EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 12
337-344
Year: 2018

# PRELIMINARY PHARMACOGNOSTIC AND PHYTOCHEMICAL SCREENING OF MARKET SAMPLES OF KARKATAKASRINGI (GALLS OF PISTACIA INTEGERRIMA STEW.EX BRAND.) IN KERALA

**Dr. Sunitha S. Nair*[1], Dr. Indhulekha V. C.[2] and Dr. A. Shahul Hameed[3]**

*[1]PG Scholar, Dept. of Dravyagunavijnanam, Govt. Ayurveda College, Thiruvananthapuram.
[2]Guide, MD(Ay), Assistant Professor, Dept. of Dravyagunavijnanam, Govt. Ayurveda College, Thiruvananthapuram.
[3]Guide, MD(Ay), Associate Professor, Dept. of Dravyagunavijnanam, Govt. Ayurveda College, Thiruvananthapuram

***Corresponding Author: Dr. Sunitha S. Nair**
PG Scholar, Dept. of Dravyagunavijnanam, Govt. Ayurveda College, Thiruvananthapuram.

**Article Received on 04/10/2018** **Article Revised on 24/10/2018** **Article Accepted on 14/11/2018**

## ABSTRACT
Karkatakasringi is an important ayurvedic drug indicated in many diseases and a constituent of preparations like Dasamularista, Chyavanaprasa, Shringyadi curna etc. It is a drug of vegetable origin and the source is identified as leaf galls of Pistacia integerrima Stew. Ex Brand as per API. This tree grows in Himalayas and often cultivated in Punjab. There was one pubished report of suspected adulteration of Karkatakasringi in South India. A market survey conducted showed that cost of Karkatakasringi in North India is  3000/Kg and that in Kerala is  400. So it is hypothesized that there is adulteration of Karkatakasringi in Kerala markets. Karkatakasringi was purchased from one market from Jammu, one market from Punjab, and from two market of south, middle and north zones of Kerala. Samples were labeled and evaluated both pharmacognostically and phyto-chemically as per standard procedures. Morphologically Kerala samples were entirely different in shape, size, color from the samples obtained from North India. They resemble Terminalia chebula galls in shape size and appearance. Phytochemical analysis showed that intensity of presence of tannin, flavonoids and phenols were far less in the Kerala market samples. In HPTLC analysis with the solvent system chloroform**:** ethyl acetate**:** formic acid (23:22:0.5), two spots were found less in Kerala samples. Quantitative estimation using HPTLC with solvent system Toluene**:**Ethyl acetate**:**Formic acid(6:5:1) and with gallic acid marker showed far less percentage of gallic acid in Kerala samples. Present study demonstrated marked adulteration of Karkatakasringi in Kerala raw drug stores and that the samples from Jammu and Punjab were genuine as per API standards.

**KEYWORDS**: Karkatakasringi, Pistacia integerrima Terminalia chebula, Galls, Adulteration.

## INTRODUCTION
Ayurveda is a well-documented ethnomedical system of India. Herbal drugs are the main source of medicines. Ayurvedic medicines are prescribed as compound formulations or single herbal remedies. Both are available in markets. Compound formulations are usually available as labelled products from pharmaceutical companies. Single drugs procured directly from the local drug market are made into the dosage forms like powders, decoctions etc. Karkatakasringi is such a single drug widely used and having therapeutic indications like asthma, tuberculosis, indigestion, heart diseases, fevers and liver disorders.[3] It is an ingredient of famous ayurvedic formulations like Dasamularista, Cyavanaprasa and Shringyadi curna etc. So there is a lot of demand for this galls. Raw drugs which are not locally available are said to have some adulteration and substitution. The API source of the drug Karkatakasringi is the galls of Pistacia integerrima Stew. Ex Brand.[1] P. integerrima is a tree which is nearly glabrous growing on the steeps of Western Himalayas from Indus to Kumaon at an altitude of 350-2400 m, often cultivated in Punjab plains. The tree when get infested by an insect Dasia aedifactor[2] produces a horn like outgrowth. These galls are called as karkadakasringi. These are horn shaped curved elongated, hollow, twisted, broader at one end and tapering to other. It has longitudinal ridges externally and has round opening at the wider end. Its colour is greyish brown externally and reddish brown internally.[1] Gall gives out an aromatic odour on crushing and the powder is brown in colour, strongly astringent, slightly bitter. Previous studies report that 'Kadukka poovu' (galls of *Terminalia chebula* Retz), galls of Udumbara (*Ficus glomerata* Roxb) and Tintidika (*Rhus succedanea* Linn) are sold in market as Karkatakasringi.[4] Our preliminary market screening also revealed that rounded, irregular shaped structures are sold in the name of sringi. No studies regarding the difference of cost or

