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ANALYTICAL STUDY OF *BILWA*(AEGLE MARMELOS) ROOT 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 *Bilwa* root collected during different seasons.For the present study, *Bilwa* root was collected in different season from Shri C.B.Guttal herbal garden, Dharwad is used for analysis.Phyto-chemical analysis of *Bilwa* root was done to screen for various component.From the observation of preliminary Phytochemical study in *Bilwa* root 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 values.

Keywords :*Bilwa*, Root, Phytochemical, Tannin, Physicochemical.

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[1].

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[2].

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[3], 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.[4,5]

Acharya Sushruta and *Acharya Charaka* explained method and time of collection of root in *Pravrit Rutu* and *Greeshma,Shishira* respectively[6].

In the present era, Pharma industries give least importance of SOP[7](standard operating procedure) because of cost effective, easily availability in local market *Bilwa* is mentioned profusely in *Yajurveda Samhita, Atharvaveda,Brahmana's* and *Kalpasutra's* which show its popularity. The fruit was regarded as auspicious and the wood also was used in religious sacrifices and ceremonies[8].

Its scientific name is *Aegle Marmelos* and belongs to Family – *Rutaceae*. It is a small

or medium sized deciduous tree and native of India, Srilanka and other Asian countries.

Bilwa Has Kashaya, Tikta Rasa, Laghu, Rooksha Guna, Katu Vipaka and *Ushna Veerya And Kapha Vata Shamaka*. It has karmas viz *Pakwa Phala Durjara,Doshakara* and *Putimarutakara*. and that of *Apakwaphala* are *Snigdha, Deepana, Pachana, Ushna Veerya* and *Kapha Vatahara.*[9]

Aegle Marmelos is known to contain a large number of alkaloids, coumarins, terpenoids, fatty acids and amino acids from different parts of the plant. Root contains umballiferon, skimmianine, halopine and alpa-amyrin etc.[10]

AIMS AND OBJECTIVE OF STUDY

To evaluate and compare the phyto chemical changes in *Bilwa* root 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 & RESULTS

Table.1 Results of Standardization parameters of powder of *Bilwa* root. Results n= 3% w/w

Parameters	***Varsha***	***Sharat***	***Hemant***	***Shishira***	***Vasanta***	***Grishma***
Loss on drying	10.36	9.71	5.78	11.36	0.80	0.58
Total ash	8.97	5.03	2.11	8.13	5.63	7.23
Acid insoluble ash	2.69	1.40	0.45	2.89	0.80	0.35
Water soluble ash	0.80	0.89	0.50	1.09	1.02	2.0
Alcohol soluble extractive values	6.98	1.98	2.13	8.59	9.11	10.11
Water soluble extractive values	9.21	3.27	4.44	13.03	8.03	9.23

Table.2 Results of Preliminary Phyto Chemical Tests.

Test	*Varsha*	*Sharat*	*Hemant*	*Shishira*	*Vasanta*	*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.[11]

Drug Biwa is small to medium sized deciduous tree, armed with straight, sharp axilllary thorns. It is one among sacred tree used in many rituals ceremony and

festivals in india. It is mainly used in Atisara, Arsha, Grahani, Kamala, Pandu, Shotahara, etc. It is having more than 47 different synonyms like Shreephala, Sadaphala, Maloora, Vatasara, etc. The root, unripen and ripen fruit are mainly used for therapeutic values. The major chemical constituents are umbelliferone and halopine etc.[12]

In the Physicochemical analysis the percentage of loss on drying was very less(0.58) in *Greeshma Rutu* when compared to other *Rutu's* like in *Shishira* it is11.36 and in *Varsha* it is 10.36 and similarly the acid insoluble ash is also less(0.35)but in *Varsha Rutu*(2.69)and*Shishira*(2.89)which was little more in comparison to other seasons.Alcohol soluble extractive value(10.11)and Water soluble extractive value(9.23)were more in *Greeshm*a *Rutu*.

Looking into Physicochemicals analysis values, it was clear that the collected samples of the drug were genuine and matches with standards of API and do not contain any of the foreign matter or adulterants in it.

Preliminary phytochemical analysis of *Bilwa* root reveals the presence of phytoconstituents like alkaloids,steroids,tannin,flavonoids,saponins and coumarins were seen in *Greeshma,Vasanta* and *Shishira Rutu*which suggests the possible activity of the drug due to presence of chemical constituents in it.

Based on the Phytochemical analysis some changes were observed in collected samples of different seasons but that is not strongly correlating with seasons which are mentioned in the classics. Howeverit shows that there can be seasonal variations.

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 *Bilwa* root 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.

REFERENCES

1. *Agnivesha*, revised by *Charaka* and Dridbala " *Charaka Samhita*" Vidyotinitika, Kashinath Shastri, Goraknath hindi commentary Chaukhambha Bharati Academy, Varanasi, reprint 2009, *Sutrasthana* Chap 9 Verse 7 p.no.63.

2. *Agnivesha*, revised by *Charaka* and Dridbala " *Charaka Samhita*" Vidyotinitika, Kashinath Shastri, Goraknath hindi commentary Chaukhambha Bharati Academy, Varanasi, reprint 2009, *Sutrasthana* Chap 1 Verse 120-123 p.no.13.

3. Breslin, Andrew (2017). "The Chemical Composition of Green Plants" Sciencing, Leaf Group Ltd

4. Sushruta samhita of Shri Maharshi Sushruta edited with the Nibhandasangraha commentary of Shri Dalhanacharya/8th edition year 2005 published by Choukambha Orientalia Varanasi

5. Charaka samhita of Shri Agnivesha edited by Vaidya Yadavji Trikamji

Acharya with Ayurveda dipika commentary /edition 2004 published by Choukhamba Sanskrita Samsthana Varanasi,

6. *Agnivesha*, *Charaka Samhita*, *Kalpasthana*, Chapter 1/10, Brahmananda Tripathi editor, 1st edition, Varanasi: Chaukhambha Surabharati Prakashana; 1983.*Sushruta*, *SushrutaSamhita*, *Sutrasthana* 36/5, Ambika datta Shastri editor, 11th Edition Varanasi: Chaukambha Sanskrita Sansthana:1997.

7. The Ayurvedic Pharmacopoeia of India Volume-7 / edition @1999 Published by The Controller of Publication Civil lines, Delhi-110054

8. Sharma P.V "*Dravyaguna Vignana*(Vedic plants and History of Dravyaguna) Chaukambha Bharathi Academy, Varanasi, Reprint1999,P NO 123.

9. *Agnivesha*, *Charaka Samhita*, *Sutrasthana*, Chapter 27/138, Brahmananda Tripathi editor, 1st edition,Varanasi: Chaukhambha Surabharati Prakashana; 1983.

10. Gupta A.K, Tondon Neeraj, Sharma Madhu(Ed), Quality Standards of Indian Medicinal Plants ,Vol 2 , ICMR NewDelhi, 2005,P-215.

11. "Phytochemicals" Micronutrient Information Center, Linus Pauling Institute, Oregon State University, Corvallis, Oregon, 2017, Retrieved 12 February 2017.

12. Iyer Narayana and M. Kolamal, Pharmacognasy of Ayurvedic drugs, Department of Pharmacognosy, University of Kerala, Trivendrum, 1960, P-15-20.

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