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Role of thin-layer chromatography in ascertaining *Kashaya Rasa* (astringent taste) in medicinal plants on the concept of *Samana* and *Vichitra Pratyayarabdha* principles of Ayurveda

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Abstract

Background: Pharmacodynamics, in Ayurveda has been described in terms of *Rasadipanchaka*. *Rasa*, on one side indicates the *Bhautika* composition of the drug and on the other side predicts the action. Different analytical techniques, pharmaceutical processes are being used in Ayurveda for the purpose of standardization of raw drugs. **Aim:** In this study an attempt has been made to apply chromatographic technique in determination of *Kashaya* (astringent) *Rasa* (taste). **Materials and Methods:** Two important *Kashaya* dominant drugs *Kulattha* (*Dolichos biflorus* Linn.) and *Kanchanara* (*Bauhinia variegata* Linn.), falling under *Vichitra* and *Samana Pratyayarabdha* category respectively, were subjected to physicochemical parameters and qualitative tests followed by High-Performance Thin-Layer Chromatography (HPTLC). In light of chromatographic fingerprinting; sample preparation protocol is modified to incorporate taste threshold in correlation. Column chromatography is used for first-level discrimination technique followed by HPTLC. *Kashaya Rasa* Dominant Zone (KsRDZ) was separated and subjected to TLC fingerprinting. The KsRDZ fraction was designated as Botanical Reference Material (BRM) in further analysis. **Results:** Ash value, Alcohol and water soluble extract value were more in *B variegata* as compared to *D biflorus*. Presence of tannin in both the samples was confirmed through qualitative test. The KsRDZ fraction separated at Rf 0.46 and 0.48 for *Kulattha* and *Kanchanara* respectively. **Conclusion:** The results showed that the planner chromatography technique seems very useful when BRM hypothesis was adjunct to method that explains the categorization according to traditional *Rasa* domain classification method.

Key words: Column chromatography, high-performance thin-layer chromatography, *Rasa*, spectral comparison, taste threshold

Introduction

For the utilization of *Dravyas*; proper identification, collection, storage, processing, knowledge of their properties and actions are required, which come under the wide umbrella of *Dravyaguna*. In relation to pharmacodynamics; the properties of drugs have been described in terms of *Rasadipanchaka (Rasa, Guna, Virya, Vipaka* and *Prabhava)* in Ayurveda.[1]

Rasa (taste) is the most important *Gunas* (properties) among different *Gunas* of a *Dravya*,[2] which on one side indicates *Bhautika*[3] composition (proto elements) of the drug and on the other side predicts the action on *Dosha*, *Dhatu* and *Mala*.

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Ayurveda classifies *Rasas* into six types, namely *Madhura* (sweet), *Amla* (sour), *Lavana* (salty), *Tikta* (bitter), *Katu* (pungent), and *Kashaya* (astringent).[4] The taste perception and taste sensibility are complex bio-physical and psychological events, and translation of *Rasa* cannot be exactly evaluated without the help of tongue. In Ayurveda, drugs are used as a whole than selected extractive component form. Hence, there is no contextual relationship in between Selective Reactive Moiety (SRM) and Ayurvedic *Rasadipanchaka*. There is a gap in prevailing pharmacopoeial system between the parameter and contextual correlation with *Rasa* for quality control of raw herbal drugs. Considering above all matter, here an effort has been done to develop Botanical Reference Material (BRM) for *Kashaya Rasa* drugs with *Samana Pratyayarabdha* (proto-elemental composition of *Virya-Vipaka* according to the composition of *Rasa*) and *Vichitra Pratyayarabdha* (peculiar proto-elemental composition of *Virya-Vipaka* not according to the composition of *Rasa*) principle using chromatographic technique in which some

modifications have been done at the level of sample preparation. Specific chemical groups are responsible for specific taste and action viz., sweetness is often connected to aldehydes and ketones, cations (such as Na^+, K^+ or Li^+) produce saltiness; the amino acid and glutamic acid are responsible for umami taste,[5] wide range of different phenolic and nonphenolic compounds are responsible for astringency,[6] which is known by its astringent action in modern pharmacology.[7] Taking this into consideration, separation method of chromatography was performed on two *Kashaya Rasa* dominant drugs to develop a moral methodology for identification of *Rasa* of *Dravya*, especially for astringent taste (*Kashaya Rasa*).

