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RESEARCH ARTICLE

A PHYTOCHEMICAL STUDY OF TWO FORMULATIONS OF ALOE BARBADENSIS MILLER TO SUPPORT THEIR PHARMACOTHERAPEUTIC DIFFERENCE AS PER CLINICAL STUDY W.S.R. DYSMENORRHOEA

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ABSTRACT

Aloe barbadensis Miller showed significant results in management of dysmenorrhoea in the form of Musabbar i.e. 25.11% more relief than Swarasa, significant at $p<0.05$. ($t = 3.283$). To evaluate the reason of pharmacotherapeutic difference, analytical study was done to analyse phytochemicals in both formulations. Kumari contain properties as: - Guru, Snigdha, Pichhial Guna; Tikta, Madhur Rasa; Sheeta Veerya whereas Musabbar has: - Laghu Ruksha, Teekshna Guna; Katu Rasa; Ushna Veerya. Swarasa was exuded from pulp and sap of leaves. Musabbar was prepared by heating sap at low temperature up to complete evaporation of water. Qualitative tests, T.L.C. and UV spectral analysis were done on different extracts of both formulations to assess phytochemicals. Drug was curacoa aloe, anthraquinones, cardiac glycosides, protein; sapponins were present in both formulations. Phytosterols were present in juice not in Musabbar, Polysaccharides were present only in juice but monosaccharides and disaccharides were present in Musabbar, Flavanoides were present in both extracts of Musabbar, T.L.C. suggested presence of aloin, polyphenols, cholesterols, proteins and glycosides in Musabbar. Spectrum of acetyl groups and Phenolic compound were observed in UV spectral analysis of both formulations. Musabbar contains more anthraquinones having similar chemical structure to prostaglandin substrates to act as false substrate thus blocking prostaglandin synthesis, Musabbar contains glycosides, monosaccharide and disaccharides but Kumari Swarasa had only polysaccharides. So better result of Musabbar, may be due to these chemical constituents. Phytochemical study showed that drug prepared was genuine and difference in phytochemicals and Rasapanchaka supports the difference of results in clinical study.

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INTRODUCTION

Acharya Charaka has asserted that each substance on this earth is useful in combating illness when applied with planning and for a specific purpose (Shastri Kashinath and Chaturvedi Gorakhnath, 1989). Acharya Charaka has also said that for the successful management of the disease, it is essential to select proper medicine (Shastri Kashinath and Chaturvedi Gorakhnath, 1989) (Ch. Su. 20/20) and examine it in all respects (Shastri Kashinath and Chaturvedi Gorakhnath, 1989) (Ch. Vi. 8/87). Bhela Samhita (1000B.C.), an Ayurvedic text was first to mention Kumari *(Aloe barbadensis* Miller) for Vatavyadhi, whereas it was first appeared in modern literature at 2200 B.C. in Sumerian clay tablet, as wound healer and laxative. Later on it has been mentioned in almost all the literature of Ayurveda, for various ailments such as kashtartava, Rajorodha (Sen Govind dass 19th edition), Gulma, Plihavridhi, Yakritavridhi (Sharma Priya Vrata *et al*., 2004) and as food ingredient, skin protective in burns, leprosy, dermatitis, ulcers and radiation injuries; anti-inflammatory, immunomodulator & used in tuberculosis (Baldwin Gertrude, 2013) in modern literature. Various phytochemicals have been noted in the plant, such as vitamins, folic acid, choline, amino acids, enzymes, calcium, chromium, selenium, magnesium, manganese, zinc, copper, iron, potassium, phosphorus, sodium, carbohydrates, anthraquinones, fatty acids, salicylic acid, lignin, saponins and therefore Aloe vera is designated as miracle plant (Baldwin Gertrude, 2013).

