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A PHARMACEUTICO-ANALYTICAL AND ANTIMICROBIAL STUDY OF SAPTAPARNA KSHARA W.S.R TO SUSHRUTA SAMHITA

K. S. Sindhura*[1], K. G. Purushotham[2], M. Harshitha[3] and Gopalakrishna N. Nayak[4]

[1]Final Year PG Scholar, Department of Rasashastra and Bhaishajya Kalpana, KVGAMC, Sullia.

[2]Professor and HOD, Department of Rasashastra and Bhaishajya Kalpana, KVGAMC, Sullia.

[3]Professor, Department of Rasashastra and Bhaishajya Kalpana, KVGAMC, Sullia.

[4]Assistant Professor, Department of Rasashastra and Bhaishajya Kalpana, KVGAMC, Sullia.

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***Corresponding Author**
K. S. Sindhura
Final Year PG Scholar, Department of Rasashastra and Bhaishajya Kalpana, KVGAMC, Sullia.

ABSTRACT

Acharya Sushruta has explained the preparation of *Saptaparna Kshara* in *Sutra Sthana, Ksharapakavidhi Adhyaya.* It is indicated in *Vrana, Vatakaphaja Roga, Kusta, Raktavikara*, ***Krimi***, *Shwasa* and *Gulma.* The method of preparation varies from other classics on the basis of ratio of water and ash, duration of soaking and filtration pattern. As *Kshara* is indicated in *Krimi Roga*, the present study is also taken up to evaluate the antimicrobial activity of *Saptaparna Kshara* on the microbes causing diseases of gastrointestinal tract viz, *Escherichia coli, Salmonella typhimurium and Candida albicans*. Pharmaceutical, Analytical and Antimicrobial study was conducted with *Saptaparna Kshara*. *Saptaparna Kshara* preparation was carried out by classical method described in *Sushruta Samhita.*[1] *Acharya Sushruta* explains about ratio of ash and water as 1:6, filter 21 times through single folded cloth. Organoleptic, Physico-chemical and Chromatographic analysis were carried out for *Saptaparna Kshara* preparation. Antimicrobial study was conducted against *Escherichia coli, Salmonella typhimurium and Candida albicans*. Organoleptically the sample *Saptaparna Kshara* had grey colour and characteristic odour of *Kshara*. The quantitative analysis reveals that the drug has presence of elements like Sodium, Potassium, Calcium, Magnesium. TLC of the sample shows around 2 prominent Rf values with almost same colour bands with similar Rf values. Antimicrobial study of Sample *Saptaparna Kshara* showed antibacterial and antifungal actions against *Escherichia coli, Salmonella typhimurium, Candida Albicans.*

KEYWORDS: *Saptaparna, Kshara, Krimi, Escherichia coli, Salmonella typhimurium, Candida Albicans*.

INTRODUCTION

Ayurveda is an *Upaveda* of *Atharvaveda* which is an ancient literature and a life science. It has its roots in antiquity, the depth of which could not be measured. *Ayurveda* is a highly evolved system of life and health science based on the unique & original fundamental principles.

Ayurveda which is also known as *Trisutra Ayurveda*, gives equal importance to *Hetu*, *Linga* and *Aushadha* for maintaining the homeostasis (*Swasthya*) of body and mind.[2] Here the *Aushadha* include various forms of preparations prepared from herbal, mineral and animal sources. With the advancement of time and due to the huge demand of medicines, availability of all the medicinal plants in the required quantity is becoming impossible leading to scarcity of many of the medicinal plants. So, it becomes the need of the day to concentrate on simple and effective preparations of easily available plant sources which are economically viable. This makes the whole treatment procedure simple, effective and accessible to people belonging to all economic status.

Acharya Sushruta has explained the preparation of *Kshara* of the plant *Saptaparna* in *Sutra Sthana.*[3] It is one of drug which is grown everywhere, easily available, possess various medicinal properties, and used for the preparation of *Kshara*. *Saptaparna Kshara* is one of the important *Kshara* which is a alkali extracted from the water-soluble ash of *Saptaparna Panchanga*.

Saptaparna (*Alstonia scholaris (L)R. Br*) is a plant having *Kashaya rasa, Snigdha Guna, Ushna Veerya, Agnideepaka* and *Saraka* in nature. It is indicated in *Vrana, Vatakaphaja Roga, Kusta, Raktavikara, **Krimi**, Shwasa* and *Gulma.*[4]

Considering all these factors in mind a simple preparation of *Kshara* prepared from a commonly available plant *Saptaparna* is selected for the study. *Kshara* is a simple preparation which can be prepared by a practitioner himself without any major infrastructure.