experiments assessing phytochemical and pharmacognostic features of the raw drugs Karkatakashringi available in Kerala market has been conducted till date. To clear the confusion regarding the authenticity of Karkatakasringi the present investigation is taken up. The samples from markets of Kerala, Jammu and Punjab states are evaluated cost wise and by analyzing morphological and anatomical[5] features in accordance with available ayurvedic and modern literature.

## MATERIALS AND METHODS

### 1. Sample Collection

Genuine samples of Karkatakasrngi galls were collected from Jammu and Punjab and the rate / Kg of the purchased samples were noted. Samples were authenticated by the pharmacognosist, drug standardization unit, Govt Ayurveda College, Trivandrum, and the voucher specimen were kept in Lab of Dept: of Dravyagunavijana, Govt Ayurveda College, Trivandrum. They were shade dried and packed in zip lock polythene bag and labeled as JKS and PS respectively.

For market study Kerala market divided into 3 zones, namely north, middle and south. Samples were collected from, one rural and one urban markets of each of three zones making the total market sample size six. The rate / Kg of the purchased samples were also noted. Samples were again dried in shade for one day and kept in air tight containers and labelled as NRS, NUS, MRS, MUS, SRS and SUS respectively.

### 2. Assessment of Organoleptic Characters

Organoleprtic characters as per API as Shape, Size, External colour, Internal colour, External surface, Fracture, Texture, Odour, and Taste were enlisted, tabulated, and the organoleprtic characters of the samples were compared with them.

### 3. Assessment of Microscopic Characters

The plant material was microscopically studied. Free hand transverse section of galls were taken and examined. The powders of drugs were studied microscopically and the characters were observed as per standard procedure.[6]

### 4. Physicochemical Screening

Physicochemical values of total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive were calculated as per Ayurvedic pharmacopoeia of India. Phytoconstituents (steroids, phenols, alkaloids, saponins, flavonoids, and tannins) were detected for the first time as it is not included in API so it can be taken as the standards for further studies.

### 5. TLC AND HPTLC

For HPTLC study, each sample (5 gm) was boiled in10 ml of methanol and boiled for one hour and extract was prepared. This extract of the samples of *karkatakasringi* were centrifuged at 3000 rpm for 5 minutes. Supernatant was used as test solution for HPTLC analysis. 2µl of each methanolic extract solution was applied as 8 mm band length in the 10 X 200 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples applied plate was kept in TLC twin through developing chamber (after saturated with solvent vapour) with respective mobile phase Chloroform: Ethyl Acetate: Formic Acid (23:22:0.5) up to 70 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm and UV 366 nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber. Before, derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm and UV 366 nm. The peak table and peak densiogram for each profile were noted. The software used was WinCATS 1.3.4 version. Another solvent system is also used for HPTLC and gallic acid was used as marker. Calculated quantities of methanolic extracts along with different concentrations of gallic acid were applied on HPTLC plate by using CAMAG Linomat applicator. Samples applied plate was kept in a solvent system Toluene: Ethyl acetate: Formic acid (6:5:1). Dried plate was scanned initially at 254 nm for qualitative analysis and then at 275 nm as $\lambda_{max}$ of gallic acid using a Camag scanner 3.

