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A comparative pharmaceutical and analytical study of *Shirisharishta* prepared by *Twak, Sara* and *Kastha* from *Shirisha*

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ABSTRACT

Shirisha (*Albizzia lebbeck* Benth.) is well known classical drug used for the treatment of various types of diseases such as *Shwasa, Kasa, Shosh* etc. In this study 3 different sample of *Shirisharista* prepared from 3 different main ingredient *Twak Sara* and *Kastha* along with herbs like *Pippali Priyangu* etc. *Shirisharishta* is one such formulation mentioned under *Visha Chikitsa*, which is in use as a mode of *Shaman Chikitsa*. The reference of this *Yoga* is adopted from *Bhaishajya Ratnavali*. To formulate *Shirisharista* from *Twak, Sara* and *Kastha* from *Shirisha* and evaluate their pharmaceutical and analytical characteristics. *Shirisharishta* was prepared from *Twak Sara* and *Kastha* of *A. lebbeck*. Organoleptic characterization pH, specific gravity, total solid content, alcohol content and TLC profile of the prepared 3 samples were determined. Heartwood is the best part of use of A. *lebbeck* for preparation of *Shirisharishta.*

Key words: *Shirisha, Shirisharishta, Twak Sara, Kastha.*

INTRODUCTION

Ayurvedic medicines are mainly derived from the plants. About eighty percent of the raw materials for preparation of Ayurvedic medicines are obtaining from the plant source. In many of the cases, the root or wood or heart wood are the used parts of the plants. For collection of the used parts, sometimes the plants are to be sacrificed. This is one of reasons for the medicinal plants become rare, endangered, and threatened (RET). It is the need of time to find the alternative part of use from the same plant having equally active phytochemical and therapeutic potential.

Shirisharishta is a well-known formulation developed by ancient scholars by applying specific pharmaceutical procedures to get maximum therapeutic effect. *Shirisha* (*Albizzia lebbeck* Benth.) is a drug that draws the attention because of its multi-prolonged utility influencing the human life. Albeit Ayurvedic classics instruct of its high utility in treating the symptom complex due to *Visha* or the venomous poison, a lot of discrete references point out its utility in a variety of diseases such as *Shwasa,* etc. The plant is reported to have various pharmacological properties like anti-asthmatic, antihistaminic, anti-protozoal, hypoglycaemic, antibacterial, antiseptic and anti-tubercular etc. properties.[1] Most of the recent studies are reported about the pharmacological actions of it's bark, leaves, pods and fruits, but almost negligible

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references about the pharmacological action of it' heartwood are available. It is commonly used since ancient period in a variety of dosage forms both externally and internally. Almost all the parts of the drug are described per various types of treatment. Therapeutic utility of almost all the parts of *Shirisha* like fruit, root, bark, flower and leaves are mentioned in the classics. Direct indication of *Puspa Swarasa* (expressed juice of flower) is indicated for the treatment of *Shwasa Roga* (Asthma) specially caused due to the vitiation of *Pitta* and *Kapha* as well as *Visha Roga Chikitsa* by *Acharya Charaka*. The available references reflect towards it's applicability for the management of disease *Shwasa*, in a suitable dosage form, which should be available whole year.[2] The indication of *Shirisha Sara*, which is nowadays supposed to be the heart wood portion of the tree, in the *Asava Yoni* (medicinal source material for fermentation) as the best suited part for fermentation.[3] But detailed description of it's *Arishta Kalpana* is not found until 18th century.

Acharyas such as *Govinda Dasa* and *Vagbhata* in their respective masterpieces viz. *Bhaishajya Ratnavali* and *Sahasra Yoga* respectively, were first time described the pharmaceutical preparation it's *Arishta Kalpana* naming *Shirisharishta*[4] whereas which part should be used is not found mentioned.

