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ANALYTICAL EVALUTION AND DETERMINATION OF *RASA* ON *TEPHROSIA* SPECIES - A SCIENTIFIC STUDY

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ABSTRACT

The plant genus *Tephrosia* (Fabaceae) is widely distributed in most tropical and sub-tropical countries. It is commonly prescribed in liver or spleen diseases. Till date the comparative *Rasanirdharana* and analytical work had not been reported. In the present study comparative study of *Tephrosia purpurea Pers.*, *Tephrosia candida DC.* & *Tephrosia jamnagarensis* Sant. has been under taken. Analytical of all the three sps. Showed Loss on drying, Total Ash, Acid insoluble ash were 7.083%, 7.624% & 7.623%; 6.079%, 6.061%, 6.72%; 0.450%, 0.150%, 0.815% respectively. Qualitative scrutiny demonstrated the presence of flavonoids and tannins. High Performance Thin Layer Chromatography (HPTLC) were carried out after organizing appropriate solvent system. Data of *Rasanirdharana* shows the highest frequency in the characteristics of *Katu rasa*, *Kashaya rasa* and *Tikta rasa* in *Tephrosia purpurea*, *Madhur rasa*, *Amla rasa*, and *Lavana rasa* in *Tephrosia candida* and *Madhur rasa*, *Amla rasa*, *Lavana rasa* in *Tephrosia jamnagarensis* as experienced by the responders.

KEYWORDS: HPTLC, Phytochemical, *Rasanirdharan*, *Tephrosia*.

INTRODUCTION

The vital responsibility played by herbal medicine in serving the therapeutic requirements of major fraction of human populace worldwide is identified since ancient times. But the quality control and standardization facets of these herbal drugs stay as a herculean task even in the

21st century. Accurate identification and guarantee of purity through pharmacognosy and pharmaceutical chemistry measures is inescapable ladder needed for the quality assurance and standardization of any of the herbal medicine whether it is single drug of formulation. *Tephrosia* species (Fabaceae) are well known medicinal plants. All the three plants commonly found in India and other tropical countries. *Tephrosia purpurea* Pers., *Tephrosia candida* DC. and *Tephrosia jamnagarensis* are herbs naturally available as weed of road side and agricultural fields. They are Laxative, Diuretic, Tonic, etc.[1]

Isolated scientific information is available regarding the phytochemical profile of this drug. Moreover, to standardize as per monographs, physic-chemical parameters are to be studied to get gist of the physical and chemical nature of the plant. Till date the comparative analytical and *Rasanirdharan* work had not been reported. Hence in the present research article an effort has been made to overcome this lacuna by studying phytochemical profile and the taste determination of whole plant powder.

In Ayurveda, *Rasapanchak* determines the actions of the drug. Among these *Rasapanchak*, *Rasa* (Taste) is one of the criteria for determining the possible action of plant drug through the basis of taste perception by tongue (sensory organ). Hence it is vital to perform *Rasanirdharan* (Determination of Taste) of the test drug to understand the possible mode of action of the drug.

MATERIALS AND METHODS

Collection of Samples

Whole plant of all the three species of *Tephrosia* are collected by the scholar from out skirts' of Jamnagar during the month of October-November 2015. Then the plant was authentified in the pharmacognosy lab. IPGT & RA, Jamnagar, herbarium submitted in the department (Specimen No.PHM 6161 *T. jamnagarensis* / 6162 *T. purpurea* / 6163 *T.candida* / 2015). Whole plant were first washed with tap water then few quantity were stored in solution of AAF (70% Ethyl alcohol: Glacial acetic acid: Formalin) in the ratio of (90:5:5) to utilize them for microscopic studies whenever needed. The remaining parts were dried under the shade and then were subjected for 60# powdering.

Preparation of powder

The whole plant was shade dried and powdered by mechanical mixer grinder then sieved through 60# sieve and stored in air tight glass container for further *Rasanirdharan* and analytical studies.[2]

Preparation of plant extract

5 g powder of each sample (root and leaf powder) was macerated with 100 ml water in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for eighteen hours. After twenty four hours samples were filtered and water extract was collected. Methanolic and extract was also prepared following same procedure. All two extracts were used for preliminary phytochemical screening.[3]

Analytical evaluation

The colour, odour and taste of whole plant powder were recorded separately through visual and sensory perceptions.[4] Physicochemical parameters.[5] preliminary phytochemical investigations.[6] quantitative estimation of Tannin.[7] and HPTLC.[8] were conducted on sample. All determinations were performed in triplicate and the results are presented as mean value.

