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PRELIMINARY PHYTOCHEMICAL AND PHYSIOCHEMICAL INVESTIGATION OF AERIAL PARTS OF *SARCOCOCCA SALIGNA*

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ABSTRACT
Ayurveda–Ancient Science of Life is alleged to be widespread for last 5000 years in India. It is one of the most important systems of medication in the earth (Kokate et al., 2007). Atharveda (around 1200 BC), Charak Samhita and Sushrut Samhita (100-500 BC) are the key classics that give full imagery of over 700 herbs. Researches on pharmacognosy, chemistry, pharmacology and clinical therapeutics have been conceded out on Ayurvedic medicinal plants and many of the major pharmaceutical corporations have transformed their strategies in favour of natural products drug innovation. Many drugs have entered the International Pharmacopoeia through the study of Ethnopharmacology and traditional medicine. The Research and Development plunge in the pharmaceutical sector is focused on progress of new innovative/indigenous plant-based drugs through exploration of leads from the established system of medicine Dr. V. E. Tyler has defined medicinal herbs as "crude drugs of vegetable beginning utilized for the treatment of disease states, often of a never-ending nature, or to attain or keep a condition of better health". In spite of the great advances observed in current medicine in recent decades, medicinal plants have played a key role in globe health. Plant based drug can be used truthfully, i.e., they may be together, dried and used as remedial agents (crude drug), or the constituents/active morality separated by various part process, which are in employment as medicines. The active principle or compounds; that may be carbohydrate, glycosides, tannins, lipids, alkaloids, etc.; with analogous structure and activity are manufactured chemically to produce the synthetic drugs used in allopathic or contemporary system of medicine.

KEYWORDS: Carbohydrate, Glycosides, Tannins, Lipids, Alkaloids.

1. INTRODUCTION

Herbal medicine

Herbal medicines are the oldest remedies known to mankind. Man's dependence on plants for health care is as old as the existence of mankind on this planet.

Dr. V. E. Tyler has defined medicinal herbs as "crude drugs of vegetable origin utilized for the treatment of disease states, often of a chronic nature, or to attain or maintain a condition of improved health". In spite of the great advances observed in modern medicine in recent decades, medicinal plants have played a key role in world health. Plant based drug can be used directly, i.e., they may be collected, dried and used as therapeutic agents (crude drug), or the constituents/active principles separated by various chemical processes, which are employed as medicines. The active principle or compounds; that may be carbohydrate, glycosides, tannins, lipids, alkaloids, etc.; with similar structure and activity are manufactured chemically to produce the synthetic drugs used in allopathic or modern system of medicine.[1]

Herbal Medicine Scenario in India

India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. There are very few medicinal herbs of commercial importance, which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine. There are about 9000 firms manufacturing traditional Ayurvedic medicines in India.[2]

Herbal Medicine- Demand in All over the World

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. In Germany and France, many herbs and herbal extracts are used as prescription drugs and their sales in the countries of European Union were around $ 6 billion in 1991 and may be over $ 20 billion now. In USA, herbal

drugs are currently sold in health food stores with a turnover of about $ 4 billion in 1996 which is anticipated to double by the turn of the century. In India, the herbal drug market is about $ one billion and the export of plant-based crude drugs is around $ 80 million. Herbal medicines also find market as nutraceuticals (health foods) whose current market is estimated at about $ 80–250 billion in USA and also in Europe.[3]

Herb- As a Dietary Supplement

Herbal supplements (a type of dietary supplement) are simple or multicomponent herb mixture used to supplement the traditional medical treatment. Dietary supplement is a product other than tobacco intended to enrich the diet containing one or more of vitamins; minerals; herbs or other botanicals; amino acids; or any combination of the above ingredients and is not used as a conventional food or as a sole item of a meal or the diet.[4]

PLANT DESCRIPTION

Sarcococca saligna is an evergreen aromatic shrub, widely distributed throughout the northern areas of Pakistan and Kashmir at 5000-9000 ft. altitudes. It is also widely distributed in western Himalayas from Afghanistan to west of Nepal. *Sarcococca* is a genus of 16-20 species of flowering plants in the family Buxaceae. They are slow growing evergreen shrubs 1-2 m tall.[5]

LEAVES

- Leaves are borne alternately and are oblong- ovate to lanceolate, acute to acuminate at both ends with entire margin.
- Leaves are 5-10cm long and 0.8-2cm wide and of deep green colour on top.
- foliage can become light green colour in high pH soils.
- leaves are 3-veined at the base, and having petioles 1.5-3mm long.

