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Histological, Histochemical and Phytochemical Studies of The Raw Drug Amrita From Different Raw Drug Markets of Kerala

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Abstract

There are two source plants for the raw drug Amrita, Chittamrita and wild amrita. The accepted source of the former is Tinospora cordifolia and Tinospora sinensis is often used as a substitute. This paper deals with the study of "Histological, Histochemical and Phytochemical studies of the Raw drug Amrita from different Raw Drug Markets of Kerala". The present study reveals that in the raw drug market of Kerala, T. cordifolia, T. sinensis and few other menispermaceae members are obtained as source plant of Amrita and concluded that about 40% of the raw drug amrita was found to be substitution/ adulteration.

Key words: Amrita, Chittamrita, Tinospora cordifolia, Tinosporia sinensis

Introduction

The term Amrita meaning immortal or eternally young is given to this plant in recognition of its capacity to impart youthfulness and longevity to the consumer. It is an important ingredient of about 68 Ayurvedic formulations like Amritharishtam, Amrithadi enna, Amrithadichoornam etc. Amrita is otherwise known as Guduchi. There are two source plants for the raw drug Amrita, Chittamrita and wild amrita. The accepted source of the former is Tinospora cordifolia and Tinospora sinensis is often used as a substitute[1,2,4,5] etc. The adulteration/substitution occurs either due to the non availability of genuine drugs in required quantities or due to the ignorance of the correct identity of the genuine drug. From the present study the author found out that most of the amrita available from the suppliers are large sized T. sinensis. Kerala physicians use T. cordifolia, even though sometimes T. sinensis is also used as a substitute/ adulterant and in dried nature it is very difficult to distinguish. The morphological profile of these two plants was reported earlier[5]. For the identification of genuine T. cordifolia this morphological character is highly useful. But the major problem that the herbal drug industry faces today is the identification of dried or fresh stem portion available through the suppliers. They mix T. cordifolia with the other species of Tinospora or similar looking menispermaceae members. It necessitates for quality standard parameters for fresh and dried portion of the stem of T. cordifolia using other parameters like histological and histochemical characterization.

Materials and methods

Tinospora cordifolia and Tinospora sinensis are the two species known as Amrita. Raw drugs were collected from different raw drugs markets, wholesalers / retailers, raw drug collectors from 13 Districts of Kerala (Alappuzha, Calicut, Eranakulam, Idukki, Kollam, Kottayam, Kuttyadi, Palakkad, Pathanamthitta, Payyannur, Thrissur, Vadakara and Trivandrum). The useful parts were fixed in Formalin Acetic acid Alcohol mixture for further study. The plant materials were also collected for the phytochemical studies.

Histological and histochemical studies.

The useful parts of the genuine plants/ substitutes/ adulterants and the market samples were fixed in Formalin Acetic acid Alcohol mixture. Cellular details of genuine plant and their substitutes/ adulterants were studied by taking sections using microtomes and double staining with appropriate staining procedure and also using histochemical techniques[3].

Phytochemical studies

General method of extraction

The first step in the phytochemical evaluation of the plant material was the extraction. The choice of extraction method depends on the nature of the plant material and the compounds to be isolated. Dried material was usually powdered before extraction. Extraction was carried out by the methods involving heating of the drug with suitable solvent.

Thin layer Chromatography (TLC) studies

Thin Layer Chromatographic (TLC) studies conducted using pre-coated plates of silica gel 60 F_{254} (E. Merck) of uniform thickness of 0.2mm.

Details of the raw drugs collected from different raw drug markets of Kerala

Districts
1. Thiruvananthapuram
2. Kollam
3. Pathanamthitta
4. Alappuzha
5. Kottayam
6. Idukki
7. Ernakulam
8. Thrissur
9. Palakkad
10. Malappuram
11. Kozhikode
12. Wayanad
13. Kannur
14. Kazargod

● Markets from where the samples collected

Results and discussion

The details of the genuineness of the materials are as follows:

Table 1 Market sample comparison of Histological and Histochemical characters of Amrita different districts of Kerala

Sl.No	Name of Districts	Amrita
1	**Alappuzha**	Genuine, T. cordifolia. Starch grains plenty in outer and inner cortex. Mucilage cavities less in number
2	**Calicut**	Genuine, T. cordifolia Starch grains absent
3	**Ernakulam**	Not genuine, Substitute- T. sinensis. Starch grains plenty. Wedge shaped vascular bundles are more in number. Mucilage cavities less in number.
4	**Idukki**	Adulterant
5	**Kollam**	Genuine, T. cordifolia. Starch grains plenty.