Kumari contain properties, as mentioned in Ayurvedic texts are:- - Guru (heavy), Snigdha (unctuous), Pichhial (slimy) Guna; Tikta (bitter), Madhur (sweet) Rasa; Katu Vipaka; Sheeta (cold) Veerya; whereas Musabbar contain properties

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as:- Laghu (light) Ruksha (dry), Teekshna (sharp) Guna; Katu (pungent) Rasa; Ushna (hot) Veerya; Katu Vipaka (Sharma and Dravyaguna Vigyana, 2003) Kumari Swarasa and Musabbar both are ingredients of Rajahpravartini Vati, indicated for Kashtartava (dysmenorrhoea) and Rajorodha (amenorrhoea) (Sen Govind dass *et al*., 19th edition), but they have different properties (Guna, Rasa, Virya). Musabbar showed 25.11% more relief than Swarasa, in the comparative clinical study, in management of Kashtartava (dysmenorrhoea) which is significant at $p<0.05$. ($t = 3.283$). To evaluate the reason of pharmacotherapeutic difference, the qualitative phytochemical study was done to analyse phytochemicals in both formulations. The goal of analytical study is drug standardization and identification. The Physico-chemical analysis provides the objective parameters to set the standards for quality of raw drugs as well as finished products.

MATERIALS AND METHODS

Collection of drug

Drug was collected in its mature stage from herbal garden Jogindernagar, after proper identification of species by botanists of herbal garden and P.G. department Dravyaguna of Rajiv Gandhi Ayurvedic College Paprola (H.P.)

Preparation of Drug

Juice (Kumari Swarasa) was exuded from pulp and sap of leaves by using choppers and then grinded it in grinder. Musabbar was prepared by cutting the lower end of leaf blade and then collecting the exuding juice/sap and thus collected juice was then pulverized and heated in low temperature up to complete evaporation of water and then Vati Kalpana was prepared. (Bentley Robert and Trimen Henry, volume-4)

Preparation of extracts (Sahira Banu and Cathrine, 2015)

5 g of the Musabbar and 5ml of the juice was weighed accurately. 100 ml of distilled water was added and kept covered overnight. It was stirred intermittently in the initial period. Next day, it was filtered. 25 ml of the filtrate was accurately measured with a pipette and transferred to the already weighed evaporating dish. The evaporating dish was placed on a water bath for evaporation of the water. After evaporation of the water it was dried in an oven, allowed cooling and weighed immediately. From the weight of the residue obtained, the percentage of water soluble extractive was calculated and expressed as % w/w. Similarly extracts of methanol, petroleum ether were prepared. Tests for Aloe (Kokate *et al*., 46th edition)

Qualitative Phytochemical Tests

Table 1. Tests for carbohydrates

Fehling's test	Equal volume of Fehling's A and Fehling's B reagents are mixed and few drops of sample was added and boiled to observe brick red colour for presence of reducing sugar.
Molisch's test	Test solution was treated with few drops of alcoholic alpha naphthol and 0.2ml of con. Sulphuric acid was added slowly through the sides of the test tube to observe a purple to violet colour ring at the junction
Benedict's test	Test solution was treated with few drops of Benedict's reagent and boiled on water bath to observe reddish brown precipitate for presence of reducing sugars.

Table 2. Test for Alkaloids

Mayer's test	Test solution was treated with 0.5 ml of Mayer's reagent to observe white precipitate.
Dragendorff's test	Test solution was treated with 0.5 ml of Dragendroff's reagent to observe reddish brown precipitate.
Wagner's test	The substance was treated with 0.5 ml Wagner's reagent to observe reddish brown precipitate.
Hager's test	The substance was treated with 0.5 ml Hager's reagent to observe yellow precipitate.

Table 3. Test for Tannins

Gelatin test	Test solution treated with 1 % gelatin solution containing 10% sodium Chloride to observe white precipitate.

Table 4. Test for Phenolic Compounds

Ferric chloride test	Test solution was treated with 2-3 drops of ferric chloride for blue green colour.

Table 5. Test for Flavonoids

Shinoda test	To the test Solution, added few fragments of Magnesium ribbon and added concentrated Hydrochloric acid drop wise, to observe pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.
Alkaline reagent test	To the test solution added few drops of sodium hydroxide solution to observe formation of an intense yellow colour, which turns to Colourless on addition of few drops of dil. acid, indicates presence of Flavonoids.

Table 6. Test for Proteins & Amino Acids

Millons test	Test solution was treated with 2ml of Millons reagent to observe white precipitate, which turns red upon gentle heating.
Ninhydrin test	Amino acids and Proteins when boiled with 0.2% solution of Ninhydrin, Violet colour appears.