## RESULTS

The price values of all the samples viz.samples of Jammu (JKS), of Punjab (PS), of south rural zone (SRS), of south urban zone (SUS), of middle rural zone (MRS), of middle urban zone (MUS), of north rural zone (NUS), and of north urban zone (NUS) were evaluated and is mentioned in table 1. From this it is clear that sample from Jammu & Kashmir and Punjab were 4 to 5 fold costlier than the Kerala market samples.

**Comparison of prices of samples (table 1)**

| Sample name | JKS | PS | SRS | SUS | MRS | MUS | NRS | NUS |
|---|---|---|---|---|---|---|---|---|
| Price of karkatakasringi | □3000 | □2500 | □400 | □600 | □500 | □400 | □500 | □400 |

### Macroscopic Evaluation

Galls are differentiated on the basis of macroscopic characters; Characters observed mentioned in table 2, table 3 and figure 1.

**Macroscopic characters of karkadakasringi samples (Table 2)**

| sample | Character as per API | | | | | | |
|---|---|---|---|---|---|---|---|
| | Shape | Size | colour | External surface | Fracture | Odour | Taste |
| | Hard, hollow, horn-like, thin-walled, generally cylindrical, tapering at both the ends | Varies from 2.5-30.0 cm or more | Greyish brown externally and reddish brown internally | Longitudinal ridges | Easy to break | Terebinthine | Strongly astringent and slightly bitter |
| JKS | + | + | + | + | + | + | + |
| PS | + | + | + | + | + | + | + |
| SRS | - | - | - | - | - | - | - |
| SUS | - | - | - | - | - | - | - |
| MRS | - | - | - | - | - | - | - |
| MUS | - | - | - | - | - | - | - |
| NRS | - | - | - | - | - | - | - |
| NUS | - | - | - | - | - | - | - |

**Macroscopy of karkatakasringi galls collected from Kerala market(table 3)**

| sample | Shape | Size | colour | External surface | Fracture | Odour | Taste |
|---|---|---|---|---|---|---|---|
| | Fan like, some oval or oblong | 1-3 cm long, 2- 4 cm wide | Externally yellowish brown, Internally dark brown | Faint granular markings | Very hard, difficult to break | Not characteristic | Not characteristic |
| SRS | + | + | + | + | + | + | + |
| SUS | + | + | + | + | + | + | + |
| MRS | + | + | + | + | + | + | + |
| MUS | + | + | + | + | + | + | + |
| NRS | + | + | + | + | + | + | + |
| NUS | + | + | + | + | + | + | + |


Galls of *karkatakasringi* collected from : (A)- JKS, (B)- PS, (C)- SRS, (D)- SUS, (E)- MRS, (F)- MUS, (G)- NRS, (H)- NUS

**Figure 1: Macroscopy of karkadakasringi samples.**

**Microscopic Evaluation**

Microscopic evaluation was done under two phases:

