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ORIGINAL RESEARCH ARTICLE

Role of Thin Layer Chromatography in Ascertaining *Tikta rasa* (bitter taste) of a Medicinal Plant on the Concept of *Samana and Vichitrapratyayarabdha* Principles of *Ayurveda*

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ABSTRACT

Ayurveda, the ancient medical science, being accepted by large group of society, stands on its own fundamental principles. *Dravyaguna vijnana,* which deals with *Ayurvedic* pharmacology, describes the drug action which is based on *Rasadi Panchaka*. It describes the classification of a drug (*dravya*) based on *rasa* (taste), *karma*(action) and *utpatti*(origin), etc. In modern science, as per Bentham & Hooker, classification of a plant is based on its morphological features. Though both the systems of classification are primarily meant for plant classification, no correlation between traditional theory of *Rasa panchaka* and Bentham & Hooker is reported yet. In present study, two important *Tikta rasa* (bitter taste) dominant drugs Katuki *(*rhizome of *Picrorrhiza kurroa)* and Guduchi *(*stem of *Tinospora cordifolia)* falling under *samana and vichitra praatyayarabdha* category, were subjected to physicochemical parameters and qualitative tests in the context of routine quality control parameters according to API followed by HPTLC study. 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 TLC. *Tikta Rasa* Dominant Zone (TRDZ) was separated and subjected to TLC fingerprinting. The TRDZ fraction was designated as Botanical Reference Material (BRM) in further analysis. The results show that the planner chromatography technique seems very important and useful when BRM hypothesis was adjunct to method that explains the categorization according to traditional classification method i.e. *Rasa* domain classification method.

Keywords: Column Chromatography, HPTLC,Rasa, Spectral comparison, Taste threshold.

INTRODUCTION

Ayurveda, the science of life, stands on its own fundamental principles. *Ayurvedic* pharmacology known as *Dravyaguna vijnana* is based on biophysical, experiential, inferential and intuitional mechanisms [1].According to it an action of a substance is based on five humors namely, 1) *Rasa* (Taste appreciation of the substance by the chemical receptors on the tongue) 2) *Guna* (Property) 3) *Veerya* (Potency) 4) *Vipaka* (Transformation) 5) *Prabhava* (Impact) [2]. Among these *Rasa* is an important quality manifested at the level of tongue which can be directly perceived but it is not only the perception of Taste but also indicator of the composition, properties and probable action of the *dravya* [3]. *Rasa* or taste which is cognized first (by impact with the tongue) is considered as prime *Rasa* (Primary taste) and the *Rasas* which are less apparent or cognizable are called *Anurasa* (Secondary Taste) [4].

Ayurveda classifies *Rasas* into 6 types, namely *Madhura*(sweet), *Amla*(sour), *Lavana*(salty), *Tikta*(bitter), *Katu*(pungent) and *Kashaya*(astringent) [5]. 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 [6].

A single drug may contain many a *Rasa* but each *Rasa* according to its concentration dominates to other present *Rasas*. Considering this, intensity of Bitterness is dissimilar in each bitter drug. A drug, prominent in one *Rasa*, is supposed to exhibit *Guna, Veerya, Vipaka* and action according to

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particular predominant *rasa*. So the drugs which exhibit structure related pharmacological activities and therapeutic effects is called as *Samana Pratyayarabdha* (Regular cause effect) *dravyas* and the drugs which do not have structural similarity among the constituents and therapeutically act differ is called *Vichitra Pratyayarabdha* (Anamalous cause effect) *dravyas*[7]. One may be cited as an example of *Samana Pratyayarabdha* where Katuki *(Picrorhiza kurroa* Royle ex Benth.*)* having *Tikta rasa, laghu ruksha guna, sheeta veerya* and *madhura vipaka* and Guduchi *(Tinospora cordifolia (*Willd.) Miers ex Hook.f. &Thomas.*)* Which having *Tikta* dominant *rasa, guru snigdha guna, ushna veerya* and *katu vipaka* may be taken as *Vichitra Pratyayarabdha dravya*.