Table 7. Test for Saponin Glycosides

Froth Test	1ml solution of drug was placed in water in a semi-micro tube and shaken well and noted for a stable froth.
Foam Test	Placed 2 ml of aqueous extract in test tube was shaken well and noted the foam which should remain as it is when test tube is allowed to stand still.

Table 8. Test for Anthraquinone Glycosides

Borntrager's test	Boiled the test material with 5ml of ferric chloride in a test tube for 5min and then added equal amount of benzene in it and shake well then allowed it to stand still for 5 min followed by separating the benzene layer and adding ammonia solution to it, to observe Rose pink to red colour by the presence of anthraquinones glycosides

Table 9. Test for Cardiac Glycosides

Legal's Test	Added sodium nitropruside in 2 ml of ammonia solution then mixed sodium hydroxide in it, allowed to stand still for few minutes then added 2 ml of sample in it to observe Pink to blood red colour.

Table 10. Test for Sterols &Triterpenoids

Salkowski test	Treated extract in Chloroform with few drops of conc. Sulphuric acid, shaken well and allow standing for some time to observe red colour for presence of Steroids and formation of yellow coloured lower layer which indicates the presence of Triterpenoids.

Tests for Aloe (Kokate *et al*., 46th edition)

Table 11. General Tests

For these tests, 1 g of aloe powder was boiled with 10 ml of water and filtered.

Bromine test	Freshly prepared bromine solution was added to a small quantity of above filtrate to observe pale yellow precipitate of tetrabromaline.
Borax test	Little quantity of above filtrate was treated with borax and shaken well till the borax dissolves then few drops of this solution were added to a test tube nearly filled with water, for appearance of a green fluorescence.

Table 12. Specific Tests

These tests are meant to distinguish the variety of Aloe (Musabbar).

Nitrous acid test	This test is due to isobarbaloin. Crystals of sodium with few drops of acetic acid were added to aqueous solution of aloes to observe sharp pink to carmine colour for Curacao aloe, faint pink colour for Cape aloe, very less change in colour for Socotrine aloes.
Nitric acid test	Test is carried out either by directly applying nitric acid to drug or to its aqueous solution to observe deep brownish- red colour for curacao aloe, brownish colour changing to green for cape aloe, pale brownish- yellow colour for socotrine aloe.
Cupraloin test	To very dilute aqueous solution of aloes, a drop of saturated copper sulphate solution was added, followed by little quantity of sodium chloride and excess of 90% alcohol to observe wine red colour persisting four hours for curacao aloe, faint colouration rapidly changing to yellow for cape aloes no colour for socotrine aloes.

- Thin Layer Chromatography (TLC) (Joseph *et al*., 1974)
- UV Visible Spectral Analysis

OBSERVATIONS AND RESULTS

Qualitative Phyto- Chemical Tests of Kumari Swarasa and Musabbar

Analytical data of phyto-chemical study of aqueous extract and alcohol extract of Kumari Swarasa and Musabbar is tabulated below-

Table 13. Extractive values of Aqueous extract and Alcohol extract of Musabbar and Swarasa

Extractive Values		
1.	Aqueous extract of Musabbar	73.55%
2.	Alcoholic extract of Musabbar	39.36%
3.	Aqueous extract of Kumari Swarasa	66.4%
4.	Alcoholic extract of Kumari Swarasa	30.6%

Table 14. Tests for Carbohydrates

	Tests for carbohydrates	Kumari Swarasa		Musabbar	
		A.E.	W.E.	A.E.	W.E.
1	Molisch's test	-	+	-	+
2	Benedict's test	-	-	+	+
3	Fehling's test	-	-	+	+

Table 15- Tests for Alkaloides

	Tests for Alkaloides	Kumari Swarasa		Musabbar	
		A.E.	W.E.	A.E.	W.E.
1.	Mayer's test	-	-	-	-
2.	Dragendorff's test	-	+	-	+
3.	Wagner's test	-	-	-	-
4.	Hager's test	-	-	-	-

Table 16- Tests for Saponins

	Tests for saponins	Kumari Swarasa	Musabbar
		A.E.	W.E.
1.	Foam test	+	+
2.	Froth test	-	-

Table 17- Tests for Flavanoides

	Tests for Flavanoides	Kumari Swarasa		Musabbar	
		A.E.	W.E.	A.E.	W.E.
1.	Shinoda test	-	-	-	-
2.	Lead acetate test	-	-	+	+
3.	Alkaline reagent test	-	+	-	+