FLOWERS

- *Sarcococca saligna* bear fragrant flowers.
- Spikes of greenish- white flowers, to ½" wide, bloom in winter and early spring.
- Flowers are unisexual, sessile, in short auxiliary raceme.
- Petals are long, ovate and acute.
- Female flowers are inserted basally, bracteoles are several, acute, ovate and densely Imbricate.[6]

FRUITS

- The fruit is a red or
- Black in colour containing 1-3 seeds.
- The fruit is about 1cm long and ovoid to globose.
- Exocarp is fleshy or subdry, endocarp is fragile.
- Fruit are become dark purple when get mature.[7]

SEEDS

- Subglobose in shape.
- Membranous testa.
- Endosperm is fleshy.

BARK

- Bark is smooth

Botanical Description

Latin name	*Sarcococca saligna* Linn.
Family	Buxaceae
Synonyms	*Sarcococca trinervia, Baxus saligna, Sarcococca salicifolia, Sarcococca pruniformis var. angustifolia.*[8]

Plant Taxonomy

Phylum	Angiospermae
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Buxales
Family	Buxaceae
Genus	*Sarcococca*
Species	*saligna*[9]

Chemical Constituents

Chemical constituents of *sarcococca saligna* are **pregnane – type steroidal** alkaloids. **Alkaloids** are the organic products of natural or synthetic origin which are basic in nature and contain one or more nitrogen atoms, normally of heterocyclic nature, and possess specific physiological actions on human or animal body, when used in small quantities.

Steroidal alkaloids form a class of compounds possessing the basic or modified steroidal skeleton within the nitrogen incorporated as an integral part of the molecule either in ring or inside the chain.

Chemical constituents have been reported from the plant *sarcococca saligna* are as follows:[10]

1. Salignarine
2. Salignarine A
3. Salignarine B
4. Salignarine C
5. Salignarine D
6. Salignarine E
7. Salignarine F
8. 2-hydroxysalignarine-E
9. Saligcinnamide
10. Dihydrosaligcinnamide

MEDICINAL AND TRADITIONAL USES

- This plant is frequently found in Kashmir and the northern parts of Pakistan has been used to cure fever and rheumatism
- This plant was also used by several tribes of American Indians to kill lice.
- It also used as a hypotensive and expectorant.
- Two antibacterial compounds isolate from ethyl acetate extract of root and stem of *Sarcococca saligna* are Saligcinnamide and Na-methyl epipachysamine-D are used for the treatment of bacterial infections.
- Leaves of *sarcococca saligna* used as laxative and blood purifier.
- The leaves and shoots are boiled and applied on swollen joints and in muscular pain.
- This plant is also used for the treatment of acute bronchitis, pneumonia and cough.
- It is reported to have antitumour and antiulcer properties.
- Aqueous-methanolic extract of *sarcococca saligna* reported to have cardiosupressant, vasodialator and tracheal relaxant effect on isolated tissues.
- Ethanolic extract of it is also reported to have an antifungal activity.
- Due to presence of steroidal alkaloids in it it shows antispasmodic, antidiarrhoeal and anti secretory properties.
- It also shows anticholinosterase activity and also shows ganglionic blocking activity by decreasing or abolishing effect on nicotine on blood pressure and smooth muscle of guinea pig ileum.[11]

MATERIALS AND METHOD

Procurement of Plant Material

In the present study, the aerial parts of *Sarcococca saligna* was collected in winter from the forest area of Patnitop,(jammu) having altitude 7000ft. The plant was

identified and authenticated by botanist. After authentication, aerial part was dried at room temperature until it was free from the moisture and subjected to physical evaluation with different parameters such as nature, odour, colour, taste, size, shape, width, length. Finally aerial part was subjected to size reduction to get coarse powder. Then the powder was subjected to extraction and determination of various parameters like foreign matter analysis, ash value, acid insoluble ash value, water soluble ash value, sulphated ash value and moisture content.

Physicochemical Investigation of Plant Material

Following parameters were used for physicochemical investigation of plant material:

Foreign matter analysis[12]

A 100 g of the plant material was spread in a thin layer and the foreign matter was sorted into groups by visual inspection and using a hand lens. The remainder of the sample was sifted through a no. 250 sieve; dust was regarded as mineral admixture. The sorted foreign matter was weighed. The content of each group was calculated in grams per 100 g of air dried sample. The observations were recorded in Table 1

Extractive Values

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists.[13]

Successive extractive value

The powdered material of the drug (50 g) was packed in a Soxhlet apparatus and was subjected to successive extraction with different solvents like Petroleum ether, Ethyl acetate and methanol. Then it was filtered rapidly taking precaution against the loss of solvent. 25 ml of filtrate was evaporated to dryness in a tarred bottom china dish. It was then dried at 105 °C and weighed. The percentage of solvent soluble extractive with reference to air dried drug was calculated. The observations were recorded in Table.

$$\text{Extractive value (\%)} = \frac{\text{Wt. of extract}}{\text{Wt. of drug taken (in gms)}} \times 100$$

Loss on Drying (LOD)

2.0 gm of powder was accurately weighed in a petridish and kept in a hot-air oven maintained at 105^{0}C for four hours. After cooling in a desiccator, the loss in weight was recorded.

