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A COMPARATIVE PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF VACHA (*ACORUS CALAMUS* LINN.) AND CLASSICALLY SHODHITA VACHA

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ABSTRACT
Vacha (*Acorus calamus* Linn.), an important and extensively used medicinal plant is advised to be internally administered after Shodhana by Ayurvedic Pharmacopoeia of India. With lack of reported data and quoted only by classical text Chakradatta, it has become an important and inquisitive subject to be scientifically evaluated. Hence in the present study, raw and classical Shodhita Vacha were subjected to pharmacognostical study, preliminary phytochemical analysis, fluorescence and thin layer chromatographic studies involving β-asarone as marker compound to lay certain standard reference parameters for future studies. Gross changes were observed in the Shodhita samples of Vacha with respect to its organoleptic characters, cytoarchitechture, oil globules and phytoconstituents which could be implied to the particular media and method of classical Shodhana.
Key words: Vacha, Acorus calamus, Shodhana, β-asrone, Fluorescence analysis.

INTRODUCTION

Vacha commonly called as sweet flag, is an important medicinal and aromatic plant being used extensively in almost all herbal based systems. It is a semi aquatic perennial plant of Acoraceae having scented rhizomes and tapered reed-like leaves. [Figure 1]. The rhizomes are considered the officinal part of the plant and have been reported to possess tranquilizing, antimicrobial, antidiarrheal, antidyslipidemic, neuroprotective, anti-inflammatory and analgesic activities.[1] The major active principles present in the calamus oil are α- asarone, β-asarone, calamene, calamenenol, calameone, α-pinene, camphene, eugenol etc. among which β-asarone is one of the most important one and has been the subject of considerable studies.[2-4] Vacha is used extensively in Ayurveda in cases of Udara roga, Vata vyadhi and Manasika rogas.[5] It is interesting to note that contraindications or toxic effects of Vacha are not observed in classical texts but still Shodhana has been advised even though it is not considered poisonous.

Shodhana methods are recommended in Ayurvedic classics with a view to modify certain poisonous plants into therapeutically safe plants.[6] The classical quotes also reveal that Shodhana not only refers to purification procedures but also to different samskaras through which there is Gunaantardhana in the primary dravya rendering it safe as well as obtaining desired qualities in it.[7] Texts like Chakradatta and Bhaishajya Ratnavali have given emphasis on Shodhana of Vacha using different media like Gomutra (Cow's urine), Mundi Kwatha (Decoction of *Sphaeranthus indicus* Linn.), Panchapallava (Decoction of a group of five leaves) and Gandhodaka (Decoction of group of aromatic herbs).[8,9] This is supported by the recommendations of API and herb directory of Indian System of Medicine and Homeopathy that rhizomes of Vacha (*Acorus calamus* Linn.) should be used after Shodhana.[10,11] Since there are no available reported data suggesting the method for Shodhana of Vacha or its outcome hence the reasoning or the objectives for the recommendation of Shodhana can be understood through detail pharmaceutical, pharmacognostical, analytical and pharmacological studies making it one of the essential subject to be scientifically evaluated. Hence in the present study, pharmacognostical and preliminary phytochemical differences between raw and Shodhita Vacha were evaluated

MATERIALS AND METHODS

Collection of plant material and grouping

After proper identification and authentication, mature rhizomes of *Acorus calamus* were collected from the forest regions of Yelagiri Hills, Tamilnadu in the month of November. The rhizomes were cleaned off the roots, attached leaves and washed thoroughly in water to remove the soil adhered and dried in partial shade for 10 days. The voucher specimen was deposited in the institute's Pharmacognosy department vide voucher specimen No. PhM. 6002. The rhizomes were cut into pieces of one inch length and equally partitioned into three groups. The first group (RV) consisted of raw Vacha. The second group (SV) was subjected to Shodhana through classical method and the third group (WV) was subjected to boiling in water and taken as a control group for comparison.

Method of Shodhana

For the classical method, the rhizomes were tied in a cotton cloth made into a pottali. It was subjected for classical swedana procedure for three hours in Gomutra, Mundi kwatha, Panchapallava kwatha successively. Later it was subjected to bashpa swedana (fomentation) with Gandhodaka. After each swedana, the rhizomes were

washed in warm water and dried for four days in sunlight. The third group (WV) was subjected to pottali swedana procedure for three hours in water (procured from a reverse osmosis plant).