Acharya Charaka and *Acharya Sushruta* have utilized its activity for various purposes and have included it into various classes of drugs like *Shiro-Virechana, Vishaghna and Pitta-Nasaka Gana.*[5,6] The *Sara* (heart wood) is the main part of use of this plant. The heart wood is included in *Asava Yoni* (source for fermentation) for the preparation of its *Asava - Arishta* preparation.[7] Some recent studies reported various phytochemical present and pharmacological actions like antiasthamatic, antiinflamatory, and others, from the heartwood of *Shirisha.*[8,9] Although the formulation *Shirisharishta* is mentioned in the context of *Visha Chikitsa* by Acharya Govinda Das in Bhaishajya Ratnavali (72/72-74),[10] various recent studies proved the effectiveness of this formulation in the ailments of respiratory system also, especially allergic in origin.[11]

Aim and Objective

1. To prepare three samples of *Shirisharishta* by using three parts i.e., *Twak* (bark), *Kastha* (sapwood) and *Sara* (heartwood).
2. To analyze all the three samples in terms of their pharmaceutical and analytical parameters to develop fingerprint profile for the *Shirisharishta.*

Materials and Methods

Shirisharista is prepared as per method described in Bhaishajya Ratnavali[12]

SN	Ingredient	Botanical Name	Part used	Form used	Ratio
1.	*Shirisha*	*Albizia lebbeck* Bebth	*Twak/ Kastha /Sara*	*Yavakuta*	50 Pala
2.	*Pippali*	*Piper longum*	Fruit	*Churna*	1 Pala
3.	*Priyangu*	*Callicarpa macrophylla*	Flower	*Churna*	1 Pala
4.	*Kushtha*	*Saussurea lappa*	Root	Churna	1 Pala
5.	*Ela*	*Elettaria cardemos*	Seed	Churna	1 Pala
6.	*Nilini*	*Indigo feratinctoria*	Roots	Churna	1 Pala
7.	*Haridra*	*Curcuma longa*	Rhizome	Churna	1 Pala
8.	*Daruharidra*	*Berberis aristata*	Wood	Churna	1 Pala
9.	*Nagar (Shunthi)*	*Zingiber officinale*	Rhizome	Churna	1 Pala
10.	*Nagkeshar*	*Mesua ferrea*	Male stamens	Churna	1 Pala
11.	*Guda*	Jaggery	Organic	-	200 Pala

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1 2.	*Jala* (w/w)	-	Portable water	-	512 Pala

Preparation of *Kwatha*

The raw drugs *Shirisha Twaka, Kastha* and *Sara* were collected separately. The fresh collected were subjected to shade drying up to constant weight obtained then size reduction (*Yavakuta* preparation). Then the *Yavakuta* of the raw drug were mixed with the mentioned quantity of water in a stainless steel vessel and subjected to overnight soaking of 12 h after that constant mild heat was applied to the vessel sufficient to facilitate the evaporation on continuous stirring up to the volume reduced 1/4th of the initial quantity. Then it was strained with double folded cotton cloth and collected in a separate vessel.

Preparation of *Prakshepa Dravya* (Adjuvants)

The *Prakshepa Dravya* (*Krishna, Priyangu, Ela, Nagkeshar, Daruharidra, Shunthi, Haridra, Nilini, Kustha*) was dried shade, cleaned and processed to coarse powder form individually. Weighed the mentioned quantity of the *Prakshepa Dravya* and mixed well.

Preparation of *Sandhana Patra* (Fermenting Vessel)

The fermenting vessels (Porcelain and Glass Jars) were properly washed with detergent, rinsed well with sufficient quantity of warm water. After cleaning, the vessels were properly dried to avoid any contamination. Dried vessels were subjected to *Dhoopana* (Fumigation) for 20 minutes.

Preparation of *Sandhan Drava* (Fermenting media)

The *Kwatha* prepared was allowed for self-cooling. *Guda* (Jaggery) was added in the *Kwatha* in three equal batches, 1/3rd was added on the same day. This solution was filtered through a double folded cotton cloth. The rest amount of *Guda* (Jaggery) was added at the interval of 15 days in two parts.

