clothing, etc., psychological ergogenic aids include hypnosis, psychotheraphy, imagery, etc. several substances previously used as ergogenic aids have been banned due to a variety of reasons with athletes being stripped of their titles. A popular example is the American athlete Marion Jones, who won five Olympic medals at the 2000 Olympics but forfeited all medals and prizes dating back to September 2000 after she admitted in October 2007 that she took performance-enhancing drugs (Millard, 2013). Several other cases abound in the sporting world. Hence, the need for alternative ergogenic substances which are neither harmful nor illegal. Sportsmen and women as well as research organizations and sports companies/agencies now turned to better herbal ergogenic aids.

2.0 Materials and Methods

Sphenocetrum jollyanum is a short evergreen shrub (dioecious in nature) found in the undergrowth of dense forest and it grows to a height of 1.5m (fig. 2.1). The plant is characterized by few branches, bright yellow roots, grey coloured bark, a stem that is thinly short-hairy when young and is widely distributed in West Africa from Sierra Leone to Nigeria (Nia *et al.*, 2004). *S. jollyanum* flowers and bears fruit either irregularly or continuously all through the year while it is pollinated by certain insects or ants and seeds are usually dispersed within a short distance from the parent plant.

Classification

Kingdom: Plantae

Division: Magnoliophyta (Conquist)

Subdivision: Maynoliophytina (Frohne and Jensen)

Class: Ranunculopsida (Bronogn)

Subclass: Ranunculidae (Takht)

Superorder: Ranunculanae (Takht)

Order: Menispermales (Bromhead)

Family: *Menispermaceae (Juss)*

Genus: *Sphenocentrum (Pierre)*

Species: *Jollyanum*

Fig. 2.10: *Sphenocentrum jollyanum*

Medicinal importance

S. jollyanum belongs to the family Menispermaceae and is known locally in Ghana as aduro kokoo (red medicine) or okramakote (dog's penis). It is called *burantashi* in hausa language. Virtually every part of the plant is used for one medicinal purpose or the other. It bears fruit that is yellowish in colour when ripe and contains a single large oval shaped seed (Mbaka & Adeyemi, 2010). The roots which are bright yellow with a sour taste are used as chewing sticks, relief for constipation, as a stomachic, as a cough medicine, for sickle cell disease, rheumatism and other inflammatory conditions (Moody *et al.*, 2006).

S. jollyanum has been extensively researched medicinal plant. Research has shown that ethanolic extracts of *S. jollyanum* enhanced sexual behavior and increased libido in male mice; also, daily administration of the extract to adult male rats for 3 weeks resulted in elevated levels of testosterone and FSH (Owiredu *et al.*, 2007). Ethanolic root extract of *S. jollyanum* has been shown to exhibit significant reduction in blood glucose level when administered to normal and alloxan-induced diabetic rabbits" (Mbaka *et al.*, 2010a). Methanolic root extract of *S. jollyanum* have also been shown to possess hypoglycaemic and hypolipidamic properties on streptozocin-induced diabetic wistar rats

(Mbaka *et al.*, 2010a). Ethanolic root extract of *S. jollyanum* have been shown to have protective effect on the morphology of pancreatic beta cells of alloxan challenged rabbits (Mbaka & Owolabi, 2011). In a another research (Mbaka & Adeyemi, 2010), it was shown that ethanolic root extracts of *S. jollyanum* were relatively non-toxic following oral administration. Methanolic stem bark extracts of *S. jollyanum* have been shown to exhibit significant antioxidant and hepatoprotective properties (Olorunnisola *et al.*, 2011). Ethanolic leaf extracts of *S. jollyanum* have been shown to have anti-hyperglycaemic property on normal and alloxan-induced diabetic rabbits (Mbaka *et al.*, 2010a). In another related study, ethanolic root extract of *S. jollyanum* were shown to produce significant increases in HGB, HCT and MCHC in a dose dependent manner which imply that the extract probably has a haemopietic effect on fischer rats following a 90-day administration period (Amidu *et al.*, 2008). An increase in the HGB and HCT concentrations indicates the extract enhances the oxygen-transport capacity of the blood (Massey, 1992), whereas the increase in the MCH and MCHC may be further evidence of the haematopoietic effect of the extract. Research indicates that the seed oil of *S. jollyanum* have beneficial effect on the plasma lipid profile of the animals by reducing the total cholesterol, triglyceride and LDL-cholesterol (‘bad cholesterol’) while increasing HDL-cholesterol (‘good’ cholesterol); thus *S. jollyanum* could be very important in the prevention and management of heart-related diseases such as atherosclerosis and coronary heart disease (Mbaka & Owolabi, 2011). The seed oils of *S. jollyanum* also resulted in significant increase in RBC count, Hb and PCV in a dose dependent manner showing that the oil extracts stimulate erythropoiesis in the kidney (Mbaka & Owolabi, 2011).