Rasanirdharan

The participants who volunteered for the study were explained about the study and their role in the study. Every volunteer was then asked to cleanse their mouth with water, prior to the onset of the experiment. Five minutes after cleansing the mouth, they were given powder of the test drug and asked to record their inputs in the questionnaire.[9]

RESULTS

Physicochemical parameters and qualitative analysis:

Whole plant powder of all the three sps. were subjected to physicochemical parameters like loss on drying, total ash, acid insoluble ash, alcohol soluble extractive value, pH value, etc. Results are depicted in table 2.

TABLE – 2: Results of physicochemical parameters of all three species:

S. No.	Parameters	*T. purpurea* (Mean±SEM)	*T. candida* (Mean±SEM)	*T. jamnagarensis* (Mean±SEM)
1.	Foreign matter	NIL	NIL	NIL
2.	Loss on drying % w/w	7.083± 0.08	7.624± 0.59	7.623± 0.03
3.	Total ash content % w/w	6.079± 0.19	6.061± 0.21	6.724± 0.835
4.	Acid insoluble ash % w/w	0.450± 0.15	0.150± 0.20	0.815± 0.16
5.	Water soluble extractive value % w/w	9.637± 0.58	14.651± 0.59	12.253± 0.75
6.	Alcohol soluble extractive value % w/w	14.581± 1.15	20.477± 0.44	19.463± 1.05
7.	pH (Aqueous 5%)	7	7	7

PCA analysis

Principle Component Analysis (PCA) is a dimensionality-reduction technique that is often used to transform a high-dimensional dataset into a smaller-dimensional subspace prior to running a machine learning algorithm on the data. When applying it on the values of physico-chemical parameters, result in Bi-plot shown in Fig. 1.

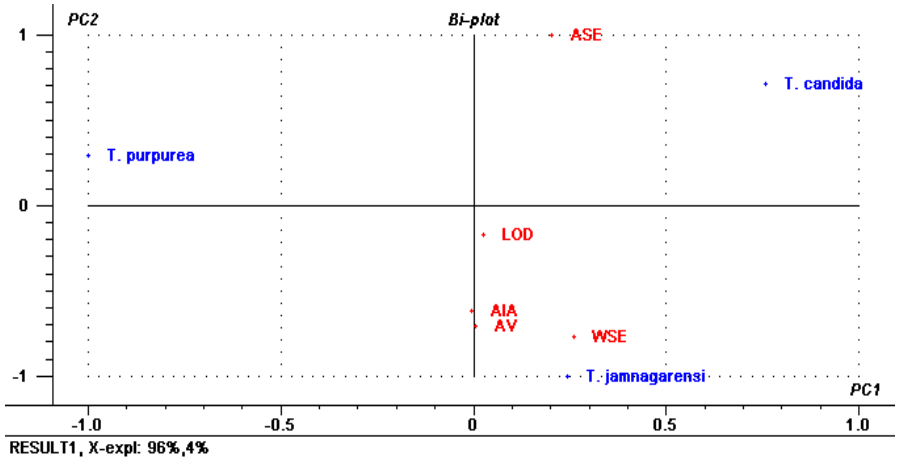

Fig. 1

Preliminary qualitative tests

Whole plant powder of all three sps. were qualitatively tested for the presence of different phytoconstituents. Carbohydrate, Cardiac glycoside, Flavonoid and Tannin are present in both the extracts. The other observed results in methanolic extracts are shown in the table 3.

TABLE – 3: Results of preliminary qualitative tests of all three species

Sr. No.	Phyto-constituents	Tests	*T. purpurea*	*T. candida*	*T. jamnagarensis*
1	Carbohydrates	Molish's	-	-	-
2	Reducing Sugar	Fehling's	+	+	+
3	Proteins	Biuret	-	-	-
4	Tannins	F_ecl_3	+	+	+
5	Steroids	Salkowski	-	-	+
6	Alkaloids	Dragendroff's	-	-	-
7	Flavanoid glycoside	Lead Acetate	+	+	+
8	Saponin glycoside	Foam	+	+	+
9	Cardiac glycoside	Keller-killiani	+	+	+
10	Amino acids	Ninhydrin	-	-	-

'+' Present, '-' Absent

Quantitative Analysis

The sample powders of all three sps. were subjected to quantitative estimation for total tannin. Results are described in table 4.