Materials and Methods

Collection of drugs

Stem bark of *Kanchanara* (*Bauhinia variegata* Linn.) was collected from matured plant in the month of March 2011 from the periphery of study center and seeds of *Kulattha* (*Dolochos biflorous* Linn.) were purchased from the local market in the month of February 2011. Authenticity of these samples was done in the Pharmacognosy laboratory and confirmed by comparing their characters with various available floras.

Sample preparation stage was followed by sensory confirmation of astringent taste by volunteers and chromatographic separation. This produced conceptual line in the chromatographic fingerprinting rather than generating the fingerprinting profile using extracts, like alcohol etc., selected drugs in the present study were categorized in the same group (i.e. *Kashaya Rasa*) with differences in constitution of *Mahabhuta* (i.e. *Samana* and *Vichitra Pratyayarabdhatva*) hence, similarity–dissimilarity index among studied drugs plays an important role. Linear model is well-known in photometric analysis which is based on SRM as standard and prediction is based on linear equation "y = mx + C" is a good curve fitting in a linear model.

Physico-chemical analysis

Total Ash value,[8] loss on drying,[9] acid insoluble ash,[10] water soluble extractive,[11] alcohol soluble extractive[12] were performed following standard procedures.

High-performance thin-layer chromatography study

Chromatographic conditions

- Application mode: Camag Linomate V
- Development chamber: Camag Twin trough Chamber
- Plates: Precoated Silica Gel GF254 plates
- Chamber Saturation: 30 min
- Development time: 30 min
- Development distance: 7 cm
- Scanner: Camag Scanner III
- Detection: Deuterium Lamp and Mercury Lamp
- Photo documentation: Camag Reprostar
- Data system: Win cats software
- Drying device: Oven
- Solvent system: Benzene.

Visualization

- Short UV under 254 nm
- Long UV under 366 nm
- Stationary phase: Silica gel GF 254
- Mobile phase: Benzene (100%)

Spray reagent: Liebermann-Buchard (Acetic anhydride-sulphufuric acid spray reagent).

Sample preparation

Methanolic extract of both the drugs was prepared by 30 min sonication followed by centrifugation. Benzene was used as mobile phase for fine-tune separation according to eluotropic series and chromatography was developed on the basis of *Rasa* dominancy in drugs. The migration distance of some groups was illustrated. Ketones and aldehydes approximately in the middle, alcohols behind them, and the acids still at the starting point. The sequence of separation thus, follows the polarity of the compounds, that is, in the following sequence.[13,14]

CH_3< -O Alkyl < > C=O < -NH_2< -OH < -COOH

Identification of the *Kashaya Rasa* fraction with gustatory evaluation

Aldehyde and ketone group is separated on upper and middle zone of eluotropic series in High-Performance Thin-Layer Chromatography (HPTLC) study. Separated zone was collected and taken for sensory evaluation of the taste. The upper and middle zone of collected fractions was determined by sensory taste determination method for confirmation of *Rasa*. It was attributed with *Kashaya Rasa* which was considered as BRM. Since the procured samples were less in amount, only five volunteers were taken for sensory evaluation of *Kashaya Rasa* domain.

Column chromatography

On the basis of planar chromatography results; column chromatography was used to isolate the selected component on the basis of *Rasa* dominance drugs as concept of Ayurvedic hypothesis. Then the isolated fractions (*Kashaya* dominant zone) were visualized further with the chromatographic separation. Hence, the optimized solvent system was used for the fine-tune separation.[15,16]

Profile of botanical reference material on planar chromatography

Kashaya Rasa Dominant Zone (KsRDZ) was subjected to optimization procedures for maximum separation of component using the simplex algorithm with respect to mobile phase selectivity.[17] On the basis of this planar chromatographic technique; *Kashaya Rasa* fraction was separated from the column. The separated fractions were further analyzed by HPTLC technique [Figures 1 and 2] to show the separation of component of interest, i.e. *Kashaya Rasa* dominant character. The separated component was further examined by developing an optimized solvent system [Figures 3 and 4].