Table 18- Tests for Glycosides

	Tests for Glycosides	Kumari Swarasa		Musabbar	
		A.E.	W.E.	A.E.	W.E.
1	Borntrager's test	+	+	+	+
2	Legal's test	+	-	+	-

Table 19- Tests for Tannins & Phenolic compounds

	Tests for Tannins & phenolic compounds	Kumari Swarasa		Musabbar	
		A.E.	W.E.	A.E.	W.E.
1	Gelatin test	-	-	-	-
2	Ferric chloride test	-	-	-	-

Table 20- Tests for Phytosterols

	Tests for phytosterol	Kumari Swarasa		Musabbar	
		A.E.	W.E.	A.E.	W.E.
1.	Salkowski test	+	-	-	-

Table 21- Tests for Protiens & Aminoacids

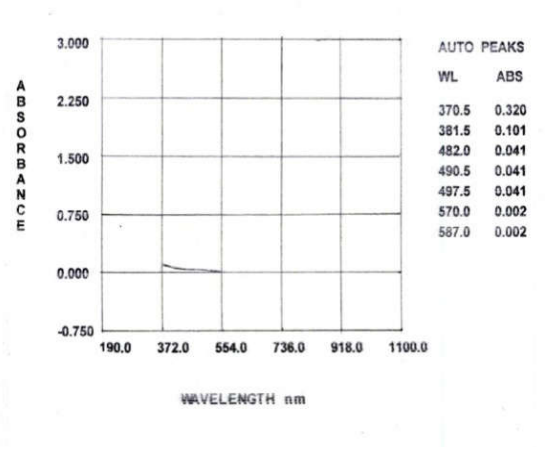
	Tests for Proteins & aminoacids	Kumari Swarasa		Musabbar	
		A.E.	W.E.	A.E.	W.E.
1.	Millon's test	-	-	-	-
2.	Ninhydrin test	+	-	+	+

Table 22- Specific tests for Aloe in Musabbar

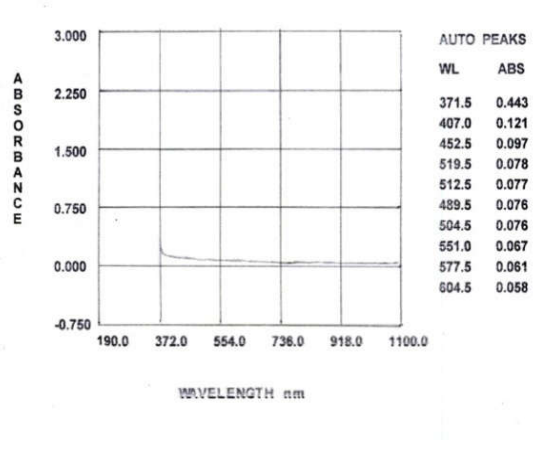
	Specific tests for Aloe in Musabbar	Observations	Results
1.	Nitrous acid test	Sharp pink colour	Curacao aloes
2.	Nitric acid test	Brownish –red colour	Curacao aloes
3.	Cupraloin tests	Wine red colour	Curacao aloes

Table 23- General tests for Aloe

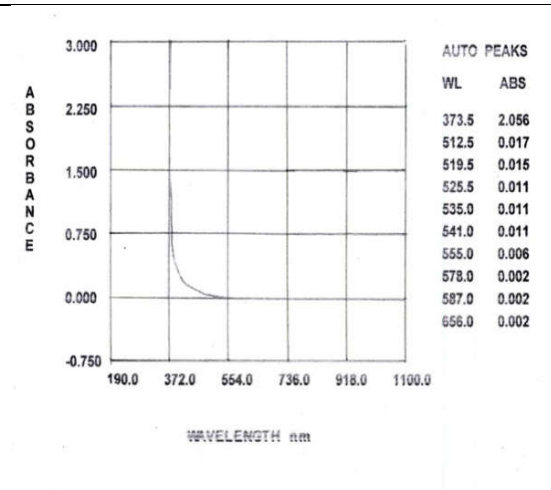
	General tests for aloe	Kumari Swarasa	Musabbar
1.	Bromine test	-	-
2.	Borax test	+	+



