wjpmr, 2018,4(2), 147-152

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

DETERMINATION OF PROXIMATE COMPOSITION, PHYTOCHEMICAL SCREENING AND MINERAL ANALYSIS OF *SANTALUM RUBRUM* SEED

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Article Received on 19/12/2017 **Article Revised on 09/01/2018** **Article Accepted on 30/01/2018**

ABSTRACT

The purpose of this present study was to investigate the proximate composition, phytochemical and mineral content of *Santalum rubrum* seed. The results obtained from proximate analysis were: moisture content (10.17%), dry matter (89.83%), ash (4.95%), organic matter (95.05%), crude protein (19.81%), crude fiber (6.87%), carbohydrate (21.39%), and nitrogen (3.17 %). From total ash we also estimated (39.97%) water soluble and (60.03%) water insoluble while (92.01%) acid soluble and (7.99%) acid insoluble ash. The calorific values of the seed were found 390.6cal/gm. The presence of some phytochemicals like alkoloids, saponins and flavonoids explained the medicinal action of the plant encountered in its therapeutic uses. The seed samples contains reasonable amount of essential mineral needed for the body such as potassium, sodium, calcium, iron, magnesium, aluminum, zinc, copper, manganese, lead, nickel, chromium and cadmium. The mineral elemental concentration carried out showed potassium having the highest concentration of 91.89 mg/kg while Cd was the lowest (0.0001 mg/kg).

KEYWORDS: *Santalum rubrum*, Proximate analysis, Atomic Absorption Spectrophotometer, mineral compositions.

INTRODUCTION

Now days, the people of developing countries especially the rural pregnant women and children are suffering for malnutrition. Food contains essential ingredients for sustenance of plants and animals.[1] Small but mighty, seeds can supply various life-enhancing nutrients such as protein, iron, fiber and vitamins that can help the body fight diseases and promote good healthy living. Studies have shown that seeds do not only contain nutritionally important bio-compounds but are also sources of other phyto-compounds which at certain critical levels have significant anti-nutritional effects.[2]

Santalum rubrum, with the common names red sanders, red sandalwood, and saunders wood, is a species of Rubrum to the southern Eastern Ghats mountain range of South India.[3] This tree is valued for the rich red color of its wood. Demand for red sandalwood is mainly in the overseas market, said a trader and it comes mainly from countries like China, Japan, Myanmar and other others in East Asia.

The red sandalwood has medical advantages. According to Institute of Wood Science & Technology the wood gives cooling effect when applied externally for inflammations, head-ache, bilious affections and skin diseases and improves treating headache, skin diseases.

This plant, *Santalum rubrum*, is widespread across Bangladesh, India, Malaysia, Canada, and Australia. Bangladesh is a good repository of medicinal plants belonging to various families. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safely and effectiveness, leads for treating different diseases.[4]

Few interesting compounds could be found out through the investigation which may be unknown, pharmacology active and highly potent. Results obtained, if significant, can be used as a treatment option against some diseases. This may provide cost effective treatment due to its availability in Bangladesh. Hence the objective is to explore the possibility of developing new drug candidates from this plant for the treatment of various diseases.

Proximate analysis is a system of analysis of nutrients also termed “conventional analysis” in which the gross components (protein, fat, carbohydrate, ash) of the food material rather than individual nutrients (amino acid, fatty acid, monosacharides) are determined.[5]

Phytochemical are chemical compounds derived from plants that are non-nutritive secondary metabolic compounds occurring in different parts of plants. They are important as protective and disease fighting compounds which help the body to prevent of fight against diseases and so are required by the human body to sustain life. Their therapeutic use in prevention or fighting a number of diseases is the basis of their extensive use in traditional medicine. Some of the phytochemicals are water soluble while others are not.[6]

The aim of current study was to determine proximate composition, phytochemical screening and mineral concentration present in *Santalum rubrum* seeds.

MATERIALS AND METHODS

Collection of sample

Fully matured fresh seeds of *Santalum rubrum* were collected from local area of Rajshahi district, Bangladesh in the month of April 2016 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. =43205) has been deposited.

Preparation of sample

The matured seeds were washed to remove dirt. Then it was oven-dried at reduced temperature less than 45^0C to make it suitable for grinding purpose. The screened (20 mesh) powder was then stored in air-tight container with marking for future experiment.