Preparation for Fermentation

The Jaggery was poured in to the fumigated fermenting vessels *Prakshepa Dravya* (adjuvants) was added in the vessels accordingly and stirred properly till they get wetted completely with the fermenting media. Vessels were closed by respective lids to prevent entry of any contaminant. Determination of proper initiation of fermentation was done by regular examination on 3rd, 5th, 8th, 15th, 30th, 45th and 60th day without disturbing the fermenting media.

List of *Rasapanchaka* (factors determining the function of this formulations) of ingredients of *Shirisharishta*

SN	Name	*Rasa*	*Guna*	*Virya*	*Vipaka*	*Karma*
1.	*Shirisha*[13] (*Albizia lebbeck)*	*Kashaya, Tikta, Katu*	*Laghu, Ruksha, Tikshna*	*Ishad Ushna*	*Katu*	*Tridoshahara, Vishaghna, Shwasahara*
2.	*Pippali*[14] (*Piper longum)*	*Katu, Madhura*	*Laghu, Snigdha, Tikshna*	*Anushnasheeta*	*Madhura*	*Kapha- Vatashamaka, Kushthaghna, Shwasahara, Kasahara*
3.	*Priyangu*[15] (*Callicarpa macrophylla)*	*Tikta, Kashaya, Madhura*	*Guru, Ruksha*	*Sheeta*	*Katu*	*Tridoshahara, Rakthashodhaka, Sthambhana*
4.	*Kushtha*[16] (*Saussurea lappa)*	*Tikta, Katu, Madhura*	*Laghu, Ruksha, Tikshna*	*Ushna*	*Katu*	*Vaatakapha Shamaka, Lekhaniya, Kaasahara, Shwasahara, Hikka Shamaka*