- Histological evaluation
- Powder microscopy

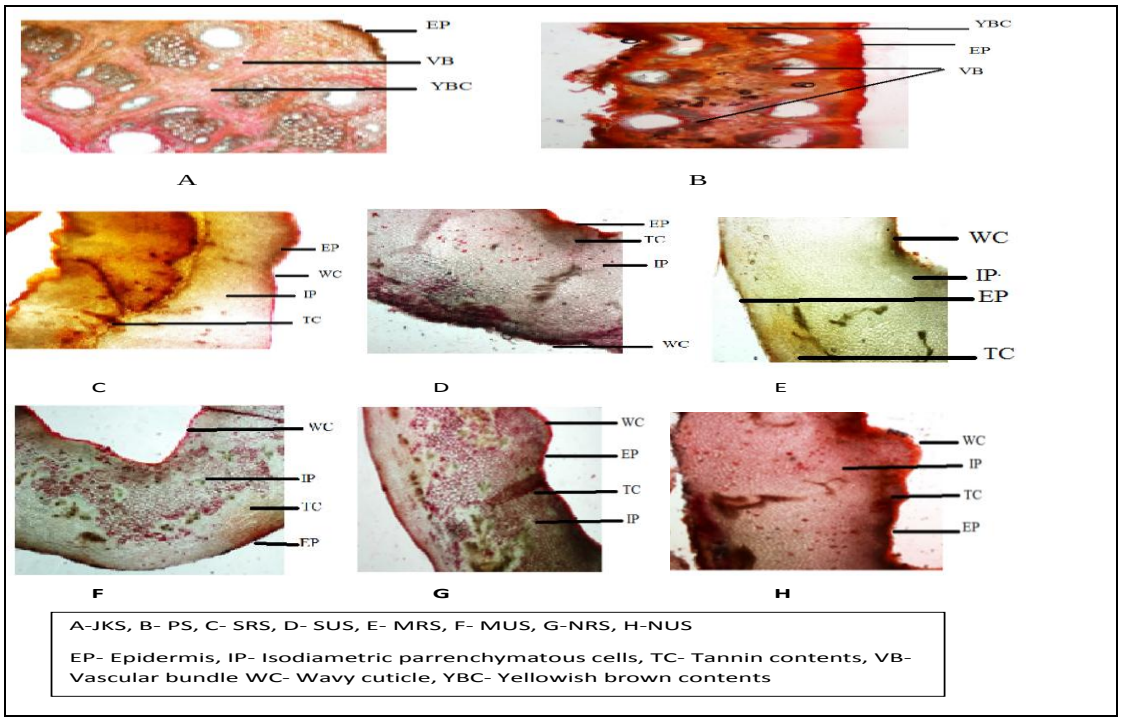
**Histological evaluation**

Galls are differentiated on the basis of microscopic characters, Characters observed were mentioned below.

T S of galls of Jammu & Kashmir and Punjab showed collapsed epidermis on both the sides, thin-walled tangentially elongated epidermal cells, ground tissues thin-walled and oval or circular, cells of ground tissue filled with yellowish brown contents, vascular bundle scattered throughout the ground tissues in two rows, phloem with large tannin sac in each vascular bundle. These characters are matches with that mentioned in API. But these features are not found in any of the samples of Kerala market.

**Microscopy of karkatakasringi galls collected from Kerala market**

T. S. of the gall of Kerala market showed single layered parenchymatous cuticular lower and upper epidermis; and has wavy cuticle. Below upper epidermis, many layered thin-walled oval to isodiametric parenchyamtous cells constitutes the ground tissue; Presence of reddish – brown or blackish contents of tannin; Vascular bundles can be seen scattered in ground tissue. (figure 2).




**Microscopy of karkadakasringi samples (figure 2).**

**Powder microscopy**

Galls are differentiated on the basis of powder microscopic characters, Character observed were mentioned.(figure 3).

Powder of all the samples are greyish brown, under microscope. Fibres present in JKS and PS are with sharp ends, and in Kerala market samples the fibres are having blunt end.

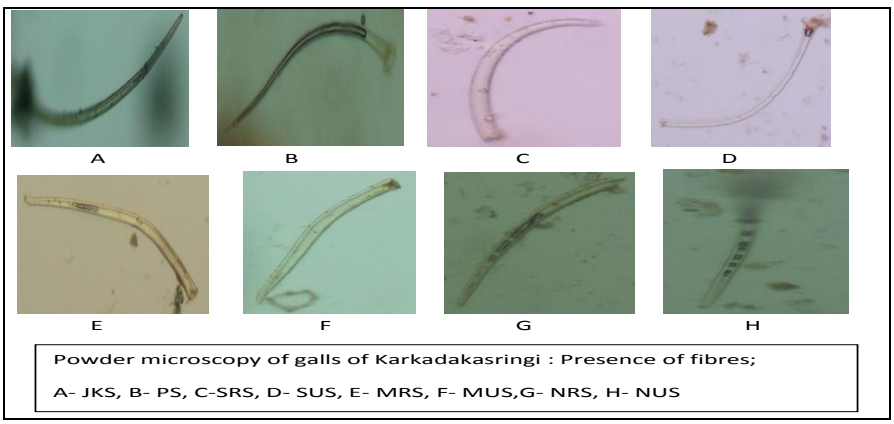



**Figure 3: Powder microscopy of samples.**

**Results of Qualitative tests done in karkadakasringi samples**

Galls are differentiated on the basis of Qualitative tests by its ethanolic extracts, Characters observed were mentioned in table 4.