This procedure was repeated till constant weight was obtained.[14]

$$\text{Loss on drying (\%) (LOD)} = \frac{\text{Loss in weight}}{\text{Weight of the drug (in gms)}} \times 100$$

Ash Value

The ash remaining following ignition of medicinal plant materials is determined by 3 different methods which measure total ash, acid insoluble ash and water soluble ash.

The **total ash** is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash" which is derived from the plant tissue itself and "non-physiological ash" which is the residue of the extraneous matter (sand and soil) adhering to the plant surface.

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

a. Total ash

Accurately weighed 2g of ground dried material was taken in a previously ignited and tared crucible (usually of platinum and silica). The material was spreaded in an even layer and ignited by gradually increasing the heat to 500-600^{0}C until it was white, indicating the absence of carbon. Then it was cool in a dessicator and weighed. The content of total ash was calculated in mg per g of air dried material.

$$\text{Total ash (\% w/w)} = \frac{(z - x)}{Y} \times 100$$

z = weight of the crucible + ash (after complete incineration)

x = weight of the empty crucible

y = weight of the material taken

b. Acid- insoluble ash

To the crucible containing total ash, 25ml of hydrochloric acid was added. Crucible was covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper (Whatman No. 41) and was washed with hot water until the filterate was neutral. The filter paper containing the insoluble matter was transfered to the original crucible, dried on hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccator for 30 minutes, and was weighed. The content of acid-insoluble ash was calculated in mg per gm of air dried material.

$$\text{Acid-insoluble ash (\% w/w)} = \frac{a}{y} \times 100$$

a = weight of the residue

y = weight of material taken

c. Water- soluble ash

To the crucible containing the total ash, 25ml of distilled water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible or on an ashless filter-paper. It was washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450^0 C. The weight of this residue in mg was subtracted from the weight of total ash. The content of water soluble ash was calculated in mg per gm of dried material.

$$\text{Water soluble ash (\%w/w)} = \frac{w - a}{y} \times 100$$

w = Weight of total ash
a = Weight of the residue
y = Weight of the material taken (WHO, 1998)

d. Sulphated ash value

Take about 2g, accurately weighed crude drug powder in a tared platinum or silica crucible previously ignited and weighed. The drug is treated with dil. Sulphuric acid before ignition. In this all oxides and carbonates are converted to sulphates and ignition is carried out at a higher temp. (600^0c). After ignition then it was cool in a dessicator and weighed. The content of sulphated ash was calculated in mg per g of air dried material.[15]

$$\text{Sulphated ash (\% w/w)} = \frac{(z - x)}{Y} \times 100$$

z = weight of the crucible + ash (after complete incineration)
x = weight of the empty crucible
y = weight of the material taken

Phytochemical screening[16]

Chloroform extract was subjected to preliminary phytochemical investigation for detection of Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Proteins and Amino acids, Saponins, Phytosterols, Acidic compounds, Resins and Reducing sugars.

RESULTS AND DISCUSSION

Foreign matter analysis

Table 1: Foreign matter analysis value of aerial parts powder of *sarcococca saligna*

S.No.	Wt. of crude Drug (g)	Wt. of drug after removal of foreign matter (g)	Wt. of foreign matter (g)	Foreign matter (% w/w)
1.	100	99.114	0 .886	0.886
2.	100	99.046	0.954	0.954
Mean				**0.920**

Foreign matter of aerial parts powder of *Sarcococca saligna* was found to be 0.920%.w/w

Loss on drying

Table 2: Loss on drying of aerial parts powder of *sarcococca saligna*.

S.No.	Wt. of Petri dish + Drug (Before drying) (g) A	Wt. of Petri dish + Drug (After drying) (g) B	A-B (g)	Loss on drying (% w/w)
1	18.545	18.329	0.216	10.8
2.	20.488	20.264	0.224	11.2
Mean				11.0

Moisture content of aerial parts powder of *Sarcococca saligna* was found to be 11.0% w/w.