Observations and Results

Physicochemical tests

Ash value, alcohol and water soluble extract were more in *B. variegata* as compared to *D. biflorus* [Table 1].

Qualitative tests

The presence of astringent principle (tannin) in both the studied sample was confirmed through positive qualitative test. Starch and Amino acid were present in *D biflorus* only [Table 2].

Figure 1: High-performance thin-layer chromatography of raw drugs for *Kashaya Rasa* Dominant Zone selection (at 254 nm UV radiation), T1 - Spot of *Kulattha*, T2 - Spot of *Kanchanara* solvent system-benzene (100%)

Figure 2: High-performance thin-layer chromatography of raw drugs for *Kashaya Rasa* Dominant Zone selection (after spray with vanillin H2SO4), T1 - Spot of *Kulattha*, T2 - Spot of *Kanchanara* solvent system-benzene (100%)

Figure 3: High-performance thin-layer chromatography of optimized *Kashaya Rasa* Dominant Zone selection (at 366 nm), T1 – Spot of *Kulattha*, T2 – Spot of *Kanchanara*, Solvent system – Chloroform:MeOH (9.5:0.5)

Figure 4: High-performance thin-layer chromatography of optimized *Kashaya Rasa* Dominant Zone selection (after spray with vanillin H2SO4), T1 – Spot of *Kulattha*, T2 – Spot of *Kanchanara* solvent system–chloroform:MeOH (9.5:0.5)

Table 1: Physicochemical analysis of the powder seed of *D. biflorus* and bark of *B. variegata*

Parameters	*Kulattha* (*D. biflorus*)	*Kanchanara* (*B. variegata*)
Loss on drying	9.27	8.81
Ash value (%w/w)	4.89	8.67
Acid insoluble ash (%w/w)	0.96	0.2
Water soluble extract (%w/w)	1.94	3.25
Alcohol soluble extract (%w/w)	1.31	3.18

HPTLC Study

Figure 1 shows the separation of *Kulattha* and *Kanchanara*. *Rf* values 0.46 and 0.48 was consider as *Kashaya* (astringent) dominant zone according to eluotropic series. To confirm the dominancy of *Kashaya Rasa* of separated zones, taste determination was done with the help of volunteers.

Taste determination

All the volunteers (five) opined the presence of *Kashaya Rasa* domain in *Kanchanara*, where as in *Kulattha* only three volunteers perceived mild *Kashaya Rasa* along with *Tikta Rasa*.

The selected zone of astringent principles was again subjected to HPTLC and visualized in UV as short UV (254 nm) and long UV (366 nm) as well as the spray detection using densitometric analysis on Camag Scanner III and spectral comparison of spots generated *in situ* comparison.

Figure 5 and 6 shows the densitogram of HPTLC study. The results obtained after HPTLC study as described in densitogram are shown in Table 3. *In situ*, UV spectral comparison of selected spots showed in Figures 7-9, which shows similar spots using spectral comparison of optimized HPTLC separation on both the tracks of *Kulattha* and *Kanchanara*. Three spots that is 0.04, 0.18 and 0.46 were similar out of total seven spots.

Discussion

Both the drugs *Kulattha* (*D. biflorus*) and *Kanchanara* (*B. variegata*) qualified according to Ayurvedic pharmacopoeia