MATERIALS AND METHOD

Collection of the drug

The raw drugs required for the preparation of medicine was procured from locality of Sullia,

Karnataka. The authentication of the raw drug was done at the P.G. Department of *Dravya Guna,* K.V.G. Ayurveda Medical College and Hospital, Sullia.

Preparation of *Saptaparna Kshara*

Method of data collection

The *Saptaparna Kshara* was prepared as per the *Kshara Nirmana Vidhi* explained in *Sushruta Samhita Sutra Sthana* 11th Chapter.[5] Pre-processing of drug like cleaning, drying was done in P.G. Department of RS & BK K.V.G.A.M.C Sullia. The *Saptaparna Kshara* was prepared at P.G. Department of RS & BK K.V.G Ayurveda Medical College and Hospital, Sullia.(Table no:1)

Selection of Raw Materials

The *Pachanga* of the plant was procured. Genuinity of the drug was tested and approved by the experts, Dept. of P.G. studies in *Dravya Guna*, K.V.G.A.M.C Sullia. Properly cleaned for extraneous matter. Dried in sun light till it complete became dried, this was tested by breaking the plant part (stem/root).

Equipment's Used for preparation

Vessels, Stainless steel spatula, Clean cotton cloth, Porcelain Beaker, Potable Water, Pyrometer, Heating Device, Airtight glass container.

Method of Preparation

Saptaparna Panchanga was collected and chopped into small pieces, then dried completely under the sun light. Complete drying was ascertained by observing cracking sound on breaking. Dried pieces of *Saptaparna Panchanga* was taken, and then ignited. After complete burning of *Saptaparna Panchanga*, it is allowed for *Swangasheeta* (complete cooling on itself). The ash obtained is later dissolved in definite quantity of water; i.e., 1 part (ash): 6 parts of water, macerated well and kept undisturbed for 1 hour. Then filtered through single folded clean cloth for 21 times. The liquid obtained is called *Ksharodaka*. Then the filtrate should be treated on fire in a wide mouthed vessel, on *Madhyamagni*, while it was slowly stirred with a ladle, till it becomes semisolid. Later it is dried to obtain – ash like fine powder called *Kshara.* The *Kshara* obtained is preserved in air tight glass container. (Table no**:**2 & Figure no 1-16)

OBSERVATIONS AND RESULTS

Drying of drug: Partially dried *Saptaparna* (28kg) was taken and chopped into small slices, and took 25 days for complete drying.

Burning of *Saptaparna Pachanga*

Dried *Panchanga* of *Saptaparna* was taken and arranged in a windless place, then it was ignited. Fire was extinguished and was allowed for *Swangasheeta* and it became completely. The ash obtained was 380 g (1000ml– Volumetrically) and pH of ash was 11.4. (Table no:3)

During and after Soaking

Whole ash was immersed in water, no particles floated over water surface. After maceration with hand, it turned finer and mixed completely in water. After soaking the whole ash was completely mixed and the liquid was black in colour.

Filtration of *Ksharaodaka*

After 21 times filtration the *Ksharodaka* was measured to be 5020 ml. The difference in quantity of *Ksharodaka* before and after 21 times filtration was 980 ml. The weight of residue left after decanting *Ksharodaka* was 392 g (wet) (Table no:4)

Boiling of *Ksharodaka*

Thus, obtained 5020ml of *Ksharodaka* was treated on fire in a wide mouthed vessel, on *Madhyamagni*, while it was slowly stirred with a ladle, till all the water content was evaporated. (Table no:5)

After Completion of boiling

Ash colour *Kshara* was left at the end. And can be assessed with certain qualities.

Result - 122 gm *Saptaparna Kshara* was obtained.

Analytical Study[6,7,8,9,10,11,12]

The analysis of the sample was carried out by using different organoleptic characters, physical, chemical tests and chromatographic analysis. Physical tests like pH, loss on drying, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive was done in quality control lab of K.V.G. Ayurveda Pharma and research centre Sullia. The chemical parameter like quantitative estimation of sample was done at Care keralam research centre. Chemical tests[13,14] like estimation of sodium, potassium, calcium, magnesium shows the presence of elements with % of *Kshara*. Chromatographic

analysis[15,16] was done at quality control lab of K.V.G. Ayurveda Pharma and research centre Sullia.