The ethanolic extracts of genuine and market samples showed following constituents, it is detected for the first time as it is not included in API and can be taken as standard for further studies.

**Qualitative analysis table 4**

| SAMPLES | Steroid | Flavanoid | Alkaloid | Tannin | Saponin | Phenol |
|---|---|---|---|---|---|---|
| JKS | + + | ++ | - | ++ | ++ | +++ |
| PS | + + | + | - | ++ | ++ | ++ |
| SRS | + | - | - | + | + | + |
| SUS | + | - | - | + | + | + |
| MRS | + + | - | - | + | - | + |
| MUS | + | - | - | + | ++ | + |
| NRS | + + | - | - | + | + | + |
| NUS | + | - | - | + | - | + |

**Physico -chemical evaluation of genuine sample and market samples**

Galls are differentiated on the basis of Physico -chemical characters, Characters observed were mentioned in table 5.

**Physico chemical evaluation: table 5.**

| Samples | Character as per API | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Moisture content (%) | Volatile oil (%) | Total ash (%) | Acid insoluble ash (%) | Water soluble extractive (%) | Alcohol soluble extractive (%) | Reducing sugar (%) | Total sugar (%) |
| JKS | Traces | 0.3 | 6.47 | 0.18 | 30.93 | 32.945 | 5 | 1.2 |
| PS | Traces | 0.1 | 1.910 | .17 | 34.24 | 30.68 | 4.5 | 1.6 |
| SRS | Traces | Nil | 4.47 | 0.414 | 30.82 | 30.15 | 3.9 | 4.5 |
| SUS | Traces | Nil | 4.2 | .380 | 30.03 | 29.19 | 4 | 4.2 |
| MRS | Traces | Nil | 3.75 | .585 | 45.04 | 56.16 | 4.1 | 5 |
| MUS | Traces | Nil | 3.01 | .588 | 36.77 | 43.92 | 3.8 | 4.2 |
| NRS | Traces | Nil | 3.30 | 0.358 | 40.83 | 19.25 | 3.9 | 4.12 |
| NUS | Traces | Nil | 3.135 | 0.241 | 39.98 | 25.36 | 3.88 | 3.87 |

**Results of Heavy metal Analysis of karkadakasringi Samples**

Heavy metal content was screened in the market samples as well as in the genuine. Characters observed were mentioned in table 6.

The heavy metal analysis revealed values within normal limits for the genuine sample and the market samples.

