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Phytochemical Study on Leaves of *Sapium Insigne* Benth

KEYWORDS: *Sapium insigne*, Protein, Carbohydrate, Mineral, Sugar, Crude Fiber, Vitamin.

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ABSTRACT *Sapium insigne Benth were analyzed and identify protein, crude protein, ether extract, crude fibre, carbohydrate, sugar, reducing sugars, minerals, and trace elements in the leaves of the Sapium insigne Benth plant.*

INTRODUCTION

Plants have been used to treat or prevent illness since before recorded history and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments (Abraham 1981). Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. They played a significant role in giving the solution to systematic problems on the one hand, and in the search for additional resources of raw materials for pharmaceutical industry. Nutrient contents are the important fodder trees of Eastern Himalyas (Singh et al. 2007, Balokhra 2002, Kharwal and Rawat 2009).

Sapium insigne also known as Khinna (Hindi), belongs to plant Euphorbiaceae Family. *Sapium insigne* (Royle) Benth. Ex Hook.fil is a deciduous tree distributed throughout India, China, Nepal, Srilanka and South-east Asia (Devkota et al. 2009). There is a report of the presence of phytochemicals like phenolic compounds in *Sapium insigne* (Devkota et al. 2010).

Sapium insigne is a medium sized deciduous tree up to 30m, found in the dry rocky situations in Himalayan region, Chemical analysis of some fodder tree leaves of Garhwal at different developmental stages (Babu 1990) reported protein, carbohydrate, sugar, minerals and vitamins contents (Devendra 1991). During the dry season, the crude protein levels fall to very low critical levels, even below 7% in the dry matter. It was estimated that 12% of the total digestible nutrients come from fodder tree and shrub leaves. Phytochemical screening revealed the presence of alkaloids, steroids, triterpenoids, saponins, flavonoids, reducing sugars and protein in almost all extract (Panda et al. 2012). The phytochemical analysis reveals the minimum required crude protein for a poor quality diet with a digestibility of organic matter of 5% would be between 6.1 to 7.4% (Sinha 1989).

Ipomoea pes-tigridis Linn. is used as a feed of livestock in green state as well as hay. It is highly nutritive of 4195.20cal/g energy and 24.25 % crude protein, 0.196% phosphorus and 5.8 potassium (Sharma 2002). The eight stovers for their nutritive value, and the ranged of crude protein, calcium, phosphorus, and dry matter was found to be 2.0 to 19.23%, 0.9 to 2.32%, 0.08% to 0.26% and 24.2 to 96.0% (WHO 1998). *Sapium insigne* leaves were successively extracted with the solvents of different polarity (methanol, ethyl acetate, Hexane) and water. The crude extracts were screened for the phytochemical analysis.

MATERIALS AND METHODS

Study Area

The study area is located at Kotdwara Bhaber region in the District Pauri Garhwal nestling in the foothills of the Shiwalik. *Sapium insigne* leaves were collected from Kotdwara, in the month of May, June, September, December. The leaves were washed with water and dried in shade.

Fig. 1 Leaves of *Sapium insigne* Benth

PHYTOCHEMICAL ANALYSIS

Crude protein: - The percentage of crude protein was obtained by multiplying the total Nitrogen of sample by 6.25.

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$$\% \text{ of Protein} = \text{Total Nitrogen} \times 6.25$$

Ether extract: - 3.0 gm of the samples was weighed and poured into a continuous extractor of soxhlet. Diethyl ether was used for the extraction. The flask with its contents was heated in an oven at 1000C after some time remove the volatile oil and then cooled in desicator. The flask containing the fixed oil was weighed and calculates the percentage of ether extract using following formula.

$$\% \text{ of Ether Extract} = \frac{\text{Weight of fixed ether extract(gm)} \times 100}{\text{weight of sample (gm)}}$$

Ash:- Plant materials were converted into ash. Dry and weighted as wet ashing was performed to determine the percentage of silica and other minerals. 5.0 gm oven dried powered samples was taken in silica crucible. The material was heated and transferred into a muffle furnace. at 3000C to 5000C to get a white ash determine the percentage .

