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PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF *SARACA ASOKA* (ROXB.) DE BARK COLLECTED FROM DIFFERENT AGED PLANTS

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ABSTRACT

Plants have become integral part of the human life since the formation of the universe either for food or as a medicine. Ayurveda is such a science which solely depends on the medicinal plants. Medicinal plants are used not only for the curative but also for the preventive purpose and for the maintenance of health. Charaka, Shushrutha and other Nighantu karas mentions that this plants has to be collected in particular season and only from the plant which has attained proper age to obtain the maximum potency and health benefits. This Medicinal plants mainly acts based on the Phytochemicals present in it. Therefore this study has been conducted to screen the changes taking place in plants based on its maturity in terms of Physico Chemical and Phytochemical parameters.

KEYWORDS: Medicinal herbs, Charaka, Collection, Proper age, Phytochemicals.

INTRODUCTION

India is bestowed with rich plant diversity. India is one of the richest countries in the world in terms of biodiversity, has 15 agro-climatic zones.[1] The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare.[2]

Asoka is one of the most legendary and sacred trees of India. Asoka tree, universaly known by its Latin name *Saraca asoca* (Roxb.), De. wild or *Saraca indica* belonging to family Caesalpiniaceae. It is an ever green tree. It is also known as Kankeli (Sanskrit), Ashoka

(Assamese), Ashoka (Bengali), Ashoka (Gujarati), Ashoka (Hindi), Ashokadamara (Kannada)Ashok (Kashmiri), Asokam (Malayalam), Ashok (Marathi), Ashoka (Oriya), Ashok (Punjabi), Asogam (Tamil), Ashokapatta (Telugu). It is distributed in evergreen forests of India up to an elevation of about 750 meters. It is found throughout India, Especially in Himalaya, Kerala, Bengal and whole south region.[3]

Saraca asoca has many uses mainly in the medicine to treat the women gynecological disorders, in all types of abnormal discharges per vagina, in uterine inertia, uterine pain, urinary calculus, dysurea, etc. Saraca asoca (ashoka) plant contains the presence of glycoside, flavonoids, tannins and saponins.[4]

Acharya Charaka emphatically describes an excellent design of drug research and describes it as **"Tasyapium pariksha idamevam Prakruti"**etc.[5] He has mentioned to collect different parts of the plant, season and age of the plant to be collected. Charaka and other authors are of the opinion that plant parts will possess maximum veerya (potency) if it is collected in particular season and age.

Properties (secondary metabolites) of the plants are influenced by seasons, climate, temperature, rain fall, duration of day light, altitude, methods of cultivation, effect of lunar cycle, collection from wild area, age of the plant during collection, soil condition and methods of collection, processing and storage, ultimately which affect the therapeutic efficiency of the drug. Therefore not only season or time of the collection of drug is important but also the age of the plant during collection is important. So the aim of the present study is to analyse the Physico chemical and the phytochemical changes occurring in the bark collected in different aged plants.

MATERIALS AND METHODS

1. Collection of Plant Material

The 12 year old plant (Fig 1) Saraca asoca was collected at JSSAMC campus and old plant of age around 35 to 40 years (Fig 2) collected near Kukkarahalli lake and authenticated by dept of dravyaguna JSSAMC, Mysore. Bark was made into small pieces and shade dried, stored in cool and dry place until further use. Older sample labeled as Sample A and the Younger Sample labeled as Sample B. Both the samples were collected in Shishira rutu – January to March on 17th February 2018.

2. Macroscopical and Microscopical Studies[6]

These studies helps in the evaluation of the raw material before using for medicine. The ashoka bark sample obtained is washed to remove the adhered dirt and then it is evaluated for the morphological features like colour, shape, size, odour, taste and texture etc. Then the cross section of the same is taken and mounted on slides. Slides prepared were observed under microscope. Cell structures were observed and then the photographs were taken. Then the dried bark is powdered and this powder was analysed both Macroscopically and Microscopically.