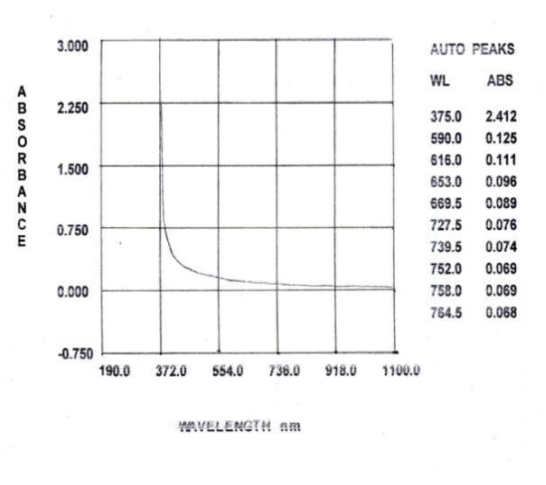
Methnolic extract of Kumari Swarasa



Aqueous extract of Kumari Swarasa



Methanolic extract of Musabbar



Aqueous extract of Musabbar

Table 24. UV Spectral Analysis

Figure 1. T.L.C. Plate in Day light

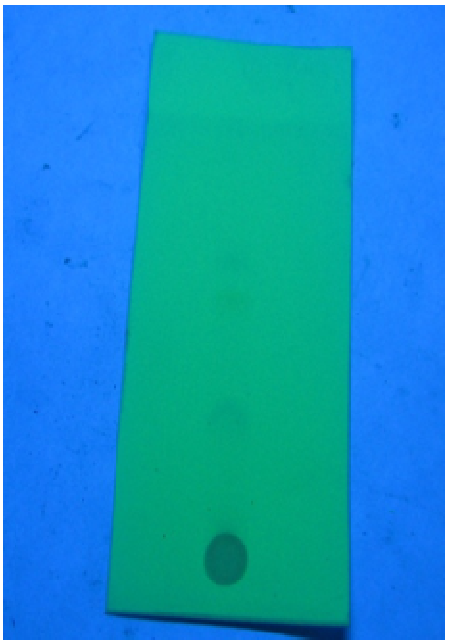


Figure 2. T.L.C. Plate in U.V. Fluorescence analyzer

Thin Layer Chromatography:

1. **Sample** - (A) Alcoholic extract of Musabbar, (B)Alcoholic extract of Kumari Swarasa
2. **Mobile Phase** - Toluene- 10 ml + Formic Acid- 9ml +Carbon tetrachloride- 1 ml

R_f Value under day Light - A= 0.19, B= 0
R_f. Value under U.V. Long Wavelength -A= 0.19, 0.36, B= 0
2. Sample - Alcoholic extract of Musabbar
Mobile Phase - Diethyl ether- 6ml + Toulene- 4 ml
R_f. Value under U.V. Short Wavelength - 0.29, 0.69, 0.75
R_f. Value under Long Wavelength- 0.54- Light pink colour, 0.75- Sky blue colour, 0.89- Red colour
R_f. Value Visualization under Iodine Vapours- 0.58, 0.64, 0.97
3. Sample - Alcoholic extract of Musabbar
Mobile Phase - Ethyl acetate- 77ml + methanol- 33 ml + water- 10ml
R_f. Value under U.V. Short Wavelength - 0.19, 0.38, 0.76, 0.47
R_f. Value under Long Wavelength- 0.54- Light pink colour, 0.47-yellow colour

UV Visible Spectral Analysis:

Methanolic extract of Kumari Swarasa-
Wavelengths- 370.5, 381.5, 482, 490.5, 497.5, 570,587 nm
Absorption peaks- 0.320, 0.101, 0.041, 0.002, 0.002

Aqueous extract of Kumari Swarasa-
Wavelengths- 371.5, 381.5, 482,490.5, 497.5, 570,587 nm
Absorption peaks- 0.443, 0.121, 0.097, 0.078, 0.077, 0.067, 0.061

Methanolic extract of Musabbar-
Wavelengths- 373.5, 512.5, 519.5, 525.5, 535.5, 541, 555, 576, 578, 587, 656nm
Absorption peaks- 2.056, 0.017, 0.015, 0.011, 0.006, 0.002, 0.002