S. jollyanum ethanolic root extract has been shown to possess anti-depressant-like effects when administered to mice (Woode *et al.*, 2009). In forced swimming test (FST) and tail suspension test (TST), administration of extracts of *S. jollyanum* were significantly less potent (20-50 times less) compared to the standards used (imipramine and fluoxetine) (Woode *et al.*, 2009).

Research indicates that methanolic extracts of the root of *S. jollyanum* may produce harmful effects on reproductive functions (reduction in progressive motility of spermatozoa, viability and total sperm counts) in male wistar rats; a significant decrease ($P< 0.05$) in serum aspartate and alanine aminotransferase activities with a significant increase ($P < 0.05$)

in testicular SOD activity at a dose of 50 mg kg bodyweight was also observed while ethanolic root extract of *S. jollyanum* significantly increased follicle-stimulating hormone (FSH) levels by the second week of treatment in a dose-dependent manner ($P<0.001$) and also the level of testosterone was greatly increased by the third week of treatment (Owiredu *et al.*, 2007). Studies on mating behavior shows that extract of *S. jollyanum* stimulated mounting and mating behaviour by increasing mounting frequency, intromission frequency and prolonged ejaculation latency (Owiredu *et al.*, 2007). Studies have been carried out on the myorelaxant effect of the ethanolic root extract of *S. jollyanum* in rabbit aortic strip and corpus cavernosurn and the result showed that the ethanolic root extract of *S. jollyanum* has myorelaxant effects (Woode *et al.*, 2009). This may prove very useful in sports and endurance exercises.

a. Proximate determination

Determination of Moisture Content: A glass petri-dish was accurately weighed, after which an approximately 1.0g of sample was added and reweighed and the weight recorded as (w_1). This was kept in a vacuum oven for 1 hour at the 105^0C, the dish was removed from the oven, cooled and re-weighed and recorded as (w_2). this process was repeated until a constant weight was attained. This process was repeated for all the samples, and the moisture content was calculated in percentage as follows:

% moisture = (w_1-w_2/weight of sample used) x 100

Determination of Ash Content: 1.0g of sample was accurately weighed in a platinum crucible and recorded as w1, this was transferred to muffle furnace at the temperature of 5500˚C for 8 hours until a white ash was obtained. The platinum crucible was removed and place in a dedicator to cool and weighed, the value was recorded as w_2, Percentage as was calculated as

% ash = (w_1-w_2/weight of sample used) x 100

This was repeated for all samples.

Determination of Fats and Oil: Cold method of extraction was used to determine fats and oil in all the four samples, 10g of samples of accurately weighed into round bottom tom flasks then 50ml of n-hexane was added to each of the samples and covered for 24 hours for proper extraction of oil after which clean and dried empty beakers were weighed and weights noted. The samples were decanted into the beakers and were heated to dryness and transferred in a desiccator to cool and weighed and new weights taken. Percentage fats were calculated thus;

% fat or oil = (w_2-w_1/weight of sample used) x 100

Crude Fibre Determination: 2.0g of samples were digested in 200ml of 1.25% H_2SO_4, the mixture was boiled for 30min. and was filtered and washed with hot water to reduce the acidity, this was tested with pH paper, the residue was again digested in 200ml of 1.25% NaOH. The mixture was heated for 30min. filtered and washed with hot water and dried in an oven, this was transferred to a platinum crucible and weighed (w_1) then heated in a furnace at 5500^C to ash and weighed again (w_2). Percentage crude fibre was calculated as:

% crude fibre = (w_1-w_2/weight of sample used) x 100

Protein determination: The protein nitrogen in 0.5g of dried samples was converted to ammonium sulphate by digestion with concentrated H_2SO_4 and in the presence of Cu_2SO_4 and Na_2SO_4. This was heated and the ammonia involved was steam distilled in 4% boric acid solution, the nitrogen from ammonia was deduced from the titration of the trapped ammonia with 0.1N H_2SO_4 with methyl red indicator until a pink colouration was observed indication the end point of titration. Protein was calculated by multiplying the deduced value of nitrogen by a protein constant 6.25mg.

Carbohydrate Determination: The carbohydrate content of the samples was estimated as the difference obtained after subtracting the values of organic protein, ash content, fat or oil, crude fibre, and moisture content from 100. That is 100- (protein + ash + oil + crude fibre + moisture content).

b. The mineral composition

Sample preparation (wet digestion method): A total volume of 100ml of H_2SO_4, HNO_3 and HClO in the ratio of 40%:40%:20% was mixed together. Next, 1-3g of the sample was weighed into a conical flask and 2ml of the mixed acid was added to each of the sample in the conical flask. Digestion was carried out in a fume cupboard with hot plate until white fumes appear. It was then cooled and filtered into a 100ml volumetric flask and make up to mark with distilled water.