Proximate analysis of Santalum rubrum seeds

Determination of moisture and dry matter content

5 grams of samples were weighed into pre-weighed petri dish and dried to constant weight in an oven at 110°C for 6 hours. The oven dried petri dish was then removed and placed in a dessicator to cool before weighing. After 30 min the petri dish was removed from desiccators and weighed. This process of heating and cooling was continued until a constant weighed was obtained. From the final weight, the moisture content of samples was determined from the mean values of triplicate determinations.

$$\% \text{ moisture} = \frac{\text{initial weight (before drying)} - \text{final weight (after drying)}}{\text{Initial weight (before drying)}} \times 100$$

% dry matter = 100 - % moisture

Determination of ash and organic matter content

The crucible was first washed, dried in an oven at 180°C for 30 minutes cooled and then weighed (W_1). Three grams of sample was placed in the crucible and weighed, (W_2) then the crucible was transferred into the muffle furnace, whose temperature was set at 650°C and allowed to stay for 3 hours, until the content became white after which the crucible was cool in a desiccators and weighed (W_3). The percentage ash content was then calculated using the relation below;

$$\% \text{ of ash} = \frac{weight\ of\ ash}{weight\ of sample} \times 100 = \frac{W_3 - W_1}{W_2 - W_1} \times 100.$$

% of organic matter = 100 - ash%

Determination of acid soluble and insoluble ash

25 ml of dilute hydrochloric acid was added to 0.5 gm of ash sample and boiled gently for 5 minutes. The insoluble matter was collected on an ash less filter paper and washed with distilled water and dried then ignited for one hour, cooled in desiccator and weighed. Continuing ignition until a constant value is obtained.

$$\% \text{ of acid insoluble ash} = \frac{(\text{weight of ash taken} - \text{wight of acid insoluble ash})}{\text{weight of ash taken}} \times 100$$

% of acid soluble ash = 100 – acid insoluble ash%

Determination of water soluble and insoluble ash

1 gm of ash sample was mixed carefully with 25 ml of distilled water and boiled gently for 5 minutes. The insoluble matter was collected on ash less filter paper and washed with distilled hot water and dried then ignited for 15 min at temperature not more than 450°C, cooled in desiccator and weighed. Continuing ignition until a constant value is obtained.

$$\% \text{ of water insoluble ash} = \frac{(\text{weight of ash taken} - \text{wight of water insoluble ash})}{\text{weight of ash taken}} \times 100$$

% of water soluble ash = 100– acid insoluble ash%

Determination of Nitrogen content

It was determined as total Kjeldahl nitrogen. Two grams of the sample were poured in a macro kjeldahl flask and 20 ml of distilled water was added. The flask was swirled for a few minutes and allowed for 30 minutes to prevent foaming. Fifty ml of concentrated H_2S0_4 was also added through an automatic pipette. The flask was heated cautiously at low temperature of 45°C on the digestion stand. When water has been removed and frothing has ceased, the temperature of the flask was increased until the digest was cleared. The digest was boiled for five hours. Heating was regulated during boiling so that H_2SO_4 condenser is about half way up the neck of the flask. The flask was allowed to cool and 50 ml of distilled water was added to the flask. 10 ml of the aliquot was carefully transferred into a macro kjeldahl flask. 20 ml H_3BO_3 indicator solution was added into 50 ml Erlemmeyer flask which was then placed under the condenser of the distillation apparatus. 20 ml of 40% NaOH was added to the macro kjeldahl flask through a funnel on the stop cork and distillation was commenced. The condenser was kept cool at 300°C allowing sufficient cold water to flow through and heat was regulated to minimize frothing and prevent suck back. 40 ml distillate was collected and distillation was stopped. NH_4-N in the distillate was determined by titrating with 0.1N standard H_2SO_4 using burette graduated at 0.1 ml intervals. The color changed at the end point from green to pink. The percentage Nitrogen in the sample was calculated as follows:

$$\% \text{ of Nitrogen} = \frac{0.14\ x(V_1 - V_0) \times N}{p}$$

Here,
V_0 = Blank determination (volume of 0.1 N H_2SO_4 in ml)
V_1 = sample determination (volume of 0.1 N H_2SO_4 in ml)
N= strength of H_2SO_4.
P = weight of sample in gm.

Determination of Protein content
The protein content was obtained by multiplying Total Kjeldahl Nitrogen (TKN) value by a conversion factor [7] of 6.25.
% Crude Protein= % N × 6.25

Determination of crude fiber
Five grams of sample was transferred into clean fitter crucibles. 200 ml of 0.255M H_2SO_4 previously pre-heated in the reagent system was added to prevent foaming. The contents of the beakers were boiled for 30 minutes and filtered through a Buchner funnel with the aid of a suction pump. The residues were washed with hot deionized water until acid free. The residues left after acid digestion were carefully transferred into a 500 ml beaker. 200 ml of 0.313M NaOH solution was added to the sample The contents of the beaker was filtered through a Buchner funnel and 15 cm diameter whatman no. 4 filter paper on cooling. The residue was washed several times with hot water and once with methylated spirit until free of alkali. The residues was finally washed three times with acetone, carefully transferred into porcelain crucibles and dried at 110°C for 2 hours. They were allowed to cool in desiccators before weighing.[8]

$$\% \text{ crude fiber} = \frac{\text{mass of dried fiber} \times 100}{\text{mass of sample used}}$$

Determination of carbohydrate contents
The content of the available carbohydrates is determined by subtracting from the sum of the values (per 100 grams) for moisture, protein, fat content, ash, and crude fiber.