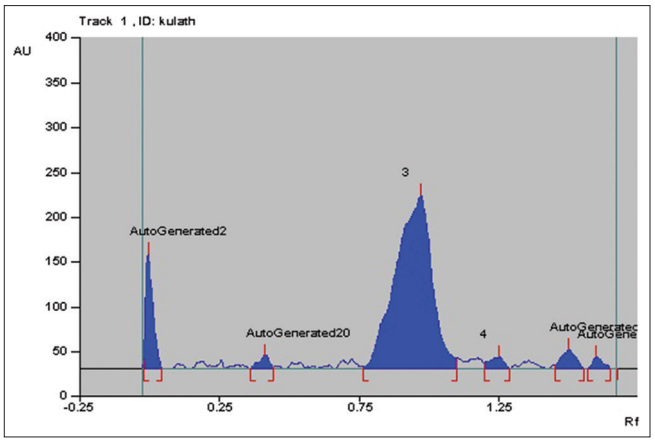

Figure 5: Densitogram of *Kulattha* at 366 nm

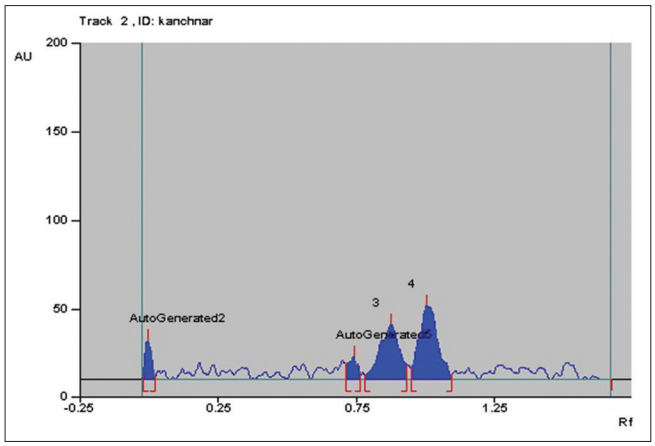

Figure 6: Densitogram of *Kanchanara* at 366 nm

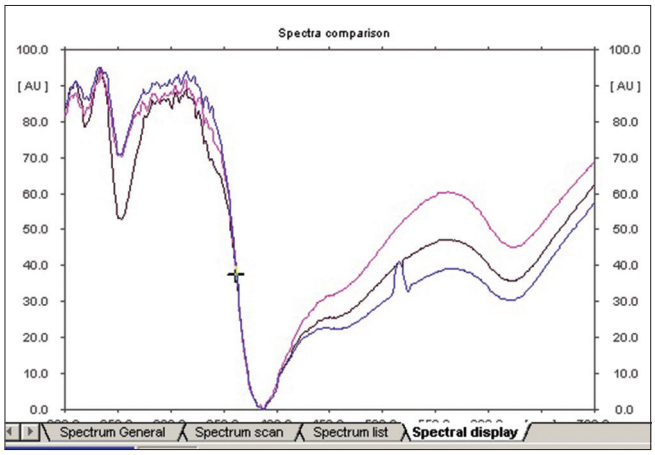

Figure 7: Spectral comparison at *Rf* 0.04

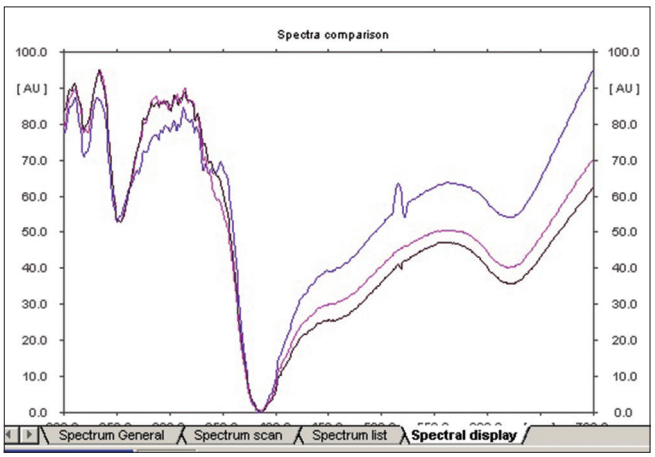

Figure 8: Spectral comparison at *Rf* 0.18

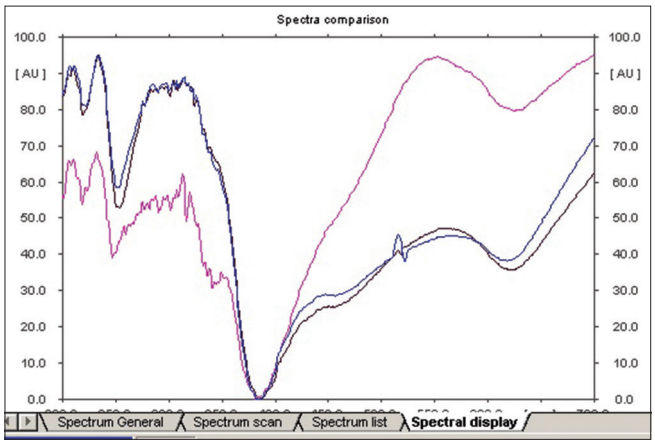

Figure 9: Spectral comparison at *Rf* 0.46

Table 2: The result of qualitative tests of both drugs

Active constituents	*Kulattha* (*D. biflorus*)	*Kanchanara* (*B. veriagata*)
Tannin	+	+
Flavonoids	–	–
Starch	+	–
Alkaloid	–	+
Amino acid	+	–
Cyanogenetic glycoside	–	+
Saponin	–	+
Carbohydrate	–	+
Phenolic compound	–	+

on the basis of suggested physicochemical taste. Qualitative testing of *Kulattha* and *Kanchanara* responds positive in the test for the presence of tannin. *Kashaya Rasa* is known for its astringent action and tannin is responsible for the astringency.[6] Out of six *Rasas*, *Kashaya Rasa* dominant drugs were selected for this study. The fraction of *Kashaya Rasa* was found out with the help of a solvent having dielectric constant, that is, benzene. Benzene falls in medium polar solvent 2.284 at 20°C having interfacial tension with water around 35.0 dynes/cm (20°C), was selected to separate KsRDZ from both the drugs. The whole course to separate out target zone is included in BRM generation from both the drug. In modern science, SRM is the basic logic to generate regression module in quantitative analysis. Mostly this module deals with univariant type of analysis. Hence, chromatographic profiling was generated using KsRDZ. This gives good differentiation and similarities among *Samana* and *Vichitra Vratyayarabdha* drugs.

Table 3: The *Rf* value comparison