Antimicrobial Study[17]

The anti-microbial study was done at Care keralam research centre.

Antimicrobial study was conducted against *Escherichia coli, Salmonella typhimurium and Candida albicans.*

Table No 1: Details of raw drug used.

Sl.no	Common Name	Latin Name	Part Used	Quantity
1.	*Saptaparna*	*Alstonia Scholaris.L.(Br)*	*Panchanga*	28Kg (Partially dried *Saptaparna Panchanga*) 9Kg (After complete drying *Saptaparna Panchanga*)

Table No 2: Details of preparation of *Saptaparna Kshara.*

Sl. No	Feature		Sample
1.	**Ash taken**		1000ml (v/v)
2.	**Quantity of water taken – soaking**		6000ml
	Drug and water ratio		1:6
	Soaking time		1 hour
3.	**No. of times of filtration**		21 times
	No. of folds of cloth		1-fold
4.	**Filtration Time**	Starting Date & Time	15/06/22 9.35am
		Ending Date & Time	15/06/22 11.30am
5.	***Ksharodaka* obtained after complete filtration**		5020ml
6.	**Weight of Residue**		392gm(wet)
7.	**Total Kshara obtained**		122gm(w/w) 130ml(v/v)
8.	**Duration of preparation**	For filtration	2hrs 5mints
		Saptaparna Kshara after boiling	2hrs 25mints

Table No 3: Observations during drying of *Saptaparna Panchanga.*

No of Days	Changes Observed
1 – 5 days	Partially dried, but presence of moisture can be noticed, flower & leaves green in colour
6 -10 days	Colour changes from green to slight brownish
11 – 15 days	Dried partially, crackling sound on breaking- absent Leaves, flowers, fruit dried, roots drying observed
16 – 20 days	Leaves & flowers dried completely, root & bark dried partially, crackling sound on breaking slightly +
21 - 25 days	Dried completely with cracking sound on breaking

Table No 4: Observations during filtration of *Ksharodaka*.

No. of filtration	Total *Ksharodaka* Before Filtration (ml)	Total *Ksharodaka* After Filtration (ml)	Observation	Total Time required	% of *Kashrodaka* obtained
1st	6000	5990	Dark blackish with slight greyish, Slimy + + +	15mints	99.83%
2nd	5990	5975	blackish with slight greyish, Slimy + +	15mints	99.58%
3rd	5975	5950	Light black with slight greyish, Slimy +	20mints	99.16%
4th	5950	5920	Slight greyish black colour, slight particles +, Slimy +	15mints	98.66%
5th	5920	5880	Slight greyish black colour, Slimy +	15mints	98%
6th	5880	5830	Dark blackish slimy reduced	15mints	97.166%
7th	5830	5770	-do-	10mints	96.16%
8th	5770	5670	-do-	10mints	94.5%
9th	5670	5670	-do-	10mints	94.5%
10th	5670	5570	-do-	8mints	92.83%
11th	5570	5470	-do-	8mints	91.16%
12th	5470	5320	-do-	6mints	88.66%
13th	5320	5320	-do-	6mints	88.66%
14th	5320	5170	-do-	6mints	86.16%
15th	5170	5170	Light greyish black colour slimy absent	5mints	86.16%
16th	5170	5170	-do-	4mints	86.16%
17th	5170	5070	-do-	4mints	84.5%
18th	5070	5020	-do-	4mints	83.66%
19th	5020	5020	-do-	4mints	83.66%
20th	5020	5020	-do-	4mints	83.66%
21th	5020	5020	Clear fluid, No Particles present Slimy – Absent	4mints	83.66%

Table No 5: Observations during boiling of *Ksharodaka.*

Time	Temperature	Observation
12.45pm (Kept for boiling)	41.3°C	Clear *Ksharodaka* without any fumes
12.56pm	66.9 °C	Appearance of fumes, Boiling started
1.15pm	78.9 °C	White froth – all over the surface Boiling- smell o*f kshara*
1.35pm	85.8 °C	White froth – all over the surface
1.50pm	88.5°C	White froth increased
2.10pm	88.5 °C	Boiling- smell of *kshara*
2.35pm	92.3 °C	Boiling continued
2.55pm	95.3 °C	White froth seen over the surface and liquid reduced
3.10pm	95.3 °C	Liquid reduced in volume and became thick consistency
3.25pm	97 °C	Whitish colour froth with thick consistency
3.40pm	99.2 °C	Semisolid thick sluggish consistency
4.05pm	90.2 °C	Light greyish ash colour powder form with moisture, Started forming grey particles at the centre of vessel
4.25pm	87.6 °C	Complete water portion reduced, moisture +
4.40pm	83.2 °C	Greyish colour observed, powder of *Kshara* obtained