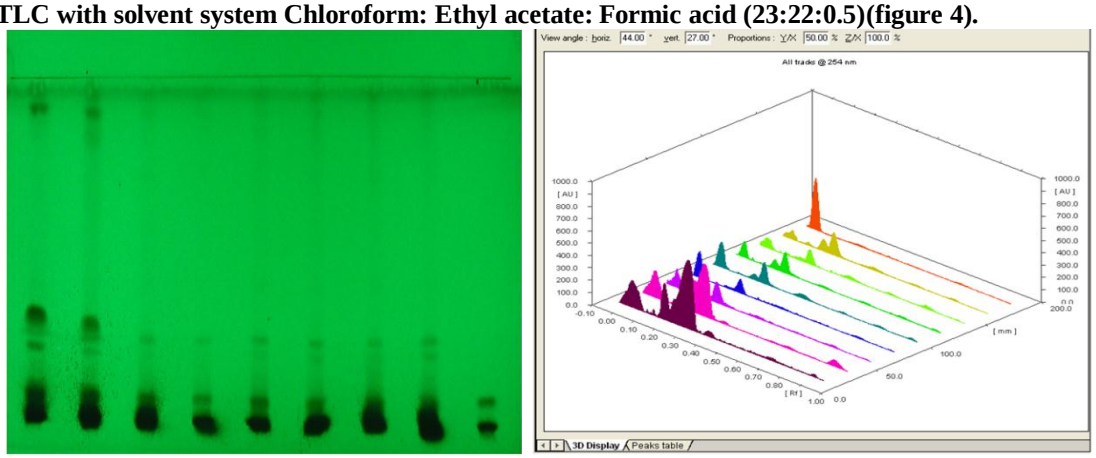
**Heavy metal analysis: table 6.**

| Samples | Heavy metal concentration (in ppm) | | | |
|---|---|---|---|---|
| | Pb | Cu | Cd | Fe |
| JKS | 0.0724 | 0.1330 | 0.0416 | 4.4309 |
| PS | 0.1352 | 0.1497 | 0.0458 | 5.7113 |
| SRS | 0.1487 | 0.1348 | 0.0491 | 3.3065 |
| SUS | 0.1382 | 0.1321 | 0.0452 | 3.2551 |
| MRS | 0.6885 | 0.1489 | 0.0678 | 5.7521 |
| MUS | 0.0676 | 0.1477 | 0.0618 | 3.7683 |
| NRS | 0.1324 | 0.2102 | 0.0667 | 3.6511 |

| | | | | |
|---|---|---|---|---|
| NUS | 0.0782 | 0.1195 | 0.0497 | 2.6414 |

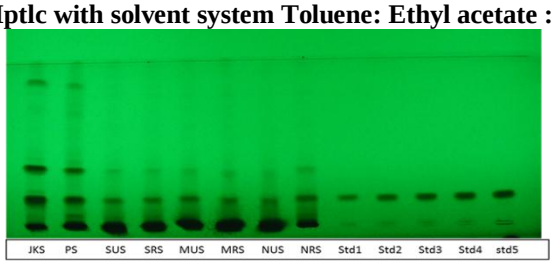
**Results of HPTLC analysis of the samples**

**a) HPTLC with solvent system Chloroform: Ethyl acetate: Formic acid (23:22:0.5)(figure 4).**

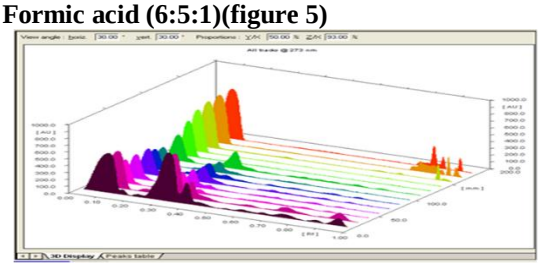


(A) Hptlc plate with solvent system chloroform : ethyl acetate : formic acid (23:22:0.5)
(B) 3D scan profile at 254 nm(densitometric)

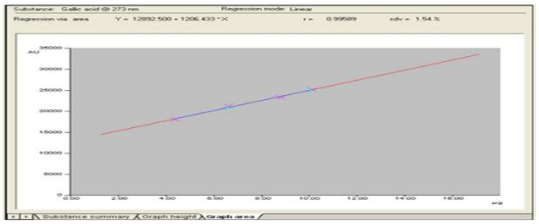
**b) Hptlc with solvent system Toluene: Ethyl acetate : Formic acid (6:5:1)(figure 5)**



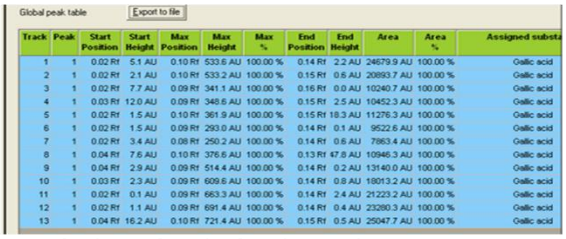

Hptlc plate with solvent system toluene : ethyl acetate : formic acid(6:5:1)
Std 1: gallic acid 2 µg, std 2 : 4 µg, std 3 : 6µg, std 4 : 8µg, std 5 : 10µg