$$\% \text{ of ash} = \frac{\text{Total ash} \times 100}{\text{Weight of sample}}$$

Carbohydrates :- Carbohydrates are the important components of storage and structural materials in the plants. The carbohydrate content can be analyzed by hydrolyzing the polysaccharides into sugars by acid hydrolysis and estimating the resultant monosaccharide.

$$\% \text{ of carbohydrate} = \frac{\text{mg of glucose} \times 100}{5}$$

Elements:-Analysis of elements was done by the method of food analysis reported by Joylon (Misra 1968). The ash solution was slipped down a rod, using several small volumes of water into a 100 ml volumetric flask make up to the mark with water. 10 ml of this solution was taken in boiling tube and added 3.0 ml acetate buffer, 2.0 ml hydroquinone, 2.0 ml 2, 2'-Dipyridyl solution. The optical density of this solution was observed at 520 nm. A blank was also performed and both readings were compared with the standard curve to obtain the concentration of iron.

Sugar: - The extraction of sugar and protein was accomplished by 80% ethanol. Extraction was performed by means of a soxhlet. The sugar solution was then heated in a water bath and centrifuged sugars were extracted from the plant material, through boiling and calculated the percentage using following formula.

$$\% \text{ of reducing sugar} = \frac{50 \times \text{volume of sugar solution} \times 100}{\text{Titer value} \times \text{weight of sample in solution (mg)}}$$

Vitamins A: - The sample was refluxed gently for atleast half an hour, with 30 ml of ethanol and 3 ml of a solution of 1 gm/ml in water. Anhydrous solution of antimony tri-chloride reagent was added in this solution. A transient blue color appears due to the presence of vitamin "A".

Fig. 2 Molecular structure of Vitamin A.

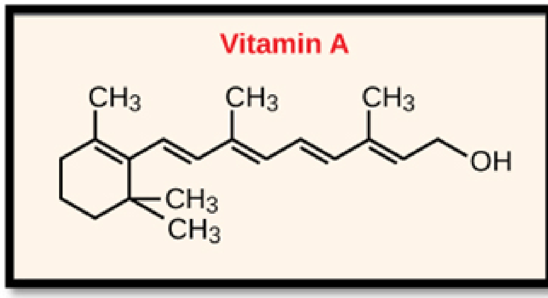
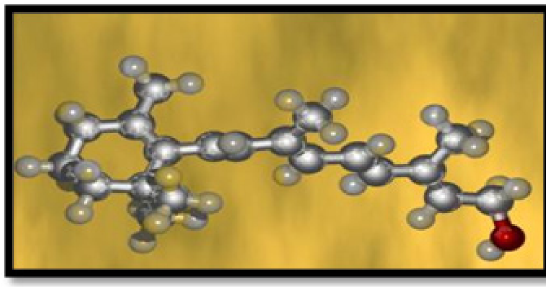


Fig. 3 3D Structure of Vitamin A



Vitamin C: - Vitamin C was determined calorimetrically. The dehydroascorbic acid was reacted with 2, 4 dinitrophenyl hydrazine (DNPH) to form osazone and dissolved in sulphuric acid to give an orange-red color solution.

Fig. 4 Molecular structure of Vitamin C.

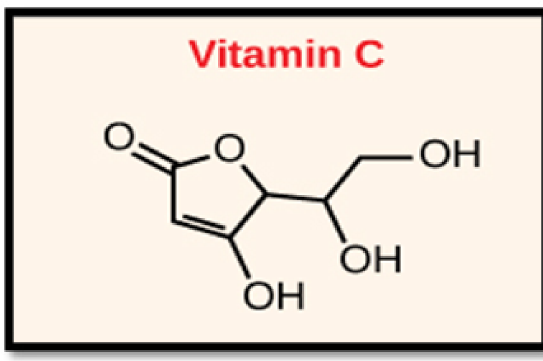
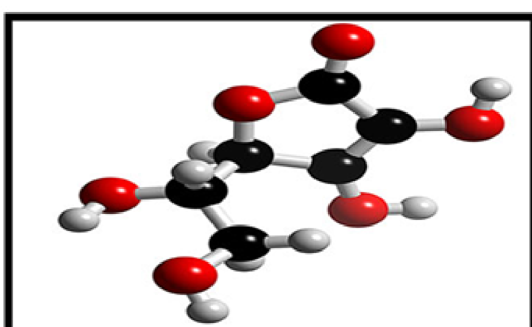