6	**Kottayam**	Genuine, T. cordifolia, Starch grains less. (young stem)
7	**Kuttyadi**	Genuine, T. cordifolia, Starch grains comparatively lesser.
8	**Palakkad**	Not genuine. Substitute - T. sinensis. Prominent stone cells are seen in the pith region.
9	**Pathanamthitta**	Not genuine. Substitute, T. sinensis. Stone cells are seen in the pith region. Starch grains are present in very little amount.
10	**Payyannur**	Genuine, T. cordifolia. Starch grains comparatively lesser.
11	**Thrissur**	Genuine, T. cordifolia. Starch grains plenty
12	**Trivandrum**	Genuine, T. cordifolia. Starch grains comparatively lesser.
13	**Vadakara**	Not genuine. Substitute- Tinospora sinensis. Prominent stone cells are seen in the pith region.

Materials collected from 12 districts of Kerala and studied their pharmacognostical and chemical characters. The anatomical comparison of the genuine sample shows there is no variation in the basic anatomical characters of T. cordifolia. Details of the anatomical studies are given in Table 1. Differences were observed in the depositions of lignin and starch according to the place of collection. (Plate 1-3). Samples collected from Vadakara, Pathanamthitta, Palakkad and Ernakulam raw drug markets were confirmed that it is T. sinensis.

In this case in addition to the common bands with T. cordifolia, a few additional bands are also present and it is used as substitute. Anatomical and chemical profiling of samples collected from Idukki raw drug market is totally different from the genuine drug T. cordifolia and substitute T. sinensis. So it is confirmed that it is an adulterant. A band at R_f 0.07 corresponding to berberine is visible in all the market samples except Idukki sample at 254 nm 366 nm. Details of the R_f values of the prominent bands of TLC profile of the market samples are given in Tables 2 and 3 (Plate 4 & 5).

Conclusion

Using histological, histochemical and phytochemical studies to check the extend of variability of source plant of Amrita from different markets of Kerala. Difference were observed in the deposition of lignin and starch according to the place of collection, but the chemical profiling showed that the compounds are similar but a little difference in their concentrations.

Samples collected from Vadakara, Pathanamthitta, Palakkad and Ernakulam raw drug markets were found to be the substitute T. sinensis. The profiling of the sample collected from Idukki was entirely different from the genuine T. cordifolia and the substitute T. sinensis. So it is confirmed as an adulterant.. The study reveals that in the raw drug market of Kerala, T. cordifolia, T. sinensis and few other menispermaceae members are obtained as source plant of Amrita. The conclusion is that about 40% of the raw drug amrita was found to be substitution/ adulteration.

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Table 2 R_f values of different market samples of Amrita (UV 254 nm)

Rf value of the bands in														**Colour of the band**
BER	**ALP**	**CLT**	**EKM**	**IDK**	**KLM**	**KTM**	**KTD**	**PKD**	**PTA**	**PYR**	**TSR**	**TVM**	**VDA**	
0.07	0.07	0.07	0.07	_	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	Grey
	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	Grey
	_	_	_	0.35	_	_	_	_	_	_	_	_	_	Grey
	0.41	0.41	0.41	_	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	Grey
	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	Grey
	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	Grey
	_	_	0.62	0.62	_	_	_	0.62	0.62	_	_	_	0.62	Grey
	_	_	0.72	_	_	_	_	0.72	0.72	_	_	_	0.72	Grey

Table 3 R_f values of different market samples of Amrita (UV 366 nm)

Rf value of the bands in														Colour of the band
BER	**ALP**	**CLT**	**EKM**	**IDK**	**KLM**	**KTM**	**KTD**	**PKD**	**PTA**	**PYR**	**TSR**	**TVM**	**VDA**	
0.07	0.07	0.07	0.07	_	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	Green
	_	_	_	0.09	_	_	_	_	_	_	_	_	_	Blue
	_	_	_	0.16	_	_	_	_	_	_	_	_	_	Blue
	0.20	0.20	_	_	0.20	0.20	0.20	_	_	0.20	0.20	0.20	_	Green
	0.31	0.31	_	0.31	0.31	0.31	0.31	_	_	0.31	0.31	0.31	_	Blue
	_	_	_	0.37	_	_	_	_	_	_	_	_	_	Blue
	0.44	0.44	_	_	0.44	0.44	0.44	_	_	0.44	0.44	0.44	_	Green
	_	_	_	0.49	_	_	_	_	_	_	_	_	_	Blue
	0.53	0.53	0.53	_	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	Red
	0.60	0.60	_	0.60	0.60	0.60	0.60	_	_	0.60	0.60	0.60	_	Red
	_	_	0.63	_	_	_	_	0.63	0.63	_	_	_	0.63	Green
	_	_	_	0.67	_	_	_	_	_	_	_	_	_	Blue
	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	Blue
	_	_	0.79	_	_	_	_	0.79	0.79	_	_	_	0.79	Blue
	0.82	0.82	_	0.82	0.82	0.82	0.82	_	_	0.82	0.82	0.82	_	Red