Theoretically therapeutics in *Ayurveda* practice, drug is used as whole or compound form rather selected component extraction in pure form hence, no contextual relationship between Selective Reactive Moiety (SRM) and *Ayurvedic Rasapanchaka* index of drug given by *Acharyas.* There is a gap in prevailing pharmacopoeial system between the parameter and contextual correlation with *Rasa* for quality control of raw herbal drug. Modern analytical technique like i.e. planner chromatography should be developed with modification in sample preparation stage which is connotative to the *Rasa* of drug which is generally used as a base for classification of drugs in *Ayurveda*. Considering above all matter here an effort has been made to develop Botanical Reference Material (BRM) for *Tikta Rasa* drugs with *samanapratyayarabdha* and *vichitrapratyayarabdha* principle by using chromatographic technique in which some modification has been done at level of sample preparation stage.

In sample preparation stage prior to Chromatographic separation followed by Sensory confirmation produce conceptual line in the chromatographic fingerprinting rather generating fingerprinting profile using extracts e.g. alcohol extract. Selected drugs in present study categorized in same group with different humor hence similarity-dissimilarity index among studied drugs play important role. Liniar model is well known in photometric analysis is based on Selective Reactive Moiety (SRM) as standard and prediction is based on linear equation y= mx+C is good curve fitting in linear model.

MATERIAL AND METHODS

Collection of Drugs:

After proper identification fresh stem of Guduchi- *Tinospora cordifolia* (Willd.) Miers. were collected from IPGT& RA campus, Jamnagar and root rhizome of Katuki- *Picrorhiza kurroa* Royle ex. Benth. were purchased from Pathankot, Punjab.

Physico-chemical analysis:

Total Ash value [8], Loss on drying [9], Acid insoluble ash [10], Water soluble extractive [11], Alcohol soluble extractive [12]

HPTLC study [13]:

Chromatographic conditions:

Application mode	: Camag Linomat 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, Tungstun lamp
Data System	: Win cats software.

Sample preparation:

Sample prepared by 30 min sonication of drugs with alcoholic medium and filtration was used for experimental task. Selection of the mobile phase may be applied to fine-tune separation according to “elutropic series”. ‘Benzene’ was selected as the mobile phase and chromatography was developed on the basis of *Rasa* dominancy in drugs which was determined by threshold test, the migration distance of some groups were illustrated by E. Stahl. 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.

If functional groups are introduced into a hydrocarbon, the adsorption affinity is increased in the following sequence [14].

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

So elutes were taken and collected after column chromatography and then dried. The dried fractions were desorbed in water for sensory evaluation of the taste. The fraction having *Tikta rasa* conformation through taste threshold method was designed as BRM and the chromatographic pattern was developed.

Column Chromatography [15]**:**

On the basis of planner chromatography results, Column chromatography was used to isolate the selected component on the base of *rasa* dominance drugs as concept of *Ayurvedic* hypothesis. Then the isolated fractions were visualized further with the chromatographic separation. So we used the optimized solvent system for the fine-tune separation [16].

Profile of BRM on planar Chromatography:

TRDZ was subjected to optimization procedures for maximum separation of component using simplex algorithum [17] with respect to mobile phase selectivity. On the basis of this planner chromatographic technique, *Tikta rasa* fraction was separated from column. The separated fractions were further analysed by HPTLC technique. (**Fig 1**) shows the separation of component of interest i.e. *Tikta rasa* dominant character. The separated component was further examined by developing an optimised solvent system. The same has been shown in (**Fig 2**).

Taste determination:

The finally purified fractions were marked as BRM and are subjected for taste determination. Since the procured samples were less in amount, only 5 volunteers were taken for sensory evaluation of *Tikta rasa* domain.