Macroscopic and microscopic evaluation

The rhizomes of raw as well as Shodhita Vacha samples were subjected to macroscopical and microscopical evaluation as per standard procedure.[12,13] The sample powder passed through sieve no 60 was used for powder microscopy. Both stained and unstained specimens were used to identify and confirm the microscopic structures.[14] Photomicrographs were taken using Carl Zeiss binocular microscope.

Phytochemical evaluation

Different physicochemical tests like loss on drying at 105°C, ash value, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value, pH value as well as qualitative test for various functional groups like alkaloids, glycosides etc were also carried out for all the three samples. Heavy metal analysis and pesticide residue analysis was done only for raw Vacha to check the contamination. Histochemical tests were carried out by treating the transverse sections of all the samples using specific reagent to detect the colour changes and localization of chemicals. Fluorescence analysis was carried out with the powder of the rhizome sieved through 60 mesh and treated with various reagents. The supernatants were examined under day light and ultraviolet light (254nm and 366nm) [15-18].

Thin layer chromatography

TLC of methanol extracts of sample RV, SV and WV was carried out in comparison to standard marker compound β-asarone (1mg dissolved in 2ml of methanol)**.** Prepared silica gel G plate of thickness 0.3mm activated at 105°C for 30 minutes was used along with Toluene:Ethyl acetate (9:1) as mobile phase. 10µl of test solution and 5µl of standard solution was applied on the TLC plate and were developed in the solvent phase till the solvent front run was 9.6 cm. Later they were visualized under UV light at 254nm and 366 nm and after derivatization with Vanillin – Sulphuric acid reagent[19].

Table 1: Important microscopic characteristics observed in the rhizomes of both raw and Shodhita Vacha

Microscopic character	Sample RV	Sample SV	Sample WV
Region	The rhizome is differentiated into cortical region constituting around 1/3rd of the area and stelar region constituting around 2/3rd.	There is no change in the regional differentiation but the areas are compressed than RV	The size and structure of cortex and stellar region is similar to raw Vacha
Cork, Cortex and Stele	The periphery of the cortex consists of single layered dark brown corky tissue followed by single layered epidermis having radially elongated cells with thickened walls. Under the epidermis 2 to 3 layers of closely arranged collenchymatous cells form the hypodermis followed by spherical to oblong thick walled parenchymatous cells covering the rest of the cortex. These cells are arranged to form a network leaving large intercellular spaces. The lower boundary of the cortex is characterised by a distinct endodermis having barrel shaped, thin walled cells which separates it from the stellar region. The stellar region also consists of parenchymatous cells similar to cortex.	The cork tissue is blackish in colour. The epidermis, collenchyma as well as parenchyma cells could not be differentiated from one another very clearly because of the gelatinous, compressed, patchy starch grains covering the cells with the intercellular spaces also being reduced to a large extent. This is also observed in the parenchyma cells of stellar region. There is no gross change in the endodermal cells.	Light brown coloured corky tissue formed the periphery of the cortex followed by the epidermis. The collenchyma cells are slightly compressed when compared to RV. There are no changes in the parenchyma and endodermal cells.
Vascular bundles	The vascular bundles are fairly large, collateral with spiral and annular, scalariform thickening. They have large air spaces and found scattered in the cortex. Stele also consists of vascular bundles in large numbers arranged in the form of rings especially near the endodermis. They are mostly leptocentric (amphivasal) but irregular types of bundles are also found to occur. Few yellow to brown oil globules are also seen scattered in both cortical and stellar vascular bundle.	The xylem vessels are vertically compressed in the cortical vascular bundle and the air spaces are smaller than raw Vacha. There are no changes in the vascular bundles of the stellar region	The vascular bundles of both cortical as well as stellar region seemed to be similar to RV. Few oil globules are also seen scattered in the vascular bundle.
Oilcells and globules	The oil cells are globose, found amongst the parenchyma cells devoid of starch grains. They are slightly larger and thin walled than the surrounding cells. They often contain yellowish brown oil. Many broken oil cells are also observed with spilled out oil droplets. The oil droplets appear crimson red and the walls of the oil cell appear light pink upon staining with Sudan red III and safranin	The oil cells are hardly visible because of the masking by patchy starch grains. The oil globules are irregularly shaped, thickened, dark brownish in colour. Staining with Sudan red III and safranin did not alter its colour.	All the oil cells are ruptured and the oil globules are smaller in size and dispersed throughout the cortical and stellar region. There is no change in the colour of the oil. Upon staining with Sudan III and Safranin they changed to crimson red.
Oleoresin content	Dark brown coloured Oleoresin deposits are found scattered throughout the cortex and the stellar region.	The oleoresin deposits are scarce and found occasionally only in the sub epidermal region. The colour is even more darker than raw Vacha	The oleoresin deposits are seen scattered throughout the cortical and stellar region. The colour is same as RV
Starch grains	The starch grains are abundant and often seen clustered in the parenchyma cells. Mostly round with occasionally oval shaped, simple, single or in aggregation. The grains are translucent white, highly refractive and blackish blue upon iodine staining.	The starch grains are flattened, pale brown coloured, gelatinous with less refractive nature, studded and covering most of the parenchyma cells. Upon staining with iodine the patches appeared light blue.	The starch grains are seen scattered in the corners of the parenchyma cells. They are rounded, slightly larger than raw Vacha. There is no change in the colour or refractive nature. The grains changed to blackish blue colour upon staining.

