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A Comparative Phytochemical Study of Ashoka (Saraca asoca) collected in different seasons

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Abstract

The observations and the inferences drawn by ancient acharyas regarding the plant material, appear very rational on the background of recently accumulated knowledge about the variation in phyto-chemical contents of plants, which is known to occur depending on place and time of collection. The study aims at comparing phyto-chemical aspect of Ashoka bark collected during different seasons. For the present study, Ashoka bark was collected in different season from Alvas herbal garden, Moodbidri is used for analysis. Phyto-chemical analysis of Ashoka bark was done to screen for various component. From the observation of preliminary Phytochemical study in Ashoka bark water soluble extractives shows some of the remarkable changes of Alkaloid, Steroid, Tannins and Flavanoids percentages and some of the changes observed in Loss on Drying, total Ash values and in Acid insoluble Ash vales.

Key words: Ashok, Bark, Phytochemical, Tannin, Kashaya.

Introduction:-

Ayurveda is the science of life practiced by ancient Aryan's which is based on Atharva-Veda, one of the oldest scriptures of Hindus'. The system of Ayurveda embraces within its fold the drugs of plant, animal and mineral origin, both single drugs and compounded formulations. Applicability, richness of quality, abundance and utility in multipurpose are said to be the best qualities of a drug. There are instructions about which parts of the plants are to be used, whether it should be fresh or dry

and what should be the time of collection. It is stressed that the pharmacognostic knowledge (Naamarupa Vijnana) is essential, along with knowledge of physico-chemical properties and effects of drugs.

The observations and the inferences drawn by ancient scientists, regarding the plant material, appear very rational on the background of recently accumulated knowledge about the variation in phytochemical content of plants [1,], which is known to occur depending on place and time of collection The drug or plant shows its effect properly when it is collected in prescribed time. it is said that collection of part of plant / drugs in specific season show good efficacy. Selection of appropriate raw drug is an essential aspect to have an effective medicament.[2,3]

Acharya Sushruta and Acharya Charaka explained method and time of collection of barks in Sharat rutu. In the present era, Pharma industries give least importance of SOP[4] (standard operating procedure) because of cost effective, easily availability in local market. Ashoka is one of the sacred trees of Hindus and Buddish. As the name signifies the tree is believed to be 'Capable of reducing the sorrows of people'. It is considered as a symbol of love andis dedicated to kama. The Indian god of love. Its scientific name is Saraca asoka and belongs to Family – Leguminaceae. It is medium size tree and native of India, Srilanka and other Asian countries.

Ashoka has Kashaya, Tikta rasa, Laghu, Rooksha guna, Katu vipaka and Sheeta veerya and Kapha pitta shamaka. It has karmas viz Sheetal, Grahi, Varnya, Apachi, Krimighna, Shoshahara, Vishahara, Asrajit, Hrudya, Shramaapaha, Gulmahara, Shulahara, Udarahara, Adhmanahara.[5]

Bark contains Tannins and catechol. Phenoliz glycoside has been found to vary depending upon the place time of and collection storage condition. It contains helmato xylem and also iron and other substances ferrous compounds. Sterols and organs calcium compound are also present in the drug

Aims and objective of Study

To evaluate and compare the phyto chemical changes in Ashoka bark collected during different seasons.

Research centre

The Phytochemical study was done at Shri Dharmasthala Manjunatheswara Centre for Research In Ayurveda and Allied Sciences, Udupi.

Methodology

Loss on drying at 105 o C

10 g of sample was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

Water soluble ash

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash with reference to the air-dried sample.

Alcohol soluble extractive

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to

stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water soluble extractive:

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

Preliminary phytochemical tests:

Tests for alkaloids

a. Dragendroff's test: To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

b. Wagners's test: To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.

c. Mayer's test: To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

d. Hager's test: To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

Tests for carbohydrates

a. Molisch's test: To the extract, 1 ml of α-naphthol solution and conc. Sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

b. Fehling's test: A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was

warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

c. Benedict's test: To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

Test for steroids

a. Libermann-Burchard test: To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

b. Salkowski test: The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for saponins

a. To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

Test for tannins

a. To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

Test for flavonoids

a. Shinoda's test: To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

Test for phenol

a. To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for coumarins

a. To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

Test for Triterpenoids

a. The extract was warmed with tin bits and few drops of thionyl chloride.