Successive extractive value

Table 3: Petroleum ether extractive value (successive) of aerial parts of *sarcococca saligna.*

S.No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	% Extractive value
1.	50.0	34.366	35.606	1.24	2.48
2.	50.0	37.484	38.644	1.16	2.32
Mean					**2.40**

Petroleum ether extractive value of aerial parts of *Sarcococca saligna* (successive extractive) was found to be 2.40%w/w

Table 4: Ethyl acetate extractive value (successive) of aerial parts of *Sarcococca saligna.*

S.No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	% Extractive value
1.	50.0	34.366	35.600	1.234	2.468
2.	50.0	37.484	38.700	1.216	2.432
Mean					**2.45**

Ethyl acetate extractive value of aerial parts of *Sarcococca saligna* (successive extractive) was found to be 2.45%w/w

Table 5: Methanolic extractive value (successive) of aerial parts of *Sarcococca saligna*

S.No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	% Extractive value
1.	50.0	34.366	40.584	6.218	12.436
2.	50.0	37.484	43.766	6.282	12.564
Mean					**12.5**

Methanolic extractive value of aerial parts of *Sarcococca saligna* (successive extractive) was found to be12.5%w/w.

Ash values

Table 6: Total ash value of aerial parts of *Sarcococca saligna.*

S.No.	Wt. of empty crucible (g)	Wt. of crucible+ Wt of crude drug (before ignition) (g)	Wt. of crucible after ignition (g)	Wt. of total ash (g)	% Total ash value
1.	26.276	28.276	26.372	0.096	4.80
2.	28.458	30.458	28.558	0.100	5.00
Mean					**4.90**

Total ash value of aerial parts of *Sarcococca saligna* was found to be 4.90%w/w

Table 7: Acid insoluble ash value of aerial parts of *Sarcococca saligna.*

S.No.	Wt. of drug taken (g)	Wt. of empty crucible (g)	Wt. of crucible + acid insoluble ash (g)	Wt. of acid insoluble Ash (g)	% Acid insoluble ash value
1.	2.000	26.276	26.2893	0.0133	0.665
2.	2.000	28.458	28.4719	0.0139	0.695
Mean					**0.680**

Acid insoluble ash value of aerial parts of *Sarcococca saligna* was found to be 0.680%w/w.

Table 8: Water soluble ash value of aerial parts of *Sarcococca saligna.*

S.No.	Wt. of empty crucible (g)	Wt. of total ash (g)	Wt. of water insoluble ash (g)	Wt. of water soluble ash (g)	% Water soluble ash value
1.	26.276	0.099	0.053	0.046	2.30
2..	28.458	0.097	0.047	0.050	2.50
Mean					**2.40**

Water soluble ash value of aerial parts of *Sarcococca saligna* was found to be 2.40%w/w.

Table 9: Sulphated ash value of aerial parts of *Sarcococca saligna.*

S.No.	Wt. of empty crucible (g)	Wt. of crucible + Wt of crude drug (before ignition) (g)	Wt. of crucible after ignition (g)	Wt. of total ash (g)	% Total ash value
1.	26.276	28.276	26.3344	0.0584	2.92
2.	28.458	30.458	28.5196	0.0616	3.08
Mean					**3.00**

Sulphated ash value of aerial parts of *Sarcococca saligna* was found to be 3.00%w/w

Phytochemical screening

Table 10: Phytochemical screening of aerial parts of sarcococca saligna.

S.No.	Constituent	Observation
1.	Alkaloids	+
2.	Carbohydrates	_
3.	Glycosides	_
4.	Phenolic Compounds and Tannins	–
5.	Flavonoids	_
6.	Proteins	+
7.	Saponins	_
8.	PhytoSterol	+
9.	Amino acids	+
10.	Fixed oils	_
11.	Resin	_
12.	Reducing sugar	_

+ = Present, -- = Absent

CONCLUSION

Plant materials and herbal remedies are being used from decades for cure and treatment of various disease and disorders. Finding healing powers in plants is an ancient idea. Herbal medicines have the ability to affect body system. The effects are dependent on the chemical compounds present in the plant used. Human being have been benefited from these compounds for many years in both medical and nutritional context. The plant natural product has been studied by combination of chemical, biochemical, and molecular, biological and genetic approaches. Now days they represent substantial portion of the global drug market. As per the World Bank Report, the international market for herbal drug is estimated to be more than 60 billion US$ which is expected to grow up to 6 trillion US$ by the end of 2050.

Medicinal plants still remain the mainstay of primary health care because of better compatibility with human body and lesser side effects. Medicinal plants belong to the oldest known health care products that have been used by the mankind all over the world in the form of folklore medicines. The World Health Organization estimates that 80% of the World's population still relies on herbal medicines as its major source of medicinal products.

For global positioning of herbal drugs, it is necessary to do the standardization and characterization of herbal extracts. A herbal drug may be standardized on the basis of its organoleptic characters, Macroscopy, Microscopy and Chemical constituents. In present investigation the bioactive phytoconstiuents have been made the basis of standardizing selected herbal drugs.

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