Table 2: Physicochemical parameters of raw and Shodhita Vacha samples

Parameters	Results		
	RV	SV	WV
Loss on drying at 105°C	14.8	11.7	13.8
Ash value	6.2	13.47	11.34
Acid insoluble Ash	0.56	0.85	0.44
Water soluble extractive	21.02	11.11	18.92
Methanol soluble extractive	15.54	4.83	8.2
pH	4.23	5.75	4.72

Table 3: Phytochemical tests for various functional groups

Functional groups	Tests Performed	RV	SV	WV
Carbohydrates	Molish's test	+	+	+
Reducing sugars	Fehling's test	+	+	+
Non reducing polysaccharides	Iodine test	-	-	-
Proteins	Biuret test	+	+	+
Amino acids	Ninhydrin test	+	+	+
Flavonoids	Shinoda test	+	+	+
Steroids	L. Burchard test	+	+	+
Cardiac Glycosides	Legal's test	--	+	--
Anthraquinone Glycosides	Modified Borntrager's test	+	+	+
Saponin glycosides	Honey comb shape stable froth test	-	+	-
Alkaloids	Dragendorff's test	+	+	+
Tannin	Neutral $FeCl_3$ test	+	+	+

Table 4: Histochemical tests of raw and Shodhita Vacha samples

Test	Reagent	Colour observed		
		RV	SV	WV
Starch	Iodine	Dark blue	Blackish	Dark blue
Tannin	Ferric chloride	Brown	Brown	Brown
Saponin	Conc. H_2SO_4	Light yellow	Light yellow	Light yellow
Fat	Sudan III	Crimson red	Dull red	Crimson red
Sugar	20%aq NAOH	Yellow	Yellow	Yellow
Alkaloids	Dragendroff's reagent	Orange	Orange	Orange

Table 5: Fluorescence analysis of rhizome powders of different Acorus samples in daylight

SN	Treatment category	Acorus samples		
		RV	SV	WV
1.	Powder + 1N NaOH	Fossil	Central stage	Fossil
2.	Powder + 1N NaOH (alcohol)	Peach rose	Apple cream	Peach rose
3.	Powder + 1N Hcl	Pale Dawn	Corn cob	Pale Dawn
4.	Powder + 1:1 $H2SO_4$	Hip purple	Burnt violet	Hip purple
5.	Powder + 1:1 HNO_3	Wild yellow	Spice	Wild yellow
6.	Powder + Acetone	Cool grey	Iced silver	Cool grey
7.	Powder + Alcohol (ethanol)	Cool grey	Pale pearl	Cool grey
8.	Powder + Benzene	Cool grey	Sky mimic	Cool grey
9.	Powder + Chloroform	Cool grey	Water	Cool grey
10.	Powder + Ammonia	Majestic purple	Crimson depth	Majestic purple

NOTE: The colour mentioned in the table are based on the "Asian paints" colour spectra, Asian paints limited, Mumbai (www. asianpaints.com)

Table 6: Fluorescence analysis of rhizome powders of different Acorus samples in Short UV (254nm)