3. Physico Chemical Studies[7]

Bark is tested for physico chemical constants like Ash values, Moisture content, P^h, Specific gravity, Fluorescence analysis as per prescribed methods described in Indian pharmacopia.

4. Extraction of the plant material

Extraction process was conducted using both cold and hot (reflux) extraction techniques. 2g of bark powder was accurately weighed and soaked in 60ml solvents: Methanol, Water, Chloroform, Hexane separately for both cold and hot extraction.

Cold extraction: Mixture was kept undisturbed for 48hrs followed by filtration using Whatman No 1 filter paper.

Hot (reflux) extraction: Mixture was boiled in round bottom flask which was connected to the reflux condenser for 30 min and after cooling filtered using Whatman No 1 filter paper. Then both the filtered cold and hot extracts are subjected to evaporation in water bath and then extraction are collected in clean and dry container,weighed and then stored in cool and dry place.

5. Qualitative Phytochemical Analysis[8,9,10]

Methanolic and Water extracts of both the samples i.e, Young and Old samples were tested for the presence of biological compounds by using following standard methods.

Test for Carbohydrates

Fehling's test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Iodine test: Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for Phenols and Tannins: Crude extracts were mixed with 2ml of 2% solution of FeCl3. A blue–green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoid

Alkaline reagent test Crude extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Saponins

Crude extracts were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponin.

Test for Glycosides

Liebermann's test: Crude extracts were mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H2SO4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test: Crude extracts were mixed with 2ml of chloroform. Then 2ml of concentrated H2SO4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test: Crude extracts were mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H2SO4. A brown ring at the inter phase indicated the presence of cardiac glycoside.

RESULTS AND DISCUSSION

Fig. 1.

Fig. 2.

Fig. 3: (Just after collection). Fig. 4: (After few minutes of collection).

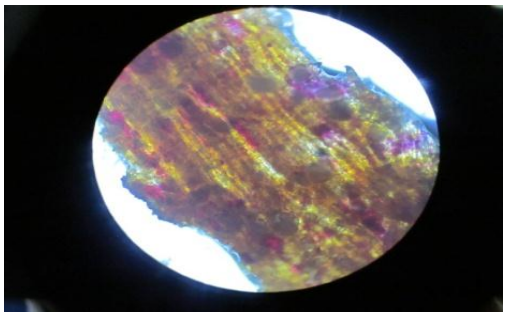

Fig. 5: (Sample A).

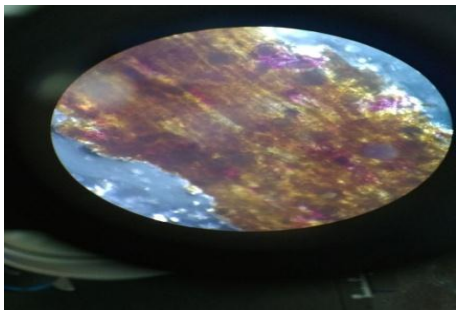

Fig. 6: (Sample B).

Fig. 6: (Sample B).

Fig. 7: (Sample A).

Table. 1: Fluoroscence characteristics of powdered bark under UV light.

Powders	Day Light		Short UV Light (254nm)		Long UV Light (365nm)	
	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
Powder as such	Light Brown	Light Brown	Light Brown	Light Brown	Light Green	Light Green
Powder treated with NaOH in methanol	Dark brown	Dark brown	Brown	Brown	Violet	Violet

Table. 2: Physico Chemical Analysis.

	Sample A	Sample B
Loss on Drying	10.50%(C), 11.4%(F)	10.20%(C), 10.50%(F)
Total Ash value	10.6%	9.4%
Acid insoluble ash	0.74%	0.89%
Water soluble ash	10.82%	10.76%
P^H	5.2	4.8
Specific gravity	1.005	1.002

C – Coarse powder, F – Fine powder.

Table. 3: Extractive Values.