TABLE – 4: Results of quantitative analysis of all three species

Phyto-constituents	Observation		
Total Tannin Content	***T. purpurea***	***T. Candida***	***T. jamnagarensis***
	2.93 % w/w	2.51 %w/w	2.09 %w/w

High-performance thin layer chromatography

HPTLC study of methanolic extract of all the three sps. were carried out by using solvent system of Toluene : Chloroform : Acetone (4:2.5:3.5) v/v was used. Results are shown in table 5, plates are shown in Fig. 2 & 3, simple and 3D peak display at 254 nm and 366 nm in Fig.4 to Fig.11 and Comparative spectra of all three sps. at R_f 0.84,0.94,0.98 shown in Fig.12 to Fig.14.

Table – 5: HPTLC Studies of alcohol extracts of all three species

Name of Samples	At 254 nm	At 366 nm
T. purpurea	0.05, 0.34, 0.96, 0.98	0.05, 0.83, 0.94
T. candida	0.06, 0.26, 0.83, 0.97	0.06, 0.83, 0.94
T.jamnagarensis	0.06, 0.19, 0.99	0.06, 0.19, 0.85, 0.95

Fig.2 254 nm

Fig.3 366 nm

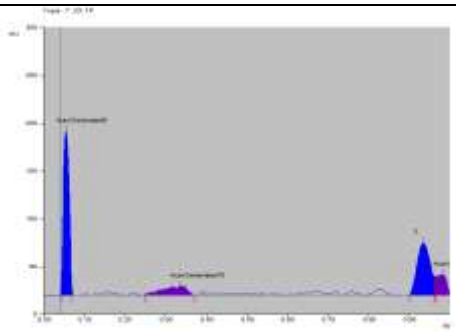

Fig.4

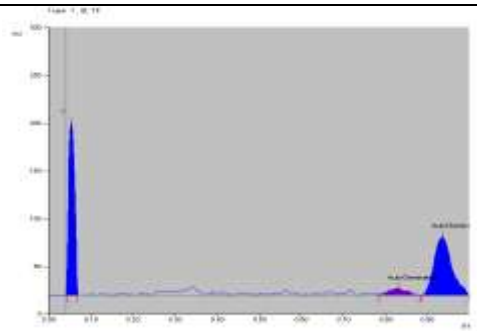

Fig.5

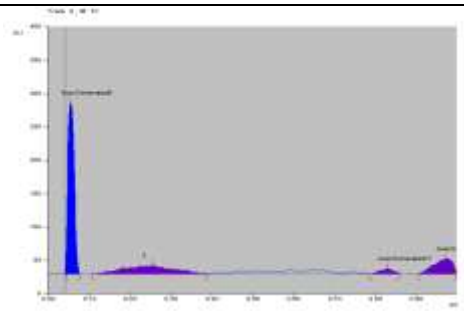

Fig.6

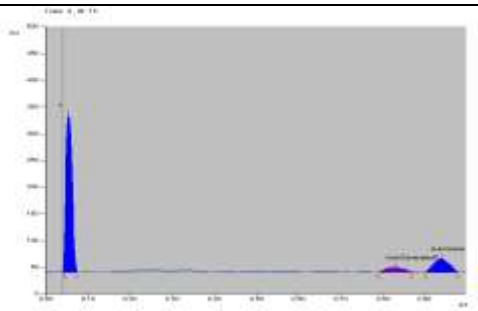

Fig.7

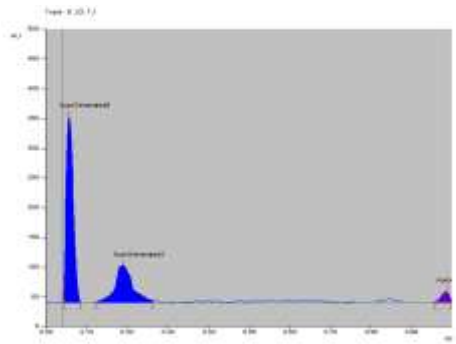

Fig.8
Simple peak display of all sps. at 254 nm

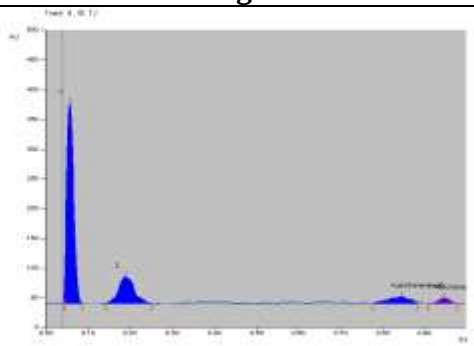