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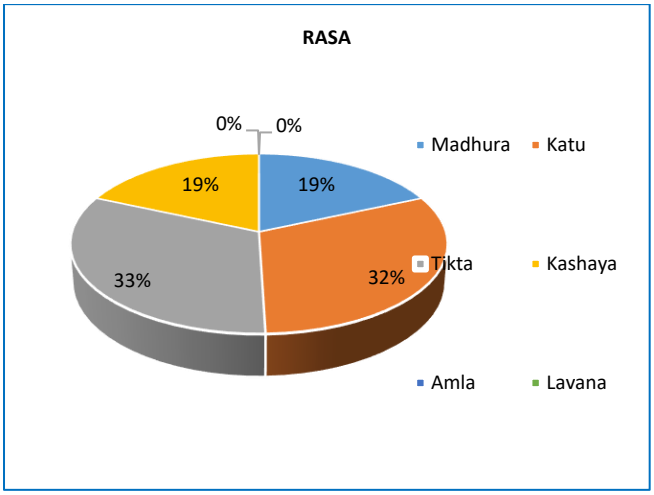
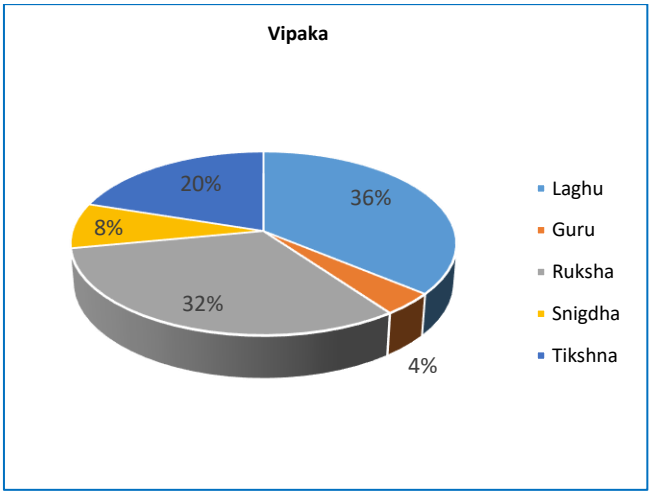
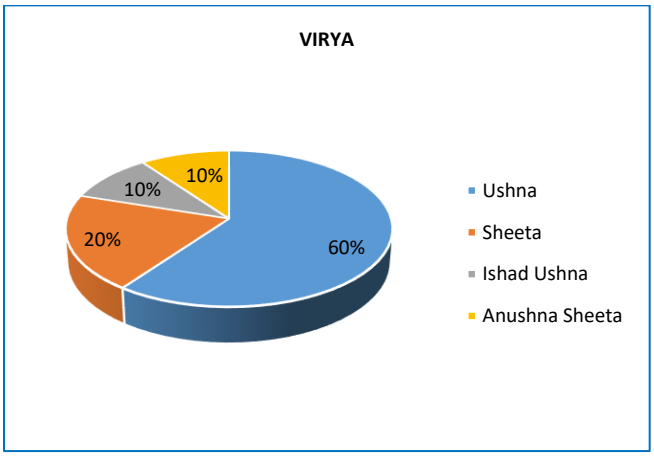
5.	*Ela*[17] (*Elettaria cardamomum*)	*Katu, Madhura*	*Laghu, Ruksha*		*Katu*	*Kapha-Vatahata, Deepana, Rochana,*
6.	*Nilini*[18] (*Indigofera tinctoria*)	*Tikta*	*Laghu, Ruksha*	*Ushna*	*Katu*	*Kapha- Vataghna, Krimihara*
7.	*Haridra*[19] (*Curcuma longa*)	*Tikta, Katu*	*Ruksha, Laghu*	*Ushna*	*Katu*	*Kapha-Vataghna, Kushthaghna, Jwaraghna*
8.	*Daruharidra*[20] (*Berberis aristate*)	*Tikta, Kashaya*	*Laghu, Ruksha*	*Ushna*	*Katu*	*Kapha-Pitta Shamaka, Krimihara*
9.	*Shunthi*[21] (*Zingiber officinale*)	*Katu*	*Laghu, Snigdha*	*Ushna*	*Katu*	*Vata-Kapha Hara, Dipana, Shwasa Hara, Kasahara, Hikka Shamaka*
10.	*Nagakeshara*[22] (*Mesua ferrea*)	*Kashaya, Tikta*	*Ruksha, Tikshna, Laghu*	*Ushna*	*Katu*	*Kapha-Pittahara, Shothahara, Dahahara*
11.	*Guda*					







Properties of *Shirisharishta*

- *Rasa - Katu, Tikta, Madhura*
- *Guna - Laghu, Ruksha, Tikshana*
- *Virya - Ushna*
- *Vipaka - Katu*
- *Karma - Tridoshahara*[23]

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The physico-chemical analysis of the different samples of *Shirisharishta* from *Twak, Sara* and *Kastha* from *Shirisha*.

1. Organoleptic Characterization

Parameters	Sara	Kastha	Twak
Color	Dark brown	Dark brown	Dark brown
Odor	Fruity, pleasant	Fruity, pleasant	Fruity, pleasant
Consistency	Good and Even	Good and Even	Good and Even
Nature of fracture	Smooth	Smooth	Smooth

2. Determination of pH

The pH value of the trial drug was tested as per the standard protocol.

Determination of pH

- pH of the Sample *Shirisharishta Sara* is 4.13
- pH of the Sample *Shirisharishta Kastha* is 4.32
- pH of the Sample *Shirisharishta Twak* is 4.09

3. Viscosity Index

100ml measuring cylinder was taken and filled with water, small weigh bead was dropped from the top and the time taken for the bead to reach the bottom was noted. The sample experiment was repeated with the sample with 1mg/ml concentration and time taken by the bead to reach the bottom was noted. Viscosity index was calculated by the given formula,

Calculation:

$$\text{Viscosity index} = \frac{\text{Flow rate of Sample}}{\text{Flow rate of Water}}$$

Results:

Time taken by bead to pass reach the bottom in water = 3.6sec

Time taken by bead to pass reach the bottom in Sample *Shirisharishta Sara* = 4.1sec