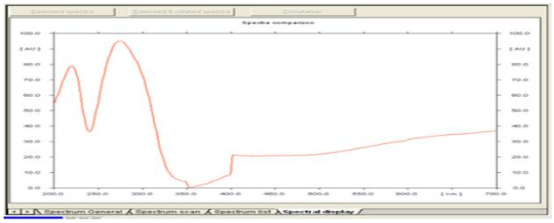
3d scan profile at 254 nm



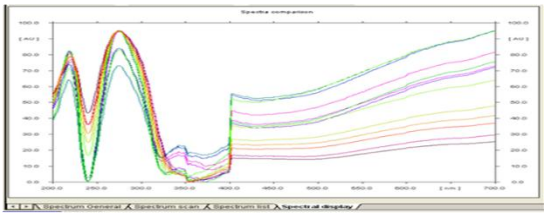
Linear regression graph of gallic acid



Rf values of peak of each samples



UV spectrum of gallic acid Lamda max 275 nm



Spectral comparison of all the samples

## DISCUSSION

To ascertain the quality, purity and genuineness of raw drugs, standardization is must. Herbs are the backbone of ayurvedic systems of medicine. Practically scarcity is the main problem facing in this industry. This scarcity can lead to adulteration and substitution. Therefore it is important to takeup studies for standardization of raw drug. The present study assess the characters of Karkadakasringi (Pistacia integerrima) available in raw drug market of Kerala with the aid of pharmacological, physio-chemical, HPTLC and AAS. The findings obtained from the results of evaluation carried out are discussed here.

Karkadakasringi is a widely used single drug, having North India as geographical source. Gall of Pistacia integerrima is mentioned as the officinal part of Karkadakasringi in API. To conduct this study genuine

samples are collected from Jammu Kaashmir and Punjab. Also two market samples of Karkadakasringi were colleceted from each of the three zones of Kerala (South, Middle, North) and compared with the API standard and with genuine sample. While purchasing from Kerala market, The price of raw drug purchased from Kerala market were somewhat similar and ranges from 400 to 600 □ / Kg. But those collected from Jammu Kashmir and Punjab were much costlier than Kerala markets, ranges from 2500-3000 □ /Kg. samples obtained were having the characters which are totally different from the characters of karkadakasringi (Pistacia integerrima) mentioned in API. So it is hypothesized that there is adulteration of Karkatakasringi in Kerala markets.

In organoleptic evaluation, samples collected from Kerala are having fan like oval shape, 1-3 cm long, externally yellowish brown and internally dark brown in color, it is difficult to break and there is no characteristic odour and taste. But the samples from Jammu Kashmir and Punjab were hard, hollow, horn like, cylindrical and tapering at both ends. Their sizes varies from 2.5 – 30 cm, greyish brown externally and reddish brown internally in colour, easy to break, having an aromatic odour and strong astringent taste. The characteristic features of Jammu Kashmir and Punjab samples matches with the standards mentioned in API. From the market we traced the source of karkadakasringi and found that they are supplied as kadukkapoovu (literally meaning flowers of Chebula) by 'Kaani' tribals. It was observed that these kadukkapoovu are galls collected from the fallen leaves of Terminalia chebula.

In histological evaluation sample collected from Kerala market are having the features like, single layered parenchyma, cuticular lower and upper epidermis, wavy cuticle, isodiametric parenchymatous cells in ground tissue, tannin contents were observed. But sample from Jammu Kashmir and Punjab are having collapsed epidermis on both sides, oval or circular ground tissue, vascular bundles are scattered in ground tissue in two rows and large tannin sac in each vascular bundles were observed. There was similarity between API standards and microscopic features of Jammu Kashmir and Punjab samples. Powder microscopy can be put as a reference standards for the identification of powder. In powder microscopy, the powder of all samples were appears greyish brown under microscope. In Kerala market samples the fibres are having blunt end and of Jammu Kashmir and Punjab samples are with sharp ended fibres.