Sample	Solvent system		Visualization of the derivatization with vanillin sulfuric acid at the 400 nm invisible view
Kulattha (D. biflorus)	Chloroform: MeOH (9.5:0.5)	7	0.04, 0.09, 0.14, 0.18, 0.32, 0.46, 0.55
Kanchanara (B. veriagata)	Chloroform: MeOH (9.5:0.5)	7	0.04, 0.12, 0.18, 0.34, 0.46,0.48, 0.58

Conclusion

According to the traditional classification; *Rasa* is the basic discriminator. In this study, *Kashaya Rasa* dominant zone may play a role of botanical reference material. The results showed that the planner chromatography technique seems very useful when botanical reference material hypothesis was adjunct to method that explains the categorization according to traditional *Rasa* domain classification method. The method may be considered as a major step in profiling traditional classification method of drugs with concept of *Rasa* dominancy.

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हिन्दीसारांश

समान और विचित्र प्रत्यारब्ध औषधीय वनस्पतियों के रस निर्धारण के लिए थिन लेयर क्रोमटोग्राफिक तकनीक का प्रयोग

रसिका एच. कोल्हे, रबिनारायण आचार्य, विनय जे. शुक्ला

द्रव्य की कार्मुकता ज्ञात करने के लिए, द्रव्यगत रस का ज्ञान होना आवश्यक है । पंचमहाभूतों के विविध संगठनो से निर्मित, आयुर्वेद मे छः रसों का वर्णन मिलता है । आधुनिक मतानुसार, द्रव्यगत विशिष्ट रासायनिक संगठन, रस के ज्ञान के लिए कारणभूत होता है । प्रस्तुत शोधपत्र मे कांचनार (समान प्रत्ययारब्ध) और कुलत्थ (विचित्र प्रत्यारब्ध) इन दो कषाय रस प्रधान द्रव्यों पर क्रोमँटोग्राफिक तकनीक का उपयोग कर रस निर्धारण करने का प्रयास किया गया । कोलम क्रोमटोग्राफिक तकनीक का उपयोग करके कषाय रस की निश्चिती की गई और पुनश्च इस अलग किए गए झोन पर थिन लेयर तकनिक का उपयोग किया गया । दोनो द्रव्यों मे कषाय रस होने से एक ही झोन मे अलग होते हुए भी आर.एफ. व्हॅल्यु मे पाई गई विविधता आयुर्वेद के समान और विचित्र प्रत्यारब्ध संकल्पना को पुष्टी देती है ।