- Viscosity Index of Sample *Shirisharishta Sara* = 1.13

Time taken by bead to pass reach the bottom in Sample *Shirisharishta Kastha* = 3.7sec

- Viscosity Index of Sample *Shirisharishta Kastha* = 1.02

Time taken by bead to pass reach the bottom in Sample *Shirisharishta twaka* = 3.9sec

- Viscosity Index of Sample *Shirisharishta twaka* = 1.08

4. Total solids

250ml capacity glass beaker was dried and put appropriate identification mark on it. The beaker was initially weighed and noted. 100ml of the thoroughly mixed sample was poured, measured by the measuring cylinder, in the beaker. The beaker was placed in an oven maintained at 103°C for 24hours. After 24 hours, when whole of the water has evaporated, beaker was cooled and weighed. The weight of solids in the beaker was calculated by subtracting the weight of the clean beaker determined earlier.

Total Solids = Difference of weight of the beakers / Volume of sample X 1000

Results:

Shirisharishta Sara

The total solids can be calculated using the following method

Weight of the empty beaker (x) = 128.4g

Weight of empty beaker (x) + Sample after drying (y) = 131.1g

Total solids = [(y – x) / Volume of sample x 1000]

= [(131.1 – 128.4) / 100 x1000]

= 27%

Therefore % of Total solids in Sample *Shirisharishta Sara* = 27%

Shirisharishta Kastha

Weight of the empty beaker (x) = 125.6g

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Weight of empty beaker (x) + Sample after drying (y) = 127.4g

Total solids = [(y – x) / Volume of sample x 1000]

= [(127.4 – 125.6) / 100 x1000]

= 18%

Therefore % of Total solids in Sample *Shirisharishta Kastha* = 18%

Shirisharishta Twak

Weight of the empty beaker (x) = 130.4g

Weight of empty beaker (x) + Sample after drying (y) = 132.6g

Total solids = [(y – x) / Volume of sample x 1000]

= [(132.6 – 130.4) / 100 x1000]

= 22%

Therefore % of Total solids in Sample *Shirisharishta Twak* = 22%

5. Specific Gravity

The specific gravity bottle was taken and its weight was noted down. Test sample (1mg/ml) was filled into the specific gravity bottle and its weight was noted down. The difference in weight was divided by the weight of an equal volume of water to give the specific gravity of the sample.

Calculation:

$$\text{Specific Gravity} = \frac{\text{Wt. of Sample} + \text{bottle} - \text{Wt. of Empty bottle}\frac{\text{Kg}}{\text{cm3}}}{\text{Wt. of water} + \text{bottle}}$$

Results:

Empty weight of the bottle = 10.41g

Weight of the Specific gravity bottle + water = 32.1g

Weight of the Specific gravity bottle + Sample = 33.6g (*Sara*), 32.9g (*Kastha*) and 33.1g (*Twak*)

Specific Gravity of Sample *Shirisharishta Sara* = 0.722 Kg/cm3

Specific Gravity of Sample *Shirisharishta Kastha* = 0.700 Kg/cm3

Specific Gravity of Sample *Shirisharishta Twak* = 0.706 Kg/cm3

6. TLC

10µl samples were prepared 2.5 µl of samples were spotted on TLC plate and allowed to dry. A TLC plate is made up of a thin layer of Silica gel 0.25mm with fluorescent indicator F254 with Solvent system Chloroform: methanol (9.5:0.5) was used for TLC analysis. The strip or plate is then placed with this end dipping in to the solvent mixture, taking care that the sample spot/zone is not immersed in the solvent. As the solvent moves towards the other end of the strip, the test mixture separates into various components. This is called as the development of TLC plates. The separation depends on several factors, the plate is removed after an optimal development time and dried and the spots/zones are detected using UV chamber and Rf value is calculated using.

Rf = Distance moved by compound /distance moved by solvent.