RESULTS

Physicochemical tests

Quality of the drugs were confirmed using physicochemical parameters (**Table 1**)

Table 1: Physicochemical tests

S. No	Physicochemical Parameters	Guduchi *(Tinospora cordifolia)*	Katuki *(Picrorrhiza kurroa)*
1	Loss on drying	9.65% w/w	5.20% w/w
2	Ash value	5.44% w/w	4.90% w/w
3	Acid insoluble ash	0.15% w/w	0. 44% w/w
4	Water soluble extractive	12.54% w/w	7.92% w/w
5	Alcohol soluble extractive	13.32% w/w	11.8% w/w

Qualitative tests:

Presence of bitter principle is confirmed through positive qualitative test. These results are shown in (**Table 2**)

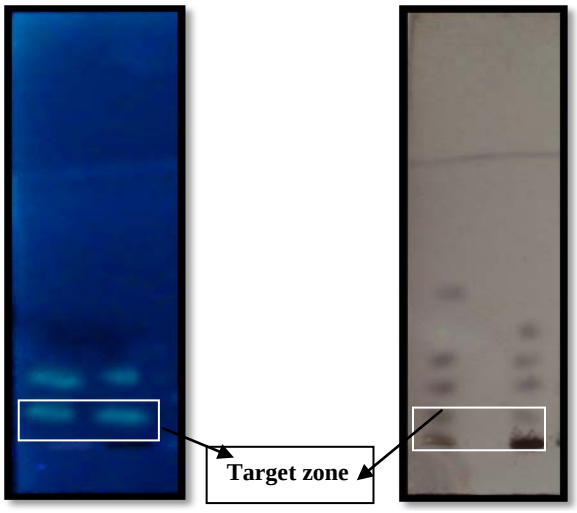
Table 2: Qualitative tests of tested drugs

S. No	Qualitative tests	Guduchi *(Tinospora cordifolia)*	Katuki *(Picrorrhiza kurroa)*
1	Alkaloids	+	+
2	Steroids	+	+
3	Amino acids	-	+
4	Carbohydrate	+	-
5	Glycosides (Bitter)	+	+
6	Tannins	+	+
7	Flavanoids	-	-

HPTLC Study:

Fig 1:HPTLC of raw drugs for TRDZ selection

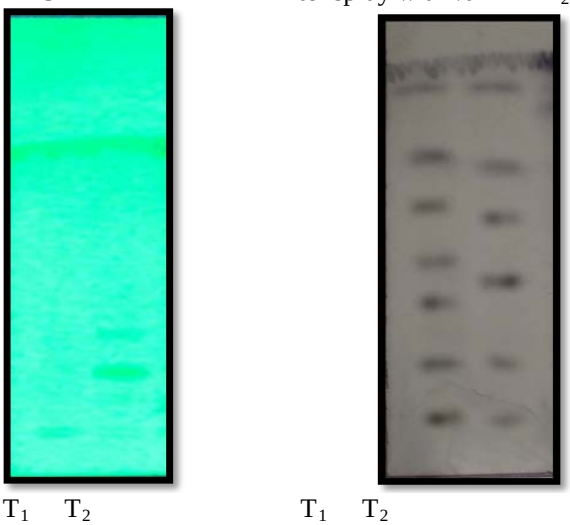
At 366 nm UV radiation After spray with vanillin H_2SO_4



T1: Spot of Guduchi; T2: Spot of Katuki
Solvent System- Benzene (100 %)
Figure 1 shows Rf value of TRDZ at 0.3

Fig 2: HPTLC of optimize TRDZ

254 nm After spray with vanillin H_2SO_4



T1 – Spot of Guduchi : T2 – Spot of Katuki
Solvent system – Chloroform: MeOH (9.5: 0.5)