SN	Treatment category	Acorus samples		
		RV	SV	WV
1.	Powder + 1N NaOH	Divine pink	Rose lace	Divine pink
2.	Powder + 1N NaOH (alcohol)	Misty meadow	Candle light	Misty meadow
3.	Powder + 1N Hcl	Sky pink	Sky pink	Sky pink
4.	Powder + 1:1 H_2SO_4	Passion fruit	Pink musing	Passion fruit
5.	Powder + 1:1 HNO_3	Soft breeze	Angel harp	Soft breeze
6.	Powder + Acetone	Twilight sky	Washout	Twilight sky
7.	Powder + Alcohol (ethanol)	Twilight sky	Twilight sky	Twilight sky
8.	Powder + Benzene	Twilight sky	Twilight sky	Twilight sky
9.	Powder + Chloroform	Twilight sky	Twilight sky	Twilight sky
10.	Powder + Ammonia	Hip purple	Wild rose	Hip purple

Table 7: Fluorescence analysis of rhizome powders of different Acorus samples in Long UV (366nm)

SN	Treatment category	Acorus samples		
		RV	SV	WV
1.	Powder + 1N NaOH	Soft focus	Pink serenade	Soft focus
2.	Powder + 1N NaOH (alcohol)	Burst of spring	Raw silk	Burst of spring
3.	Powder + 1N Hcl	Ivory coast	Nautilus	Ivory coast
4.	Powder + 1:1 H_2SO_4	Dynamic	Deep sea	Dynamic
5.	Powder + 1:1 HNO_3	Malabar hills	Fringe green	Malabar hills
6.	Powder + Acetone	Angel harp	Soft stream	Angel harp
7.	Powder + Alcohol (ethanol)	Blank canvas	Mystic sky	Blank canvas
8.	Powder + Benzene	Soft whisper	Meadow mist	Soft whisper
9.	Powder + Chloroform	Soft breeze	Blue smoke	Soft breeze
10.	Powder + Ammonia	Red wood	Iced teal	Red wood

Table 8: TLC analysis of methanolic extract of different Acorus samples

Conditions	Rf values			
	RV	SV	WV	β-asarone
Short UV (254nm)	0.05, 0.19, **0.44 [3]***	0.05, 0.16, 0.2, **0.44 [4]**	0.05, 0.16, 0.21, **0.44 [4]**	**0.44**
Long UV (366nm)	0.05, 0.10, 0.14, 0.22, 0.27, 0.31, 0.41, 0.46 **[8]**	0.06, 0.09, 0.11, 0.14, 0.31, 0.41 **[6]**	0.06, 0.1, 0.2, 0.25, 0.41 **[5]**	-
Vanillin sulphuric reagent	0.05, 0.1, 0.25, **0.44**, 0.52 **[5]**	0.05, 0.12, 0.29, **0.44,** 0.46 **[5]**	0.05, 0.15, 0.3, **0.44,** 0.52 **[5]**	**0.44**

* Parenthesis indicates total number of spots

Figure 1: Photograph showing plant profile of *Vacha*

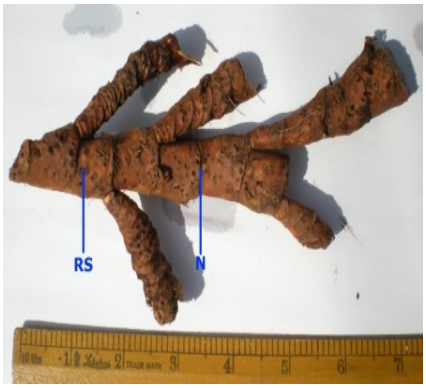

Figure 2: Rhizome of Vacha showing numerous round root scars on the ventral surface

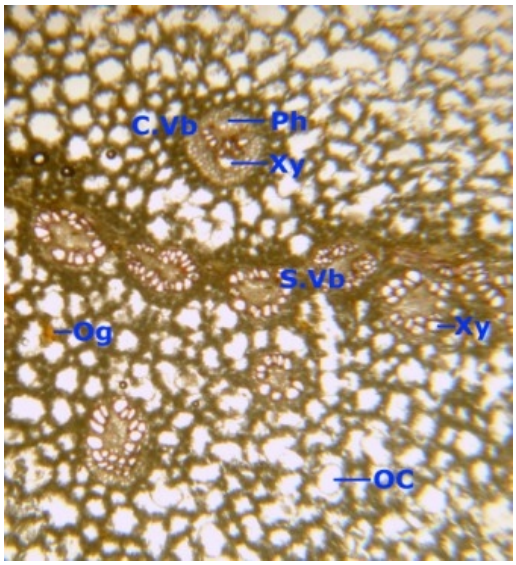

Figure 3: Transverse section of raw *Vacha* (RV) (20X)
C. Vb. – Cortical vascular bundle; S. Vb. – Stelar Vascular bundle; Ph. – Phloem; Xy. – Xylem; OC. – Oil cell; Og. – Oil globule