Fig. 5 3D Structure of Vitamin C



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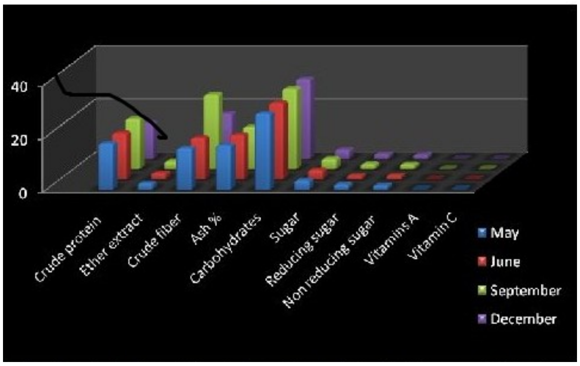

Fig. 6 Graph showing the chemical composition and nutritive value of leaf extract of *S. insigne* in different months.

RESULTS AND DISCUSSION

The leaves of the plant were collected tri-monthly for chemical examination. Nitrogen, protein, crude fiber, minerals, carbohydrates and vitamin were reported in table (a) and (b). Crude fibre, ether extract, carbohydrates, sugars and crude fibre content increases from March to September, attain maximum values 18.95%, 2.97%, 30.22%, 3.88% and 27.96%, and then decreases in December. Calcium (2.15%), phosphorus (0.79%), magnesium (2.00%), potassium (2.06%) and iron (0.072%) values are more in December in comparison to other months. Ash and silica content found more in the month of March. Copper content was found in excess in the month of June. Concentration of manganese remains constant from December to June and then decreases in September. The zinc content increase from March to June remains same till September and further decreases in December. The value of vitamin 'A' remains same during March to September and then decrease in December, while vitamin 'C' possesses same value in first six months and then decreases in September, remains constant till December.

From the above discussion, it was concluded that the plant is an average fodder, in terms of its nutritive value. However, it can be feed to the cattle in the month of September. If crude protein and amino acid content be considered as criteria of feed, then this plant may be used as a substitute of fodder in time of scarcity of fodder grasses or feeding materials. The leaves of the plant mix with wheat husk or rice straw to provide an adequate strategic feed supplementation to animals over critical periods. From October to May, leaf fodder is the major source of feeding material for livestock in the hills. Therefore, propagation techniques measure their chemical composition and feeding value reported in table given below.

Table 1.Chemical Composition and Nutritive value of *S. insigne.*

Percentage on dry matter basis	May	June	September	December
Crude protein	17.29	17.34	18.95	13.56
Ether extract	2.35	2.40	2.97	1.34
Crude fiber	15.50	15.54	27.96	17.09
Ash %	16.67	16.70	15.81	15.97
Carbohydrates	28.60	28.65	30.22	30.12
Sugar	3.46	3.50	3.88	3.52
Reducing sugar	1.92	1.45	2.00	1.96
Non reducing sugar	1.54	1.50	1.88	1.56
Vitamins A	++	++	++	+
Vitamin C	+++	+++	++	++

+: traces, ++: Moderate amount, +++: Good amount.

Table 2.Chemical Composition minerals in of S. *insigne.*

Percentage on dry matter basis	May	June	September	December
SiO2	8.16	8.20	4.14	5.68
Calcium	1.35	1.40	2.06	2.15
Phosphorus	0.69	0.74	0.72	0.79
Magnesium	1.84	1.88	1.92	2.00
Potassium	1.32	1.35	1.92	2.06
Iron	0.072	0.070	0.046	0.072
Copper	++	++	++	++
Manganese	+++	+++	++	+++
Zinc	+	+	++	+

+: traces, ++: Moderate amount, +++: Good amount.

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