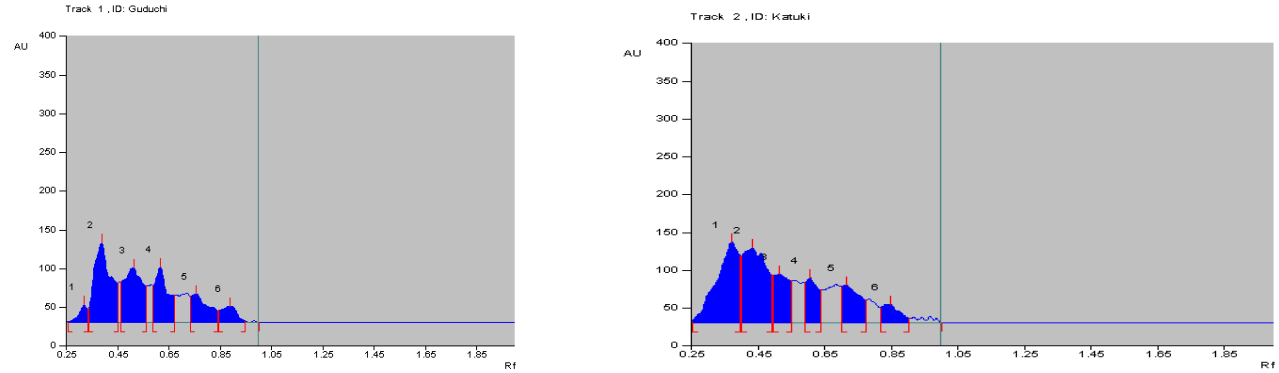
Taste determination:

All the volunteers opined the presence of *Tikta rasa* domain in Katuki where as in Guduchi only

four Volunteers perceived mild *Tikta rasa* along with *Kashaya rasa* & one volunteer perceived *Rasa* as *Madhura.*

The selected zone of bitter principles was again subjected to HPTLC and visualized in U.V. as short U.V. (254nm) and Long U.V. (366 nm) as well as the spray detection using densitometric analysis on CAMAG Scanner 3 and spectral comparison of spots generated in situ comparison.

Fig 3: Densitogram of Guduchi and Katuki at 400 nm



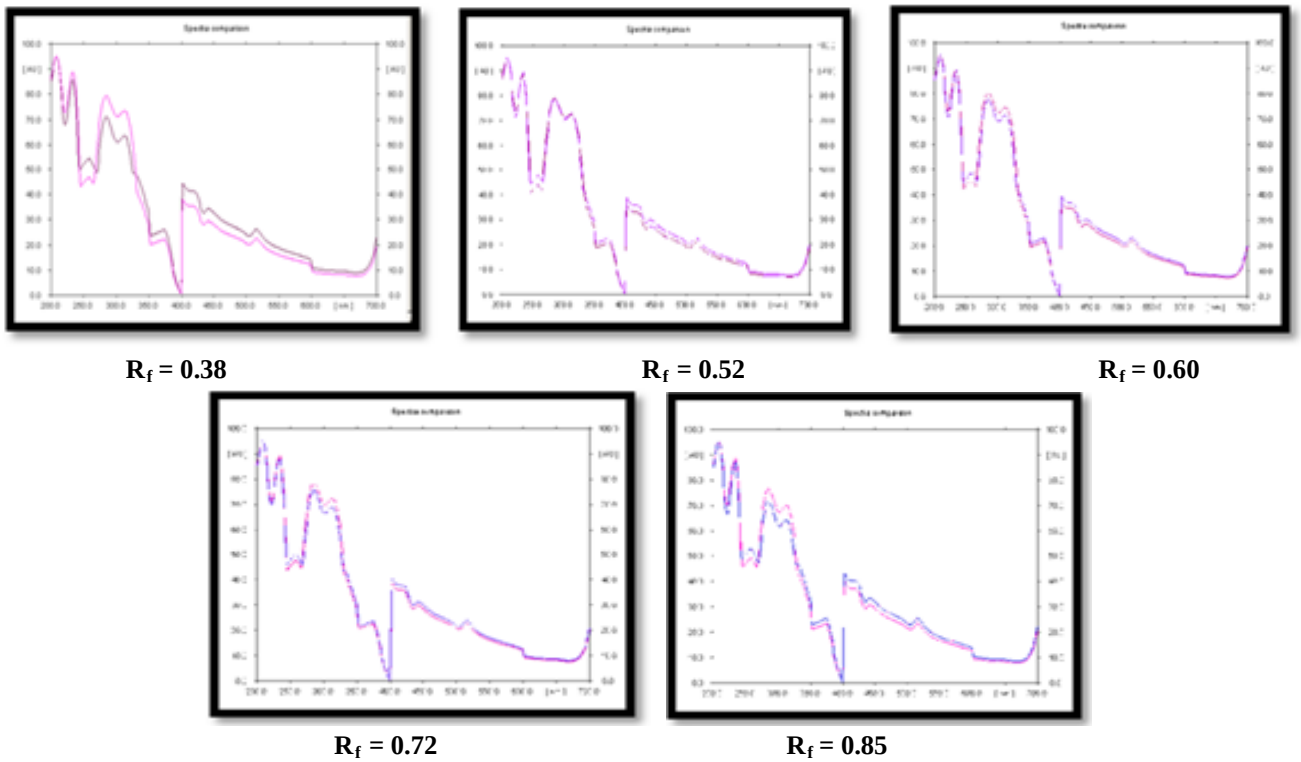
The results shown after HPTLC study as described by densitogram as showed as following (**Table 3**)