Procedure: The manufacturer's instruction was followed in carrying out the experiment. The hollow cathode lamp for the desired metal was installed and the wavelength and slit width were set as specified by the analytical methodology. The instrument was turned on and the hollow cathode lamp current was applied (as suggested by the manufacturer) and allowed for warm for 20 minutes (this enables the instrument to stabilize energy sources). The current was readjusted after

warm-up and the wavelength dial adjusted until optimum energy was obtained. Lamp was then aligned and burner head installed and adjusted. The air was turned on and flow rate adjusted to give maximum sensitivity for the metal being measured. The acetylene was then turned on and the flow rate adjusted to value specified. This was followed by flame ignition and stabilization for a few minutes. The blank was then aspirated and instrument zeroed. A standard solution was aspirated and aspiration rate of nebulizer adjusted to obtain maximum response. The blank was aspirated again and the instrument re-zeroed. A standard with a concentration near the middle of the linear range was aspirated and the absorbance recorded. The sample was then analysed and absorbance recorded. After analysis, the flame was extinguished by turning of acetylene first then air.

Calculations: The concentration of the metal in the sample can be calculated from calibration curve, or read directly from instrument the concentration in milligrams or micrograms per litre according to calibration.

3.0 Results

The results of this study are shown in the table and charts below:

Table 1: Proximate composition of *S. Jollyanum*

Parameter	Composition (%)
Ash	1.25±0.30
Moisture Content	4.93±0.75
Crude Protein	1.05±0.22
Crude Lipid	2.78±0.25
Crude Fiber	6.59±1.75
Carbohydrate	83.4±2.70

Values are expressed as mean ± S. D (n=5)

Table 2: Mineral composition of *S. Jollyanum*

Mineral	Composition (mg/kg)
Fe	31.06±0.30
Mg	5.1±0.22
Zn	3.42±0.20
Ca	8.98±80.30
K	62.62±0.50
Na	1.72±0.00
Cr	4.87±0.10

Values are expressed as mean ± S. D (n=5)

Proximate composition of *S. jollyanum*

The result of the proximate composition of *S. Jollyanum* is presented in table 1. The carbohydrate content was the highest at 83.4±0.00%, while crude protein (1.05±0.00%, crude lipid (2.78±0.00%), crude

fibre (6.59±0.00). moisture content (4.93±0.00%) and ash (1.25±0.00) also showed significant results. These results agree with the findings of Olorunnisola *et al* (2011), which reported that *S. jollyanum* have high energy content with significant amount of protein, lipids, crude fiber, ash and moisture content. These proximate values were also comparable to previous studies (Ibironke & Olusola, 2013) (Ugwu *et al.*, 2018).

Macronutrients are important for athletic performance as well as general health. Proteins rebuilds muscle tissues and are also needed for the production of different enzymes, vitamins and hormones that enhances performance. Carbohydrates are the predominant energy sources that provide energy to the muscle, brains, nerves and other body tissues.

Mineral composition of *S. jollyanum*

The mineral composition of *S. jollyanum* is presented in table 2. The following minerals were detected in varying concentrations: Fe, Mg, Zn, Ca, K, Na and Cr. K was the mineral with highest concentration (62.62±0.00mg/kg) while the lowest was Na (1.72±0.00mg/kg). other minerals had the following concentrations: Fe(31.06±0.00mg/kg), Ca (8.98±0.00mg/kg), Mg(5.1±0.00mg/kg) and Cr (4.87±0.00mg/kg).

Minerals are very important components of nutrition and these must be supplied through the diet; every mineral performs a specific function and deficiency leads to severe symptoms. The mineral composition of *S. jollyanum* in this study were comparable to other reports (Ibironke & Olusola, 2013; Ugwu *et al.,* 2018). The presence of magnesium plays a variety of roles in regulating metabolism. Athletes are particularly prone to high rates of magnesium turnover in the body because of the sweating and hormonal fluctuations that accompany physical activities. Magnesium deficiency reduces endurance performance by increasing the oxygen requirements needed to complete submaximal exercise. (Relander,2017) Potassium is a critical electrolyte that balances fluid levels nerve functions and nutrient transport systems in the body. Potassium is lost when sweating, and intense physical activities can result in immediate fatigue and muscle weakness, thus, athletes need high levels of potassium to guarantee optimal levels of performance. potassium and sodium are electrolytes that helps your body maintain fluids and blood volumes. Potassium also plays a key role in the storage of carbohydrates to fuel muscle tissues. The results of the work is in agreement with previous work done which identifies varying

concentrations of Mg, Na, K and Zn in *S. jollyanum* (Olorunnisola *et al*, 2011)

4.0 Conclusion

The ever-expanding world of sports is a very expensive and money-spinning industry, however as companies invest in athletes, the demand on these athletes to perform and deliver trophies has also increased. This has led to several interventions geared towards improving performance in sports and several of these have been banned due to their safety concerns. Nutritional and herbal aids have become increasingly more popular and has thus been researched. *S. jollyanum* (*Burantashi*) is a very useful herb in Africa (especially Nigeria) which has been used extensively in treatment and management of different conditions; its ergogenic potentials and several other parameters were investigated (Olorunnisola *et al.*, 2011; Wopara *et al.*, 2019). The crude ethanol extract of *S. jollyanum* showed significant proximate and rich mineral composition that acclaims to its folkloric use as and ergogenic in sporting activities.

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