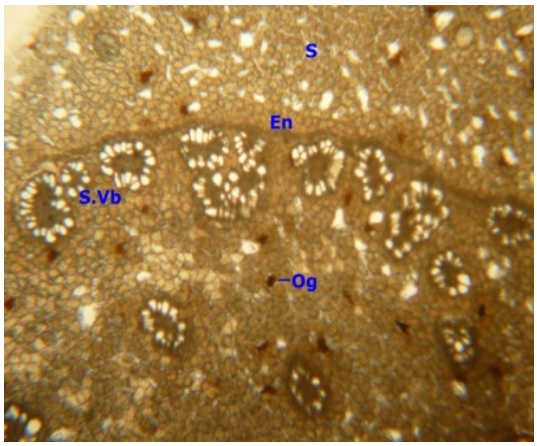

Figure 4: Transverse section of Classical *Shodhita Vacha* (SV) (20X)
En. – Endodermis; S. Vb. – Stelar Vascular bundle; Og. – Oil globule; S. – Starch grain

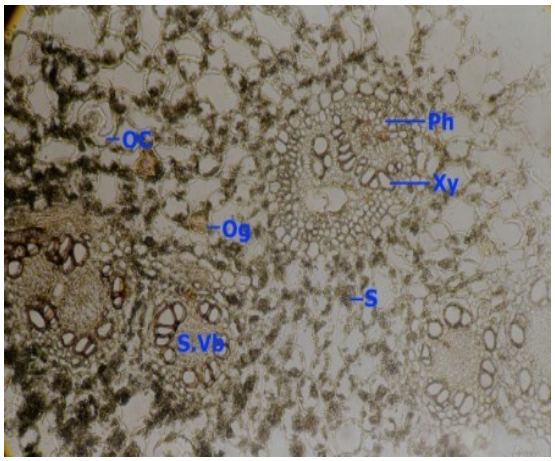

Figure 5: Transverse section of Water processed *Vacha* (WV) (20X) Ph. – Phloem; Xy. – Xylem; OC. – Oil cell; S. – Starch grain; Og. – Oil globule

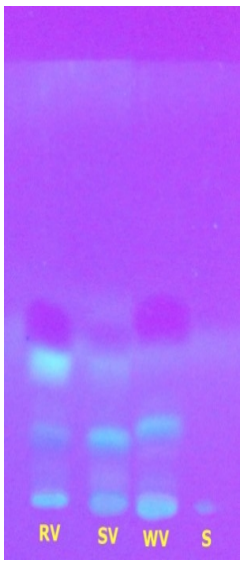

Figure 6: TLC profile of raw and *Shodhita Vacha* samples under 254nm. Track 1 - [RV]: raw *Vacha*; Track 2 - [SV]: Classical *Shodhita Vacha*; Track 3 - [WV]: Water processed *Vacha*; Track 4 - [S]: Standard marker compound β-asarone.