BER - Berberine
ALP - Alappuzha
CLT - Calicut
EKM - Ernakulam
IDK - Idukki
KLM - Kollam
KTM - Kottayam
KTD - Kuttyadi
PKD - Palakkad
PTA- Pathanamthita
PYR - Payyannur
TSR - Thrissur
TVM - Trivandrum
VDA - Vadakara

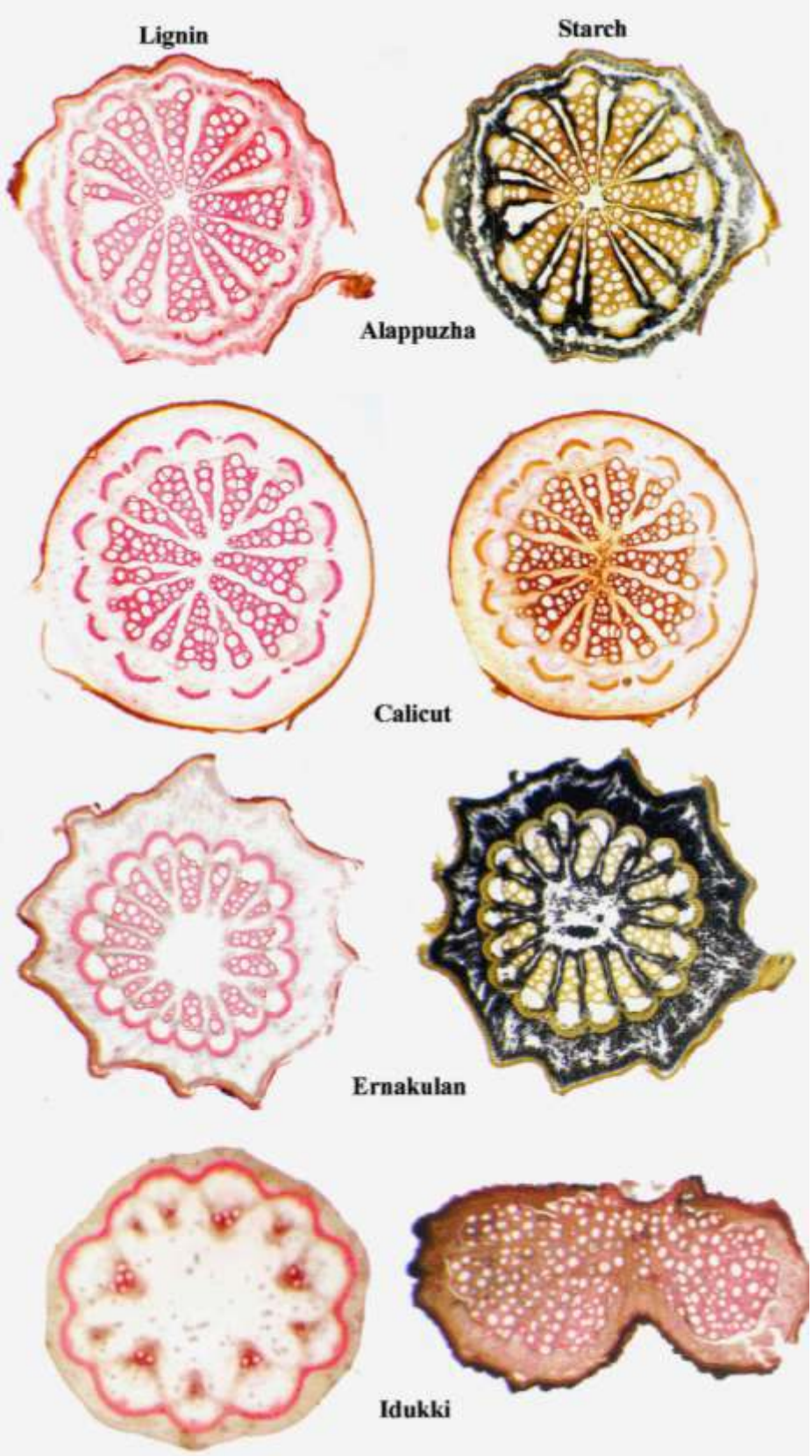

Plate: 1 Anatomical comparisons of samples from different raw drug markets

Plate: 2 Anatomical comparisons of samples from different raw drug markets

Plate: 3 Anatomical comparisons of samples from different raw drug markets [190]