So, Carbohydrates content = 100 - (moisture of dry seeds % + ash% + protein % + crude fiber% + fat content %).

Estimation of Food energy
The energy value (kcal) of the samples was estimated by multiplying percentage crude protein, fat and carbohydrate by the recommended factor (4, 9 and 4 respectively) used in vegetable and seed analysis.[9]

Phytochemical Content of* Santalum rubrum *seeds
Phytochemical screening is a process of tracing plant materials. It confirms the presence of various phytochemicals which can be seen as a potential source of useful drugs. Standard phytochemical methods Sofowora[10] were applied to detect the presence of different classes of constituents like alkaloids, flavonoids and saponins in seeds of *Santalum rubrum*.

Elemental analysis by AAS technique
The mineralized sample was used in different volume to estimate different element content. Among all elements only Sodium (Na) and Potassium (K) were estimated by using flame photometer (Model AnA-135, OSK Japan). Most of the elements like Calcium (Ca), Magnesium (Mg), Chromium (Cr), Iron(Fe), Zinc (Zn), Aluminum (Al),Copper (Cu), Nickel (Ni), Lead (Pb), Cadmium (Cd) and Manganese (Mn) in seeds of our plant samples were analyzed by using Atomic Absorption Spectrophotometer (Varian, AA240FS, Australia) which was equipped with flame and graphite furnace. For our experiment, we choose air acetylene flame mode. The condition for fixed acetylene was 1.8 l/min and air 15 l/min along with argon gas flow for inert atmosphere. The instrumental default temperature parameters were automatically fixed for each element analysis.

RESULTS AND DISCUSSION

Proximate analysis and calorific value
The proximate analysis revealed percentages of various nutrients in the seed of *Santalum rubrum*. The results for proximate composition and calorific values of *S. rubrum* seed are shown in Table 1.

Table 1: Proximate composition (%) and energy value (cal/g) of *Santalum rubrum* seed.

Parameters	Concentration (%)
Moisture	10.17
Dry matter	89.83
Total ash	4.95
Organic matter	95.05
Acid soluble ash	92.01
Acid insoluble ash	7.99
Water soluble ash	39.97
Water insoluble ash	60.03
Nitrogen content	6.37
Protein content	19.81
Crude fiber	3.17
Carbohydrate contents	21.39
Food energy	390.60 cal/gm

The percentages of moisture and dry matter were found 10.17 and 89.83, respectively. Moisture content is among the most vital factors considered in food processing, preservation and storage.[11] The low percentage of moisture obtained indicates that *Santalum rubrum* seeds have low shelf-life, implying that its long storage could lead to spoilage due to susceptibility to microbial attack.[12] Ash content is useful in assessing the quality grading of seeds and also gives an idea of the amount of mineral element present in the seed.[13] The ash and organic matter content was found 4.95% and 95.05%, respectively. The total ash is particularly important in the evaluation of purity of drug *i.e.* the presence or absence of foreign organic matter such as metallic salts or silica.[14] From total ash content we also estimated acid soluble ash 92.01% and insoluble ash 7.99% as well as water soluble ash 39.97% and insoluble ash 60.03%.

Crude fiber recorded in the present study (6.87%) indicates the level of non-digestible carbohydrate and lignin in *S. rubrum* seed.[15,16] Fiber is characterized by low or no nutritional value however, its effect on digestive system may help to fight diabetes and lower high blood cholesterol level.[17,18] Low level crude fiber is considered appropriate,[12] because high level can cause intestinal irritation, lower digestibility and decreased nutrient usage.[19]

Proteins are major source of energy. It contains essential amino acids responsible for growth and repair of worn-out tissues in humans.[20,21,22,23] It was observed that the seed sample contain 39.81% protein and 6.37% nitrogen. Seed proteins should possess the requisite functionality for their successful utilization in various food products. These functional properties are intrinsic physio-chemical characteristics that affect the behavior of properties in food systems during processing, manufacturing, storage and preparation.[24,25,26,27]

Carbohydrate gives ready source of energy to the body.[28,29] The seed contains 21.39% carbohydrate. Food energy of the seed is 390.60cal/gm. The high calorific values obtained indicate that this seed could constitute a major source of energy for many of the world's poor and least privileged people.[30,31]