	Cold Extractive Values.		Hot Extractive Values.	
	Sample A	Sample B	Sample A	Sample B
Methanol	19.4%	13.08%	20.3%	11.08%
Water	8.93%	15.7%	24.09%	14.4%
Pet.Ether	0.85%	0.49%	1.31%	0.39%
Chloroform	0.15%	0.44%	0.54%	0.48%

Table. 4: Priliminary Phytochemical Screening.

Test conducted	Sample A		Sample B	
	Methanol	Water	Methanol	Water
Alkaloids	+	+	-	-
Steroids	+	+	+	+
Terpenoids	+	+	-	-
Tannins	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	-	-	-	-
Carbohydrates	+	+	+	+
Saponins	-	+	-	-

Macroscopic Features: Younger bark was very thin and greenish brown in colour but in many places ash white patches of lichens mark was seen and Younger bark powder was light brown in colour and is fibrous. Old stem bark was dark bluish green in colour and also there was a mark of ash white patches of lichens and Old bark powder was chocolate brown and fibrous.

Fresh cut ends show 3 regions

a. A narrow outer brownish uneven layer.

b. A smooth middle yellowish red portion which is brittle.

c. Cream yellow inner region that forms the bulk part of the bark and the entire cut surface turns reddish on exposure to air (Fig 3 and 4).

Microscopic Features: Cross section of the bark was taken and observed under microscope. Bark showed Phloem and Phelloderm in the outermost layer. Cortex showed the presence of stone cells. Sclerenchymatous patches are found in phloem. Medullary rays are multisteriate. Powders of both samples under microscope showed lignin cells, stone cells, Calcium oxalate crystals (Fig 5 and 6).

Fluorescence Analysis: Fluorescence characteristics of the powdered bark of both the sample as such in the day light was Light Brown and under short UV light was Light Brown and under long UV light was Light Green in colour.

Both the samples which was treated with Naoh in Methanol and viewed as such was Dark Brown in colour and both sample viewed under short UV was brown, under long UV is Violet in colour.

Physico Chemical Analysis: Various Physico Chemical parameters viz Total Ash, Acid insoluble Ash, Water soluble Ash, Moisture content, P^H values, Specific gravity was determined according to Indian Pharmacopoeia and results are in Table 2 and 3.

P^H and Specific values of both the samples was in normal range. Ash Values of both the samples was also under the normal range which indicates the low content of Carbonates, Phosphates, Silicates and Silica which in turn indicates the low contamination with Silicon material. Moisture content of both the samples was within the normal range which indicates the stability of the drug.

Extractive Values: Cold Extractive yield of both the samples was less when compared to the Hot Extractive yield (Fig 7 and 8). The results showed that the % yield of extract increases as solvent polarity increased and maximum yield was obtained in methanol solvent. All the Extractive Values of the older bark sample was higher when compared to the younger bark sample which denotes the possible presence of the higher amount of constituents in older bark when compared to the younger bark.

Phytochemical Analysis: Phytochemical Analysis of both the samples of Ashoka showed the presence or the absence of the phytochemicals as shown in Table 4.

Saponins and Glycosides was absent in both the samples.

Alkaloids and Terpenoids was absent in Sample B and present in Sample A. Presence of both Phytochemicals in the Older bark sample indicate that appearance of these Phytochemicals takes place in the bark only after plant crossing certain age.

CONCLUSION

Herbal medicine that too Ayurveda is moving from fringe to the mainstream use with a great number of people seeking remedies. Ayurveda is such which believes in Holistic approach and follows each and every principles told by Charaka, Shushrutha. One such principle they adopted is the collection of the medicinal plants in particular season only after attaining particular age. By the above experiment it is proved either it may be the extractive yields or the presence of phytochemicals was more in Older bark sample when compared to the Younger bark sample. Hence it was emphasized to collect the medicinal plants after attainment of particular age and season. Further researches regarding the quantitative analysis and the fingerprinting of the Phytochemicals has to be conducted to understand the depth of this concept.

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