Fig.9
Simple peak display of all sps. at 366 nm

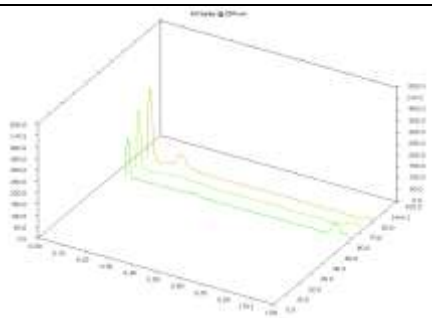

Fig.10
3D peak display of all sps. at 254 nm

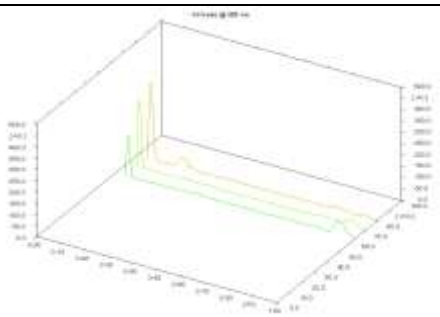

Fig.11
3D peak display of all sps. at 366 nm

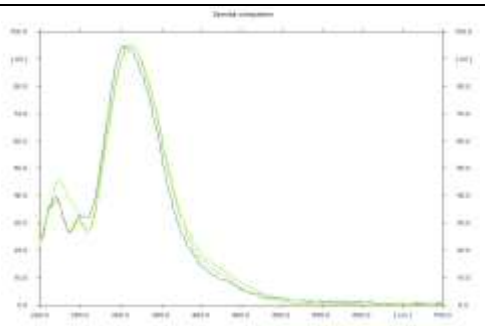

Fig.12 Comparison spectra at R_f 0.84

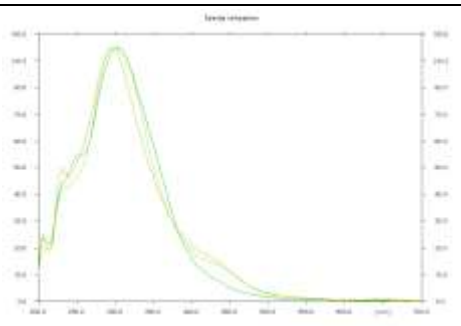

Fig.13 Comparison spectra at R_f 0.94

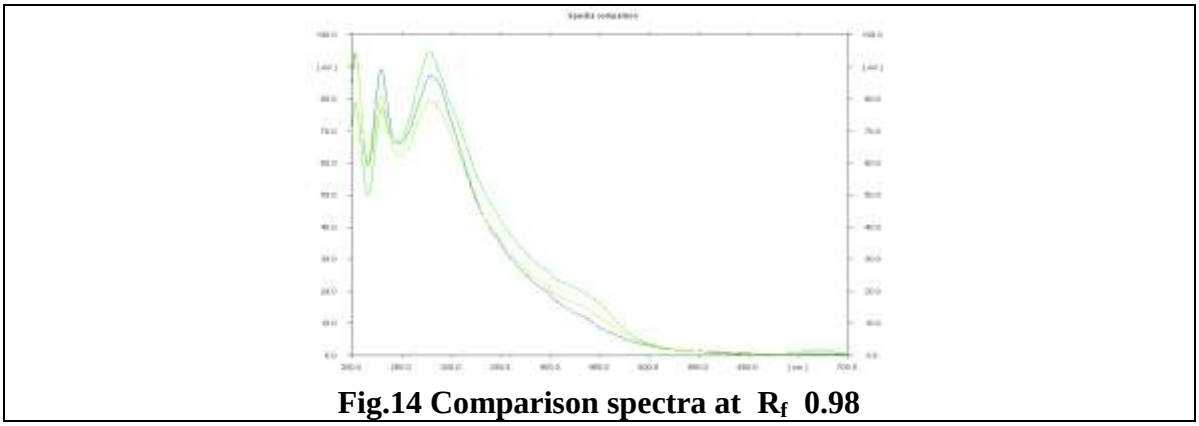

Fig.14 Comparison spectra at R_f 0.98

Rasanirdharana

Whole plant powder of all the three sps. were used in the experimental study to ascertain their *Rasa*. The study was conducted on 30 volunteers aged between 25 – 35 years, who were familiar with *Shadrasa* (six tastes) and were able to express it. The results were interpreted based on the *Rasa* perceived. Data shows the highest frequency in the characteristics of *Katu rasa* (38.29%), *Kashaya rasa* (29.78%) and *Tikta rasa* (12.76%) in *Tephrosia purpurea*, *Madhur rasa* (27.69%), *Amla rasa* (20%), and *Lavana rasa* (16.92%), in *Tephrosia candida* and *Madhur rasa* (26.08%), *Amla rasa* (20.28%), *Lavana rasa* (15.94%) in *Tephrosia jamnagarensis* as experienced by the responders and all results are depicted in table 1.