Table No 6: Assessment criteria of *Kshara.*

Criteria	Observation
Sparsha	Smooth and slimy
Rupa	Grey
Rasa	Lavana, *Kshareeya*
Gandha	*Visra Gandhi*
Karma	*Ksharana*

Table No 7: Showing observations of organoleptic characters of *Saptaparna Kshara.*

Organoleptic Characters	*Saptaparna Kshara*
Colour	Grey
Odour	Characteristic
Taste	Salty, Alkaline
Appearance	Fine Powder

Table No 8: Showing results of Analytical Study.

Physico – Chemical Parameters	***Saptaparna Kshara***
pH	11.24
Loss on drying	0.048%w/w
Total Ash	86.337%w/w
Acid insoluble ash	25.90%w/w
Water Soluble ash	63.614%w/w
Water soluble extractive	84%w/w
Alcohol soluble extractive	8.960%w/w

Table No 9: Showing results of Quantitative analysis of *Saptaparna Kshara.*

Sl. No	**Parameters**	**Result**
1.	Sodium as Na wt%	9.33
2.	Potassium as K wt%	32.48
3.	Magnesium as Mg wt%	2.22
4.	Calcium as Ca wt%	1.2

Table No 10: Showing observations of T.L.C.

Major Spot	**Colour**	**Approx.Rf**
1.	Blue	0.575
2.	Light blue	0.500

Table No: 11: Test report of *Saptaparna Kshara* over *Candida albicans, Escherichia coli* and *Salmonella typhimurium.*

Micro Organisms	**Test result (Zone of inhibition in diameter)**				**Test method**
	Sample			**Standard Drug**	
	80%	**50%**	**25%**		
***Candida albicans* (NCIM 3102)**	24mm	22mm	19mm	Clotrimazole (1000ppm -17mm)	Agar Well Diffusion Method
***Escherichia coli* (NCIM 2256)**	14mm	14mm	12mm	Streptomycin (1000ppm – 24mm)	
***Salmonella typhimurium* (NCIM 2501)**	13mm	11mm	10mm	Streptomycin (1000ppm – 25mm)	

PREPARATION OF *SAPTAPARNA KSHARA*

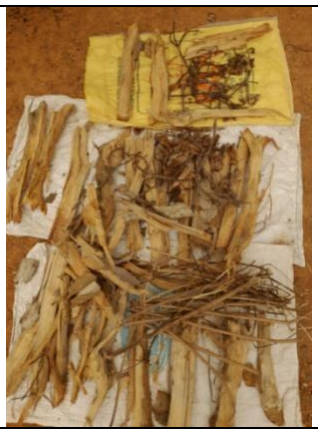

Fig 1: Drying of *Saptaparna Panchanga.*

Fig 2: Burning of *Saptaparna Panchanga.*

Fig 3: After complete burning.

Fig 4: Ash of Saptaparna.

Fig 5: Soaking of Ash.

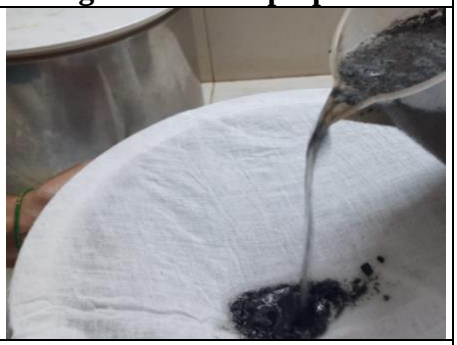

Fig 6: Filtration.

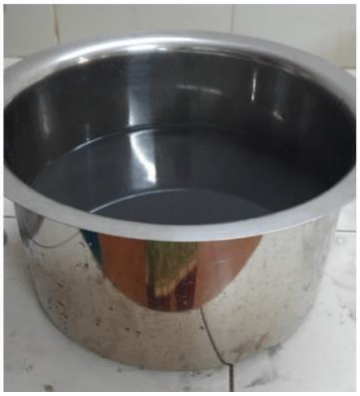

Fig 7: Ksharodaka.