Table 1: TLC Profile of Samples

Sample Name	TLC Bands	No. of Bands	Retention Factor
Shirisharishta sara	254 nm	3	0.32
			0.72
			0.76
	Visible Light	2	0.72
			0.76
	366nm	2	0.72
			0.76
Shirisharishta Kastha	254 nm	3	0.32
			0.72
			0.76
	Visible Light	2	0.72
			0.76
	366nm	2	0.72
			0.76
Shirisharishta twaka	254 nm	4	0.1
			0.32
			0.72
			0.76
	Visible Light	2	0.72
			0.76
	366nm	2	0.72
			0.76

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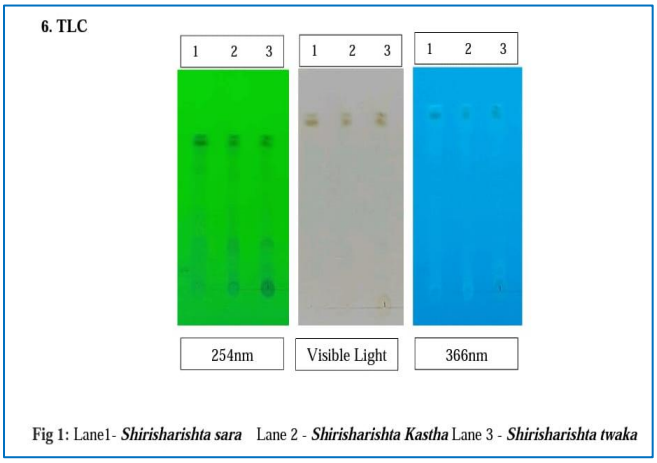


Fig 1: Lane1- *Shirisharishta sara* Lane 2 - *Shirisharishta Kastha* Lane 3 - *Shirisharishta twaka*

7. Total microbial Count

100µl of sample was homogeneously mixed with 1 ml of buffer peptone water and serial dilutions were prepared up to 10-2 following the standard protocol. An aliquot of 0.1 ml from 10-2 dilution was spread onto nutrient agar (NA) plate to enumerate the total bacterial count and potato dextrose agar (PDA) plate for the estimation of fungal count. Then the NA plate and potato dextrose agar plates were incubated at 37°C for 18 to 24 hours and at 25°C for 48 to 72 hours, respectively.

Total Bacterial count

There are few colonies seen at 10-1 dilution in *Shirisharishta Sara*, *Shirisharishta Kastha* and *Shirisharishta Twak*, but no colonies were found at 10-2 dilution in the samples.

Total Fungal count

There were no colonies seen at 100 dilutions (100 - Without dilution) in *Shirisharishta Sara*, *Shirisharishta Kastha* and *Shirisharishta Twak* samples.