Formation of pink colour indicates the presence of triterpenoids.

Test for carboxylic acid

a. Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Test for resin

a. Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of turbidity.

Test for quinine

a. A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinine

Observation and Results

Results of Standardization parameters of powder of Ashoka bark.

Results n=3%w/w

Parameters	Varsha	Sharat	Hemant	Shishir	Vasant	Grishma
Loss on drying	10.96	10.55	9.71	10.25	0.69	0.75
Total ash	10.28	10.03	10.80	10.90	3.64	4.6
Acid insoluble ash	0.40	0.0	0.5	0.1	0.5	0.43
Water soluble ash	1.09	2.19	1.96	1.49	3.39	3.39
Alcohol soluble extractive values	14.09	2.99	11.39	10.59	10.30	11.48
Water soluble extractive values	11.60	7.59	11.55	12.38	11.18	13.00

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Results of Preliminary Phyto Chemical Tests

Test	Varsha	Sharat	Hemant	Shishir	Vasant	Grishma
Alkaloid	+	+	+	+	+	+
Steroid	+	+	-	+	+	+
Carbohydrate	+	+	+	+	+	+
Tannin	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+
Saponins	-	+	+	+	+	+
Terpenoid	+	-	-	-	+	+

Coumarins	-	-	+	-	+	+
Phenols	+	+	+	+	-	-
Carboxylic acid	-	-	+	-	-	-
Amino acids	-	-	-	-	-	-
Resin	-	-	-	+	+	+
Quinine	+	+	+	-	-	-

Discussion

Phytochemical are chemicals of plant origin. They generally have biological activity. Phytochemicals generally are regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been established yet.

Ashoka is an important Ayurveda herb used mainly in bleeding gynaecological conditions and analgesic property. Its botanical name is Saraka asoca and belongs to fabaceae

Ashoka has Kashaya, Tikta rasa, Laghu, Rooksha guna, Katu vipaka and Sheeta veerya and Kapha pitta shamaka. It has karmas viz Sheetal, Grahi, Varnya, Apachi, Krimighna, Shoshahara, Vishahara, Asrajit, Hrudya, Shramaapaha, Gulmahara, Shulahara, Udarahara, Adhmanahara.

The Phytochemical estimation study was done on Asoka bark with respect to various seasons. It was observed that very minimal variations seen in chemical constituents. Presence of Alkaloid, Carbohydrate, Flavonoids, Tannins seen in seasons. Presence of quinine was seen in varsha, sharat and hemant. Presence of resins seen in shishira, vasant and grishma.

The Physico chemical analysis shows, loss on drying was more in varsha and less in vasant. Total ash value was more in shishira and less in vasant. Water soluble extractive values more in grishma and less in sharat. The drugs with kashaya rasa (astringent) contains more tannin percentage. Tannins are water soluble polyphenols that are present in many plants. The Anti microbial activities of Tannins are well documented. The

growth of many fungi, yeast, bacteria and viruses are inhibited by tannin.

Conclusion

In Ayurvedic literature, drug collection has been mentioned according to different parts of the plant in respective seasons, Nakshatras, Veeryas on the basis of therapeutic uses.

From present Phytochemical study of Ashoka bark collected in different seasons, no remarkable changes were observed in preliminary Phytochemical study.

The climate, temperature, rain fall, duration of day light, altitude, methods of cultivation, effect of lunar cycle, collection from wild area, soil condition and methods of collection, processing and storage have impact on the secondary metabolities of the plant ultimately which affect the therapeutic efficiency of the drug rather in Phytochemical screening.

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