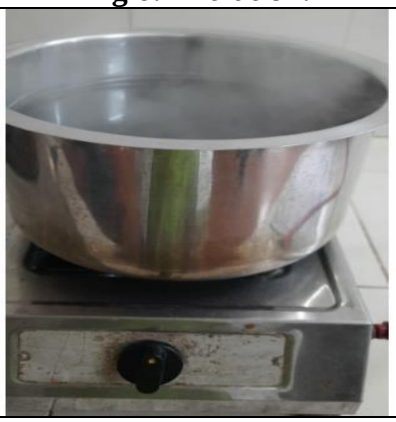

Fig 8: Boiling of Ksharodaka.

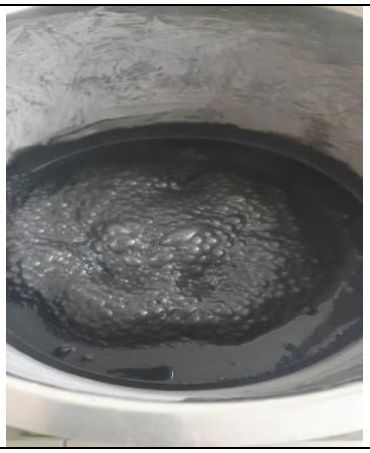

Fig 9: Froth during Boiling.

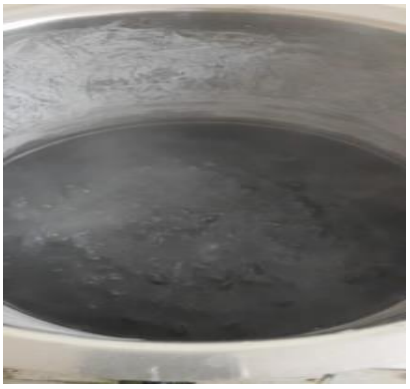

Fig 10: Evaporation of Water.

Fig 11: Semi Solid Consistency.

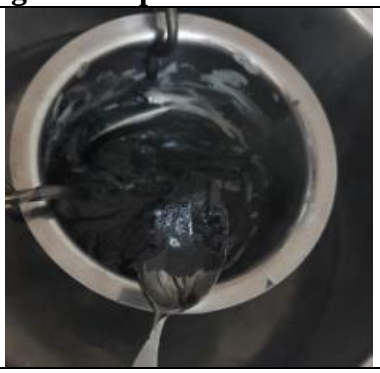

Fig 12: Sticking to Vessel.

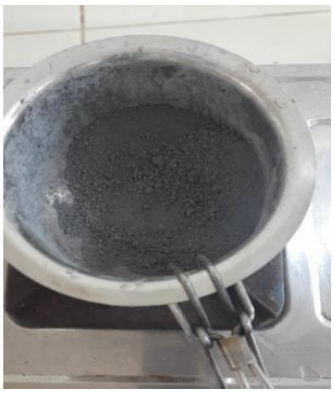

Fig 13: Powder Form.

Fig 14: Obtained final product *Saptaparna Kshara.*

DISCUSSION

Saptaparna Pachanga about 28kg was collected and it was made into slices to reduce the size. The reason behind the size reduction is to make easy and uniform drying and helps for further process like burning and preparation of ash. Fresh and matured *Saptaparna Panchanga* was collected cleaned and dried. Brown colour on stem bark denotes proper drying and produces cracking sound on breaking.

Dried *Saptaparna Pachanga* 9 kg was taken and burnt. This ash was taken for preparation of *Kshara* weighing 380 gm (1000 ml by volume). In the present study, 1 part of ash 380gm (1000ml – volumetrically) was added to 6 parts of water 6000ml-volumetrically.

Before filtration the liquid obtained was 6000 ml. After completion of 21filtrations, it turns to 5020ml with clear fluid of greyish black colour. It shows 83.66% yield of *Kshara Jala* (alkaline water) and 16.33% loss.

Kshara Jala (alkaline water) was boiled on moderate temperature till the complete evaporation of water and obtaining of *Kshara*.