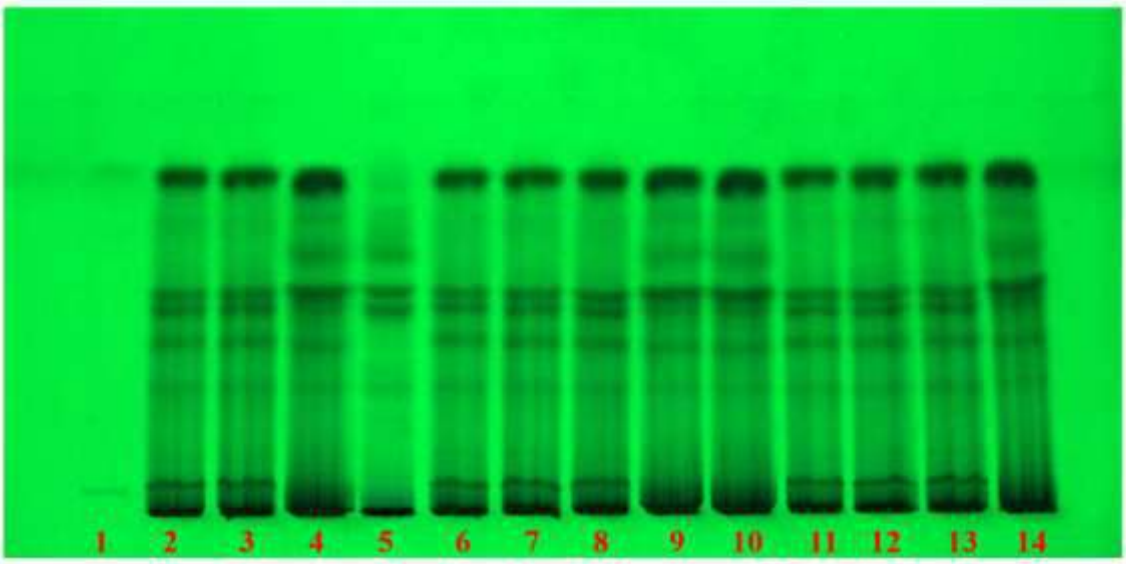

Plate: 4 Phytochemical comparison of samples from different raw drug markets (UV 254 nm)

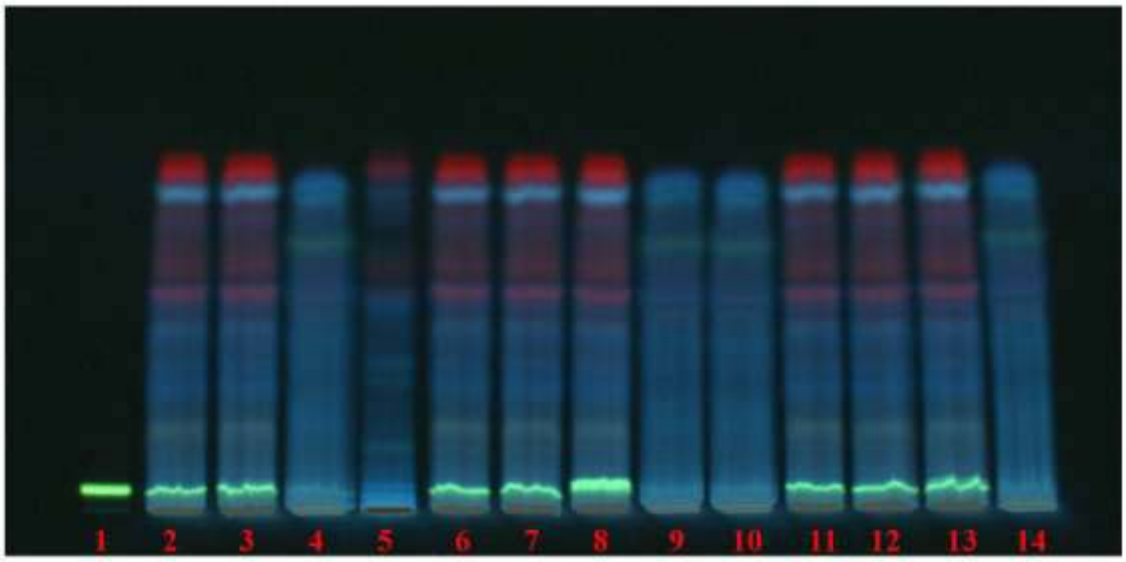

Plate:5 Phytochemical comparison of samples from different raw drug markets (UV 366 nm)

1. Berberine
2. Alappuzha
3. Calicut
4. Ernakulam
5. Idukki
6. Kollam
7. Kottayam
8. Kuttyadi
9. Palakkad
10. Pathanamthitta
11. Payyannur
12. Thrissur
13. Trivandrum
14. Vadakara