Next part of the study includes physical and phytochemical evaluation which is an important aspect in standardization. The physio chemical values of Jammu and Punjab samples are in conformity within the values mentioned in API. the kerala market samples difers with the API standards in volatile oil content and acid insoluble ash values. Alcohol soluble extractive value of NRS and NUS of Kerala samples are not within the limits prescribed in API.

In the next step qualitative analysis of samples were done to determine the physiologically active chemical constituents in it. It was detected for the first time as it is not mentioned in API. So it can be taken as standard for further studies. The result showed the presence of tannins, steroids, phenol etc. Alkaloids were absent in all the samples. Flavanoids were present only in Jammu and Punjab sample, but absent in all the Kerala market samples. Tannin and phenol content were higher in JKS and PS compared to other samples. The presence of various heavy metals was also screened in all the samples and it was found out to be in the permissible limits in all the samples.

For further authentication and identification of chemical constituents HPTLC analysis of all the samples were carried out. HPTLC analysis with the solvent system chloroform: ethyl acetate: formic acid (23:22:0.5) showed that the no of peaks in the JKS and PS samples and Kerala market samples varied. Although slight variations were seen in and HPTLC profile, the similarities suggested the presence of similar chemical constituents in it. Two spots found extra in JKS and PS may suggeste the presence of additional chemical constituents, and further study need to screen the pharmacological activity. HPTLC analysis with another solvent system Toluene: Ethyl acetate: Formic acid(6:5:1) and gallic acid as marker shows significant disparity among the samples. Quantification of gallic acid was observed from this profile. UV spectrum of gallic acid was observed from the figure, and the lamda max of gallic acid is at 275 nm. From spectral comparison of all the samples, super imposed UV spectrum can be found at 275 nm. This suggested the presence of gallic acid in all the samples. From the linear regression graph it was clear that JKS sample contains about 10.03 micrograms of gallic acid and in PS sample it is 6.57 micrograms. All other samples from Kerala market contains less than 2 micrograms of gallic acid.

## CONCLUSIONS

In the present study, pharmacognostic and phytochemical screening were carried out as per pharmacopoeia and WHO guidelines. It can be concluded that there were variations in pharmacognostical, physical phytochemical and HPTLC profile of market samples of Karkatakasringi from the genuine samples of Karkatakasringi [Pistacia integerrima Stew. Ex Brand], and from standards set by API suggesting adulteration of drug in raw drug markets of Kerala. Absence of flavonoids, less amount of tannins and phenol in Kerala market samples proved that the market samples are not genuine. First hand information also revealed that the drug being sold as karkadakasringi in kerala market is actually galls of leafs of Terminalia chebula. The findings of the primilinary qualitative and quantitative phytochemical evaluation can serve as valuable data if fix standards for genuineness of karkadakasringi(galls of Pistacia integerima).

**ACKNOWLEDGEMENT**

The author acknowledge Mr. Mahadevan, Dr. Rajesh and Dr. Binitha, Research officers, Drug Standardisation Unit, Govt. Ayurveda College, Trivandrum for the valuable suggestions and support.

**REFERENCES**

1. The Ayurveda Pharmacopoeia Of India, Part 1, Vol.1, New Delhi: Government of India Ministry of Health and Family Welfare, Dept of Ayush, 2007; 88,89.
2. http://www.planetayurveda.com/library/karkatshringi-pistacia-integerrima accessed on 23/07/2018
3. The Wealth of India, C.S.I.R., New Delhi, 1969; 8: 121.
4. Warrier PK, Nambiar VPK. Ramankutt. Indian Medicinal Plants. Vol. 4, Universities Press, Hydrabad, 304-307.
5. C.K.Kokate, A.P.Purohit, S.B. Gokhale. Pharmacognosy. 50th. Pune; Nirali prakashan, 2014; 1-15.
6. The Ayurvedic Pharmacopoeia of India. Part-1, Vol. 3, Govt. of India. Ministry of health and Family welfare, Dept. of ISM & H(AYUSH), New Delhi, 2001; 227-40.