Reduction of liquid is seen during the boiling as the water portion evaporates. White particles on the side of the vessel are observed as *Kshara* (Phyto-alkali). After the water portion completely evaporates grey powder *Kshara* (Phyto-alkali) (122 g) was obtained. It shows 32.10% yield of *Saptaparna Kshara.*

Discussion on Organoleptic Characters

Colour – Colour of *Saptaparna Kshara* was Grey in colour. Colour may be due to the evaporation of water portion during boiling, the sediments which possess the grey colour.

Taste – The taste was Salty and Alkaline, which indicates the presence of inorganic salts in *Kshara.*

Odour – Odour was characteristic and slightly pungent because of property of *Kshara*.

Touch - *Kshara* was Amorphous powder form and slightly Slimy due to the presence of salts.

Discussion on Physical parameters

Loss on drying indicates the presence of moisture content in the drug. Loss on drying in *Saptaparna Kshara* was found 0.048. The total ash figure is of importance and indicates to some extent the amount of care taken in the preparation of the drug. If more will be the content of ash in the sample more will be the presence of alkaline matter in the sample. The Total Ash value of *Saptaparna Kshara* was 86.337%w/w. The acid insoluble ash value of Sample *Saptaparna Kshara* was 25.901%w/w.

The Water soluble ash of Sample *Saptaparna Kshara* was 63.614%w/w. The sample was almost soluble in water. The pH value of the sample was 11.24 this shows the alkaline character of drug. The pH value of the *Saptaparna Panchanga* Ash was 11.40. There is mild alteration of 0.16 in the changes in pH of ash and *Kshara*, but the alkaline pH indicates the

presence of water soluble basic *Kshara*. This may depend on the amount of water added while preparing *Kshara* which changes the concentration of liquid and alteration of pH. The Water-Soluble Extractive of Sample *Saptaparna Kshara* was 84%w/w. Alcohol soluble extractive of sample was 8.960%w/w.

The presence of Sodium is 9.33%, Potassium is 32.48%, Calcium is 1.2% and Magnesium is 2.22%.

From the readings we can consider that Sample contains Na, K, Mg, as a main element.

T.L.C. study of the sample showed around 2 prominent Rf values at 0.575 (Blue) and 0.500 (Light Blue).T.L.C for *Saptaparna Kshara* was conducted as mentioned in the atlas of Ayurvedic Pharmacopoeia of India Part-1, Volume - 1.

Discussion on Antimicrobial Study

In the present study the Anti-Microbial activity of *Saptaparna Kshara* was assayed against the fungus *Candida albicans*. The sample was dissolved in 80% 50% and 25% sample concentration dissolved in sterile distilled water, and standard drug used was Clotrimazole (1000ppm) showed 24mm, 22mm and 19mm zone of inhibition in 80%, 50% and 25% sample concentration respectively.

The Anti-Microbial activity of *Saptaparna Kshara* was assayed against the bacteria *Escherichia coli.* The sample was dissolved in 80% 50% and 25% sample concentration dissolved in sterile distilled water. And standard drug used was Streptomycin (1000ppm) showed 14mm, 14mm and 12mm zone of inhibition in 80%, 50% and 25% sample concentration respectively.

The Anti-Microbial activity of *Saptaparna Kshara* was assayed against the bacteria *Salmonella typhimurium.* The sample was dissolved in 80% 50% and 25% sample concentration dissolved in sterile distilled water. And standard drug used was Streptomycin (1000ppm) showed 13mm, 11mm and 10mm zone of inhibition in 80%, 50% and 25% sample concentration respectively.

CONCLUSION

The drug *Saptaparna Panchanga* used in this study is cost effective and available throughtout India. Proper drying prior to burning of drug and proper filtation of *Ksharodaka* increases the quality and quantity of drug to prepare *Kshara*. pH value suggested to be alkaline which is

strongly alkaline in the sample. The quantitative analysis reveals that the drug has presence of elements like Sodium, Potassium, Calcium, Magnesium in traces. In TLC study of the sample showed around 2 prominent Rf values with almost same colour bands with similar Rf values.

The *Saptaparna Kshara* has the anti-bacterial activity against *Escherichia coli* and *Salmonella typhimurium*. It also has the anti-fungal activity against *Candida albicans*. The efficacy of the formulation has to be analysed in other GIT Disorders caused by other etiological factors. So, to analyse that experimental and Clinical study can be done.