Phytochemical content

Figure 1 reveals the presence of alkaloids, saponnins, and flavonoids in *S. rubrum* seed. The high degree of presence of alkaloids (25.47%) and saponins (52.90%) in *S. rubrum* seed suggests that it can be used as medicine though alkaloids are often toxic to men and may have dramatic physiological activities.[32] Saponins are group of chemicals with detergent like properties that plants produce to help resist microbial pathogens. Alkaloids and saponins prevent excessive intestinal absorption of cholesterol and reduce the risk of cardiovascular diseases such as hypertension.[33]

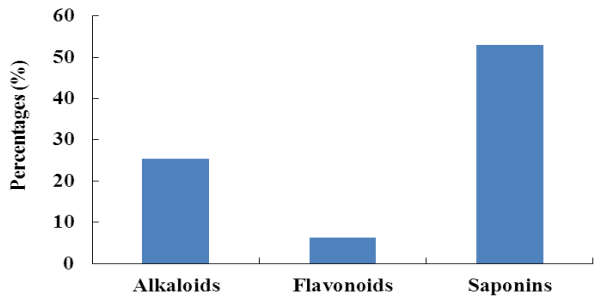

Figure 1: Phytochemical composition (%) of *Santalum rubrum* seed.

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 The quantity of flavonoid obtained in this study were 6.28%. Flavonoids are a group of polyphenolic compounds with known properties including free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action.[34] The presence of these secondary metabolites suggests that the plant might be of industrial and medicinal importance.

Elemental analysis

The result for the mineral composition is shown in Table 2. The most abundant mineral found in the sample is potassium with the concentration of 91.89 mg/kg. High concentration of potassium in the body was reported to increase iron utilization[35] and beneficial to people taking diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid.[36]

Table 2: Mineral composition of *S. rubrum* seeds.

No.	Element name	Content (mg/kg)
1.	K	91.89
2.	Ca	60.36
3.	Mg	33.72
4.	Na	20.67
5.	Zn	3.96
6.	Fe	1.42
7.	Al	1.05
8.	Mn	0.12
9.	Cu	0.11
10.	Ni	0.02
11.	Pb	0.003
12.	Cr	0.001
13.	Cd	0.0001

Calcium is the second most abundant element in the seed sample with the value of 60.36 mg/kg. Calcium is a constituent of bones and helps the body to contract correctly, blood to clot and the nerves to convey messages.[37] Magnesium and sodium are the next abundant mineral elements found in the sample of *S. rubrum* seeds with the values of 33.72 mg/kg and 20.67 mg/kg, respectively. Magnesium is beneficial to blood pressure and helps to prevent sudden heart attack, cardiac arrest and stroke. Magnesium deficiency results in uncontrolled twisting of muscles leading to convulsion, which may eventually lead to death.[38] Sodium regulates fluid balance in the body and helps in the, proper functioning of muscles and nerves.[39] However, there is a need to judiciously consider this sample, especially in sodium and potassium restricted diets. This is important since high dietary sodium is implicated in cardiovascular and renal disorders.[40]

The seed contains 3.96 mg/kg zinc and 1.42 mg/kg iron. Iron helps in the formation of blood and in the transfer of oxygen and carbon dioxide from one tissue to another.[41] Zinc boosts the health of our hairs, plays a role in the proper functioning of some sense organs such as ability to taste and smell,[39] helps in carbohydrate and protein metabolism and also assists in metabolism of vitamin A

from its storage site in the livers and facilitates the synthesis of DNA and RNA necessary for cell production.[41]

The values of aluminum, manganese, and copper in the sample are 1.05, 0.12 and 0.11 mg/kg, respectively. The concentration of lead, nickel and chromium also estimated as 0.003, 0.02 and 0.001 mg/kg, respectively. Cadmium was found to be 0.0001 mg/kg, the least of all mineral elements present in the sample.

CONCLUSION

Plants have contributed immensely to the medical field. It has been the source of most drugs used for combating infections. The proximate and phytochemical compositions of *S. rubrum* suggest that the seeds contain the important constituents needed to combat various kinds of infections in human beings. The high level of minerals elements in seeds make it useful as human diets or livestock feed and also be as raw materials in pharmaceuticals formulation.

Further investigations on the chemical compositions and possible isolation of the active ingredients for specific functions in order to standardize the formulation for efficient medical use would be carried out.

ACKNOWLEDGEMENT

We are grateful to INARS, BCSIR for giving us the opportunity to do mineral compositions analysis by AAS of plant materials. We are also thankful to the Director, BCSIR Laboratories, Dhaka for providing necessary facilities to carry out this research work.

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