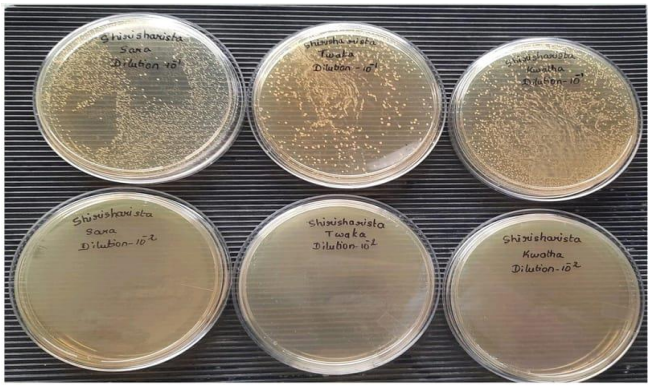


Figure 2: Total bacterial count

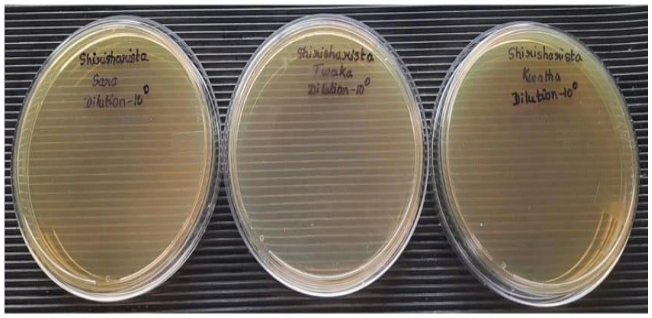


Figure 3: Total fungal count

8. Alcohol content estimation

Extraction of Ethanol from Sample

5ml of sample was taken in a distillation flask and diluted with 25ml water. Distillation is carried out till about 2ml less than the total volume was collected. Water was added to make up the volume to original test volume of liquid. Distillate was further taken for ethanol quantification.

Preparation of Dichromate Reagent

10% w/v of Potassium Dichromate was prepared in 5M of Sulfuric Acid.

Preparation of Standard

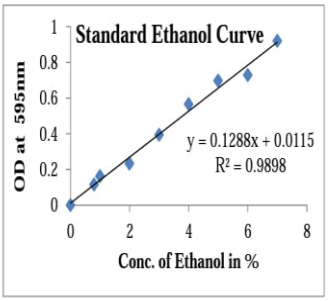
Standard Ethanol solutions were prepared from 0.8% to 7% using water.

Procedure

500µl of standard solution / sample (Distillate) was taken and 500µl of Dichromate reagent was added. The mixture was shaken gently for 1 min and incubated for 10 mins at room temperature. Absorbance of the resulting green colour reaction product was measured at 595nm. Standard Curve was plotted and alcohol content in sample was calculated.

8. Total Alcohol Content

Sample Name	Conc. %	OD @ 595nm
Blank	0	0
Ethanol	0.8	0.116
	1	0.162
	2	0.233
	3	0.394
	4	0.565
	5	0.697
	6	0.728
	7	0.919



Graph 1: Standard Ethanol Curve

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Table 2: Total Alcohol Content in Samples

Sample Name	OD @ 595nm	Ethanol Conc. %
Sara	0.932	7.15
Twaka	0.325	2.43
Kastha	0.536	4.07

9. Reducing sugar estimation

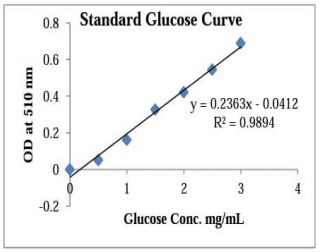
3ml of standard/sample was taken in a test tube and 3 ml of DNS reagent was added. Mixture was heated in boiling water bath for 5 mins and cooled to room temperature. Absorbance was measured at 510nm. Amount of reducing sugar present in the sample was calculated using the standard glucose curve.

Calculation

$$\text{Reducing Sugar \%} = \frac{\text{Glucose conc.} \left(\frac{\mu g}{mL}\right) \text{ x } 100}{\text{Volume of Sample (mL)}}$$

9. Reducing Sugar

Sample Name	Conc. mg/mL	OD at 510nm
Blank	0	0
Glucose	0.5	0.051
	1	0.162
	1.5	0.326
	2	0.420
	2.5	0.544
	3	0.688



Graph 2: Standard Glucose Curve – DNSA Method

Table 3: Reducing Sugar Content in Samples

Sample Name	OD at 510nm	Reducing Sugar Conc. mg/mL
Sara	0.862	0.35
Twaka	0.905	0.36
Kastha	0.954	0.38

10. Non-reducing sugar estimation

1ml of standard/sample was taken in a test tube and 4 ml of Anthrone reagent was added. Mixture was heated in boiling water bath for 8 mins and cooled rapidly under running tap water. Absorbance was measured at 630nm. Amount of total sugar present in the sample was calculated using the standard glucose curve. Total non-reducing sugar was calculated using the following formula.