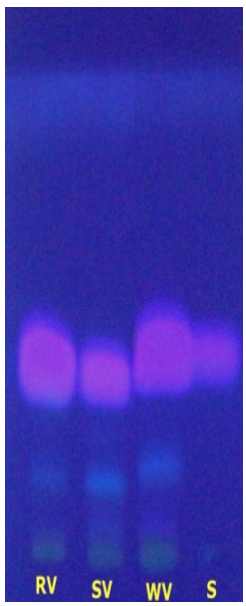

Figure 7: TLC profile of raw and *Shodhita Vacha* samples under 366nm

Figure 8: TLC profile of raw and *Shodhita Vacha* samples after derivatization

RESULTS AND DISCUSSION

Macroscopic characters

The rhizomes of Vacha attain maturity after 12 to 16 months which can be noted in the colour of the rhizome. The immature rhizomes are greenish which upon drying gets pale brown colour and the mature rhizomes attain dark reddish brown colour which deepens upon drying. [Figure 2] In the fresh state it is horizontal, woody, creeping partially underground, varying in length from 25cm to 30 cm and 1.8 to 2.5cm in diameter. It is rarely straight, vertically slightly compressed, much branched with thick long adventitious roots arising from the lower side. The dried rhizome is sub cylindrical, tortuous and 1.2 to 1.8 cm in diameter. It is much branched, brownish in colour and has distinct nodes and internodes. The nodes are broad with dry, fibrous, persistent, triangular, transverse leaf scars often attached to the upper side. The internodes are ridged, 7 to 10mm in diameter. The under surface of the rhizomes are provided with irregularly arranged, slightly elevated round root scars and short fragment of roots. Fracture short, granular and porous, emits strong aromatic odour and has a pungent taste. The fracture surface exhibits cream coloured interior with a central and peripheral region marked by a faint endodermal line. Vacha after classical Shodhana (SV) is harder than its raw counterpart, more tortuous, blackish brown in colour, having both aromatic odour and the smell of Gomutra with pungent and astringent taste. The transverse leaf scars are sparse and blackish in colour. Fracture is short but not granular or porous like raw Vacha. The fracture surface exhibits pale brown coloured interior with a compressed central and peripheral region marked by a faint endodermal line. Rhizomes of sample WV is pale brown in colour, aromatic and other features being very similar to RV.

Microscopic features

Important microscopic characteristics observed in the transverse sections of RV, SV and WV rhizomes [Figure 3, 4 and 5] have been provided in Table 1.

Powder microscopy

The powder of sample RV is pale brownish with spicy aromatic odour and pungent taste. Simple, spherical to ovoid starch granules are seen in abundance. Patches of parenchyma cells, ruptured spheroid oil cells, scattered pale yellow oil globules and oleoresin content are seen. Also lignified, simple and scalariform pitted vessels and fibres of fibrovascular bundles, occasional fragments of the epidermis and cork tissue and occasional hairs of the leaf scars in case of unpeeled rhizome are observed. The powder of sample SV is blackish brown in colour with mixed aroma of Acorus and cow's urine with astringent and pungent taste. Patches of parenchyma cells containing compressed starch granules, scattered oleoresin content, occasional fragments of the epidermis and cork tissue are seen. Spheroidal cells are rarely observed. Interestingly the powder of sample WV was very similar to sample RV and could be hardly distinguished. The observed hard consistency and change in the colour, taste and nature of the rhizome may be due to consecutive boiling in different media for many hours. The change in the consistency of the oil globules and the oleoresin deposit may be due to acquiring of oil content present in the media by the samples.

Phytochemical evaluation

Extractive values of raw and Shodhita Vacha samples have been tabulated in Table 2. Heavy metals like mercury, lead, arsenic, cadmium and pesticides like lindane, aldrin, hexa-chlorobenzene and endosulfan were not detected indicating the safety of the drug. Lowest percentage of loss on drying in SV indicates that after Shodhana the drug loses most of the moisture holding capacity. An increase in the ash value in both SV and WV samples is suggestive of remnant inorganic residue after incineration. There are also indications of presence of acid insoluble particles like silica in sample SV. A sharp decrease in methanol soluble extractive after Shodhana may suggestive of loss of polar constituents into the media during Shodhana. Functional groups like carbohydrates, flavonoids, steroids, glycosides, alkaloids and tannins were present in all the three samples. Saponin and cardiac glycosides absent in raw Vacha were observed in SV suggesting that these principles might have been transferred from the media to the drug during Shodhana [Table 3]. Histochemical tests also showed the presence of starch, tannin, sugar and glycosides [Table 4]. Fluorescence analysis of the sample powders showed the presence of florescence compounds and specific colour variations with various reagents which are tabulated in Tables 5, 6 and 7. In comparison to RV there was considerable variation in colour pattern of SV which may be due to imbibing of media used for processing; however the colour pattern of WV was same as RV.

Thin layer chromatography revealed the Rf value of marker β-asarone to be 0.44 which was observed in all three samples [Table 8] suggesting the qualitative presence of β-asarone even after Shodhana. Under short UV, both SV and WV showed extra spots with different Rf values [Figure 6] while there was reduction in the number of spots detected when observed under long UV [Figure 7]. After derivatization equal number of spots could be observed in all the samples [Figure 8]

CONCLUSION

Shodhana of a dravya using specific media has a definite role in altering the structure as well as composition of the dravya. Gomutradi Shodhita Vacha showed marked changes in comparison to its raw counterpart. Gross changes in organoleptic characters, structural changes in starch grains and change in the nature of oil globules can be implied to the media and the nature of processing involved because Vacha processed in water did not express mark able change either in cellular architecture or composition. The physiochemical standards observed in this article will be helpful in authenticating classical Shodhita Vacha and will also serve as reference material for future studies.

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