REFERENCES

1. Acharya Sushruta; Sushruta Samhita with Nibhandhasangraha Commentary of Sri Dalhanacharya and the Nyayachandrika Panjika of Sri Gayadasacharya on Nidhana Sthana edited by Vaidya Yadavji Trikamji Acharya From the beginning to the 9th *Adhyaya* of Chikitsa Sthana and the rest by Narayan Ram Acharya '*KAVYATIRTHA*', published by Chaukamba Surbharathi Prakashan, Varanasi, Print 2012, *Sutra Sthana* Verse No.11/11 Page. No. 46-47.
2. Acharya Charaka; Charaka Samhita with Ayurveda Deepika commentary by Chakrapanidatta edited by Yadavji Trikamji Acharya, published by Chaukamba Surbharathi Prakashan, Varanasi, Reprint 2011, Sutra Sthana, Verse No.1/24 Page.No.7.
3. Acharya Sushruta; Sushruta Samhita with Nibhandhasangraha Commentary of Sri Dalhanacharya and the Nyayachandrika Panjika of Sri Gayadasacharya on Nidhana Sthana edited by Vaidya Yadavji Trikamji Acharya From the beginning to the 9th *Adhyaya* of Chikitsa Sthana and the rest by Narayan Ram Acharya '*KAVYATIRTHA*', published by Chaukamba Surbharathi Prakashan, Varanasi, Print 2012, *Sutra Sthana* Verse No.11/11 Page. No. 46-47.
4. Acharya Bhavamishra; Bhavaprakasha Nighantu (Indian Materia Medica) Commentary by Prof K C Chunkar, Edited by Dr.G.S. Pandey, Published by Chaukambha Bharathi Academy, Varanasi, Vatadi Varga 74-75 Page. No. 534-535.
5. Acharya Sushruta; Sushruta Samhita with Nibhandhasangraha Commentary of Sri Dalhanacharya and the Nyayachandrika Panjika of Sri Gayadasacharya on Nidhana Sthana edited by Vaidya Yadavji Trikamji Acharya From the beginning to the 9th *Adhyaya* of Chikitsa Sthana and the rest by Narayan Ram Acharya '*KAVYATIRTHA*', published by Chaukamba Surbharathi Prakashan, Varanasi, Print 2012, *Sutra Sthana* Verse No.11/11 Page. No. 46-47.

6. Anonymous. The Ayurvedic formulary of India, Published by Govt of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and Naturopathy, Unani Siddha and Homeopathy, New Delhi, Part II, Volume I, 2008, page no:141.
7. Anonymous. The Ayurvedic formulary of India, Published by Govt of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and Naturopathy, Unani Siddha and Homeopathy, New Delhi, Part II, Volume I, 2008, page no:140.
8. Anonymous. The Ayurvedic formulary of India, Published by Govt of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and Naturopathy, Unani Siddha and Homeopathy, New Delhi, Part II, Volume I, 2008, page no:140.
9. Anonymous. The Ayurvedic formulary of India, Published by Govt of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and Naturopathy, Unani Siddha and Homeopathy, New Delhi, Part II, Volume I, 2008, page no:140.
10. Anonymous. The Ayurvedic formulary of India, Published by Govt of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and Naturopathy, Unani Siddha and Homeopathy, New Delhi, Part II, Volume I, 2008, page no:141.
11. Anonymous. The Ayurvedic formulary of India, Published by Govt of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and Naturopathy, Unani Siddha and Homeopathy, New Delhi, Part II, Volume I, 2008, page no:141.
12. Anonymous. The Ayurvedic formulary of India, Published by Govt of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and Naturopathy, Unani Siddha and Homeopathy, New Delhi, Part II, Volume II, 2008, page no:191.
13. Honward.VS, Handbook of standardization of Ayurvedic Formulation.1sted, Varanasi: Chaukahambha Orientalia, 2012.p.60 – 63.
14. Eton, F.M. (1950). Significance of carbonate in irrigation waters, soil science, 69: 123-133.
15. Lavekar GS, Padhi MM, Pant P, editors. Laboratory Guide for the Analysis of Ayurveda and Siddha Formulations. First. Jankipur, New-Delhi: Central Council for Research in Ayurveda and Siddha; 2010, Page No.49.
16. Anonymous. The Ayurvedic formulary of India, Published by Govt of India, Ministry of health and family welfare, Department of Ayurveda, Yoga and Naturopathy, Unani Siddha and Homeopathy, New Delhi, Part I, Volume VI, 2008, page no:281.
17. Microbiology an introduction by Tortora, Funke & Case – 9th Edition, Chapter 20.