Calculation

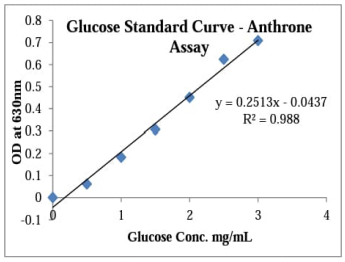
Total Sugar % = Glucose conc. (µg/mL)*100

Volume of Sample (mL)

Non-reducing sugar % = Total Sugar – Reducing Sugar

9. Non-Reducing Sugar

Sample Name	Conc. mg/mL	OD at 630nm
Blank	0	0
Glucose	0.5	0.061
	1	0.182
	1.5	0.306
	2	0.450
	2.5	0.624
	3	0.708



Graph 3 Standard Glucose Curve- Anthrone Assay

Table 4: Reducing Sugar Content in Samples

Sample Name	OD at 630nm	Total Sugar Con. mg/mL	Reducing Sugar Conc. mg/mL	Non-Reducing Sugar Conc. mg/mL
Sara	0.142	0.74	0.35	0.39
Twaka	0.145	0.75	0.36	0.39
Kastha	0.146	0.75	0.38	0.38

DISCUSSION

Medicinal plants become rare, endangered and threatened (RET) day by day due to unscientific collection and harvesting practices. One of the causes for plant death is collection of used parts like root and heartwood of the plant. The need of the time is thus to find out alternative part of use for saving the plant species. At the same time, it should also be taken in account that the prepared formulation should have equal physic-chemical properties and biological activities.

Arishta Kalpana are widely in practice because of its long shelf life and fast in action. *Shirisharishta* is popular formulation that is been used as a *Shaman Chikitsa* in *Visha, Vishaja Vyadhis, Shwasa, Kasa* etc. This formulation is help to maintain doshas in *Sama-Avastha* because of *Samavoga Visheshata* (the combination possessing special actions). Majority of the drugs are *Katu, Tiktha, Kashaya Rasas* with *Laghu* and *Rukshaguna* and has *Ushna Veerya* and *Katu Vipaka*. The drugs like *Pippali, Haridra, Nilini, Nagakeshara, Shunti* are commonly used drugs in Acute toxic pathological conditions. Because of its *Ushna Virya* and *Katuvipaka* it has quick action on *Visha. Shirisha, Pippali, Nilini, Haridra* are well known

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for its *Vishaghna* property and has been mentioned in classics. The formulation also has other properties like *Dipana, Pachana*, with *Tikshna* and *Vyavayi Guna* which helps in fast action of the drugs. The present study was planned to observe the effect of *Shirisharishta* prepared by *Twak, Sara* and *Kastha* from *Shirisha.*

- The specific gravity of *Shirisharishta* (*Sara*) sample is more due to presence of more solid in it.
- The higher total solid content in *Shirisharishta* (*Sara*) and *Shirisharishta* (heartwood) samples indicates solubility of more water and alcohol soluble active principles.
- The highest alcohol content in *Shirisharishta* (heartwood) suggests that heartwood is the best part of use for preparation of *Shirisharishta*, and it is also strengthen the view of *Acharya Charaka* for including *Sara* (heartwood) of *Shirisha* (A. *lebbeck*) as *Asava Yoni* (source for fermentation).

Conclusion

The formulation *Shirisharishta* has not been found described by name in Brihatrayi, however Acharya Charaka has used *Shirisha Pushpa Swarasa* along with *Madhu* for the treatment of disease *Shwasa*. *Shirisha* has been found described in *Sara Asava Yoni* by Acharya Charaka in his classics Charaka Samhita. Presence of highly fibrous sapwood, dark brown streaked with dark and white shaded heart wood and appreciably thick and rough dark brown to grayish bark were unique characteristic features of *Shirisha*. The adopted reference Bhaishajya Ratnavali (72/72-74) for the preparation of *Shirisharishta* should be taken as standard. The highest alcohol content in *Shirisharishta* (heartwood) suggests that heartwood is the best part of use for preparation of *Shirisharishta.* So, *Shirisharishta* prepared by *Sara* is found better.

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