Aqueous extract of Musabbar-
Wavelengths- 375,590.616,653, 669.5, 727.5, 739.5, 752, 758, 764.5 nm
Absorption peaks- 2.412, 0.125, 0,111, 0.096, 0.089, 0.076, 0.074, 0.089, 0.059

DISCUSSION

Specific tests show that Curacao aloe is the variety of Musabbar whose botanical source is *Aloe barbadensis* Miller. Presence of Phytosterols was observed in alcoholic extract of Kumari Swarasa but not in Musabbar this situation is suggestive of the solubility of phytosterols in alcohol but not in water (Jill K Winkler and Kaithleen Warner, 2008). Fehling's test and Benedict's test was negative in Kumari Swarasa and positive in both extracts of Musabbar which are significant for the presence of reducing sugars. This includes all monosaccharide and many disaccharides. Molisch's test is significant for monosaccharides, disaccharides and polysaccharides which was positive in aqueous extracts of both formulations which is suggestive of solubility of polysaccharides in water only and their conversion into monosaccharides and disaccharides by heating (https://en.wikibooks.org). Flavanoides were presents in both extracts of Musabbar and aqueous extract Kumari swarasa, alkaloids may be present in the both formulations in less quantity, Protiens and cardiac glycosides were present in alcoholic extracts of Musabbar and Kumari Swarasa, Saponins were positive in both forms of drug. Anthraquinones gave red colour in both extracts of Musabbar but pink colour in Swarasa that may be due to less quantity of anthraquinones in swarasa as it is mainly prepared of pulp but Musabbar is prepared of sap. Phenolic compounds and Tannins were absent in both forms of drug. All the three T.L.C. showed that Musabbar contains aloin (R_f=0.47) (Agaraju *et al*., 2011), polyphenols (R_f=0.54)[17], cholesterols (R_f =0.29) (http://www.pubhort.org), proteins (R_f =0.58)[17] and glycosides (R_f.=0.19 &0.36) (https://books.google.co.in). Spectrum of acetyl groups which generate a characteristic signal of absorption peak (2.00–2.415) that can be considered as the fingerprint of aloe vera (Diehl and Teichmuller, 1998) which is observed in spectrometry of Kumari Swarasa and Musabbar. Phenolic compound ranges from 350-375nm (Christiane *et al*., 2005), which is present in the graphs in highest concentration. Hence the drug was genuine and products of same species.

In cardinal symptoms like Intensity of pain, Musabbar showed 47.73% more relief than Kumari swarasa, which was statistically significant difference between the two groups at $p<0.05$ (t=2.840) and in duration of pain, Musabbar showed 38.8% more relief than group I, which was statistically significant difference between the two groups at $p<0.05$ (t=2.338). In the intergroup comparison over total criteria, Musabbar showed 25.11% more relief than Kumari swarasa which is significant at $p<0.05$. (t =3.283). On the basis of T.L.C. Musabbar contains glycosides which may not be present or present in less quantity in Kumari Swarasa, similarly phytochemical tests showed that Musabbar contains monosaccharide and disaccharides but Kumari Swarasa had only polysaccharides. So better result of Musabbar may be due to these chemical constituent Musabbar contains anthraquinone and related compounds, such as barbaloin and aloe-emodin, in sufficient quantities to act as false substrate inhibitors blocking prostaglandin synthesis, since they have a similar chemical structure to prostaglandin substrates. (Panda, 2003) Many research fellows have attributed pain-relieving properties to Aloe vera and found salicylate, lactate and magnesium in Musabbar and suggested that the anaesthetic property could either be due to an aspirin-like effect or due to the high magnesium ion content, or possibly both acting synergistically. They further postulated that anthraquinone-type compounds such as emodin and barbaloin could be broken down by the kolbe reaction to salicylates.

Conclusion

Analytical study shows that drug prepared was genuine, prepared from *Aloe barbadensis* Miller according to A.P.I. standards and contained aloin, polyphenols, cholesterols, proteins and glycosides, saponins, acetyl acemmann and carbohydrates. Musabbar contained more anthraquinone as prepared from sap of leaves and also contained reducing sugars which were not present in Swarasa and difference in phytochemicals and Rasapanchaka supports the pharmacotherapeutic difference of clinical study.

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