TABLE – 1: Results of *Rasa* perception of whole plant powder of all three species

S. No.	Characters	*RASA* (Taste)		
		T.p.	*T.c.*	*T.j.*
1.	*Madhura* (Sweet)	6.38 %	27.69 %	26.08 %
2.	*Amla* (Sour)	8.51 %	20 %	20.28 %
3.	*Lavana* (Salty)	4.26 %	16.92 %	15.94 %
4.	*Katu* (Spicy)	12.76 %	1.53 %	4.34 %
5.	*Tikta* (Bitter)	38.29 %	20 %	24.63 %
6.	*Kashaya* (Astringent)	29.78 %	13.84 %	8.69 %

"*T.p.= Tephrosia purpurea, T.c.= Tephrosia candida, T.j.= Tephrosia jamnagarensis*"

DISCUSSION

Whole plant powder of all three sps. exhibits various results viz., moisture content (L.O.D.) found more in *T. candida* (7.624 ± 0.59) in comparison to the other two species. Ash value has been found less in *T. purpurea* (6.079 ± 0.19 % w/w) than *T. candida* (6.061 ± 0.21 % w/w) and *T. jamnagarensis* (6.724 ± 0.835% w/w) sample whereas acid insoluble ash is less in *Candida* (0.150 ± 0.20 % w/w) in comparison to *T. purpurea* (0.450 ± 0.15 % w/w) and *T. jamnagarensis* (0.815 ± 0.16 % w/w) samples. Low ash value signified low level of inorganic

matter and low acid insoluble ash denotes low level of sand and silica content in sample. Neutral pH i.e. 7 has been found in all the three species. Water soluble extractive value in all samples i.e. *T. purpurea*, *T. candida* and *T. jamnagarensis* samples has been found more in compare to alcohol soluble extractive value which indicates the probability of the presence of high water soluble constituents than the alcohol soluble in all samples.

Physical manifestation of chemical moeties from the PCA analysis it is observed, chemical moieties which observed in *T. jamnagarensis* predominantly in third plot, the replicas moieties represented in the second plot. Whereas the first plot *T. purpurea* representing very least amount of characters as compare to second & third i.e. *T. jamnagarensis* showed a dominant character in our research study (Fig. 1).

Qualitative tests showed the presence of reducing sugar, tannin, glycoside and flavonoid in all three samples i.e. *T. purpurea*, *T. candida* and *T. jamnagarensis*. Whereas steroid is present only in *T. jamnagarensis* sample. So in *T. purpurea* and *T. candida* it was absent or may be present in very negligible amount (Table 3).

Qualitative analysis output showed an overall profile of chemical moieties present in plant extract. Natural products with reservoirs of structural and chemical entities will have definite therapeutic relevance. Quantitative estimation of sample powder showed presence of tannin. The presence of flavonoid and tannin content may be responsible for the taste results which are obtained from *Rasanirdharan* study *i.e. Kashaya*, *Madhura* and *Tikta rasa* dominancy amongst all three species.

HPTLC profile showed 4 spots in *T. purpurea* and *T. candida*, while *T. jamnagarensis* showed 3 spots at 254nm, whereas at 366 nm *T. purpurea* and *T. candida* showed 3 spots, while *T. jamnagarensis* showed 4 spots.

Comparative spectra of all three samples revealed the presence of common R_f. values are 0.84, 0.94 & 0.98. The result obtained, study showed the chemical profile of drug.

Whole plant powders of all the three sps. exhibits *Tikta* (Bitter), *Madhura* (Sweet), and *Kashaya* (Astringent) as *Pradhan rasa* (predominant taste) and as *Anurasa* too. Detail results of physicochemical parameters are given in Table 3.3.2.

CONCLUSION

The values obtained from analytical study including physicochemical parameters, qualitative analysis and quantitative analysis can be used for standardization of identity and purity amongst all three sps. The standard results may help for the further research works.

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