Table 3: Densitogram comparison

Sample	Solvent system	Visualization of the derivatization with Vanillin Sulfuric acid at the 400 nm in visible view	
Guduchi	Chloroform:MeOH (9.5:0.5)	6	0.32, 0.39, 0.51, 0.62, 0.76, 0.89
Katuki	Chloroform: MeOH (9.5:0.5)	6	0.37, 0.44, 0.52, 0.62, 0.71, 0.85

In Situ, U.V.Spectral Comparison of selected spots at the following in this figure shows R_f values. Five spots are similar out of the total six spots.

Fig 4: Spectral comparison



R_f = 0.38 **R_f = 0.52** **R_f = 0.60**

R_f = 0.72 **R_f = 0.85**

Fig 4 shows similar spots using spectral comparison of optimized HPTLC separation on both the tracks Guduchi & Katuki.

DISCUSSION

Both the drugs Guduchi (*Tinospora cordifolia* (Willd) Miers*)* and Katuki (*Picrorhiza kurroa* Royle ex. Benth*)* qualified according to *Ayurvedic* pharmacopoeia on the basis of suggested physicochemical taste. On the basis of qualitative testing Guduchi responds positive test for alkaloid and Katuki responds alkaloid and tannin to incorporate the hypothesis of *Ayurveda* i.e. produced the effect on the basis of dominant *Rasa*. Out of six *rasas*, *Tikta rasa* domain drugs are selected for this study. To find out the fraction of

Tikta rasa which select a solvent having dielectric constant i.e. Benzene falls in medium polar solvent 2.284 at 20^0 C having interfacial tension with water around 35.0 dynes / cm. (20^0 C) was selected to separate *Tikta* zone from both the drugs and the whole coarse to separate out target zone is included in BRM generation from both the drug. As in modern science selective reactive moiety was 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 TRDZ this gives good differentia and similarities among *samana* and *vichitra pratyayarabdha* drug.

CONCLUSION

According to the traditional classification *rasa* is the basic discriminator. Here in this study TRDZ may play a role of BRM as they stand similarity among selected zone as both the drugs belongs to same *rasa* also reported its dissimilarities at the level of Rf value due to difference in *degree of bitterness* of Guduchi under the major class of TRDZ. The method contribute major role in profiling traditional classification method of drugs with the concept of *rasa* dominancy.

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