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COMPARATIVE STUDIES OF *RUBIA CORDIFOLIA* LINN. AND *RUBIA TINCTORUM* LINN (RUBIACEAE)*

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ABSTRACT: *Comparative morphological microscopical and phytochemical studies of root and stem parts of R. cordifolia and R. tinctorum L. (Rubiaceae) have been carried out.*

INTRODUCTION

The roots of *R. cordifolia* (Rubiaceae), commonly known as Majith, **Manjistha** or **Indian Maddar,** is one of the highly reputed drug used in the Indigenous system of Medicine as an Antiinflammatory (Antarkaretal., 1983), Hemostatic (Kosuge et al., 1981), in the urinary disorders (Shah et al., 1976) and in case of number of other ailments (Mascarenhas et al., 1980; Agarwal, 1985). The Ruberythric acid, one of its major constituent, is widely used as a phytotherapeutic drug in the treatment of calcium containing stones in the urinary tract. This effect has been clinically tested and a medicine (CYSTENAL) is produced by SPOFA (Praha) (Laszlo et al., 1992).

These highly reputed therapeutic claims of the drug drew our attention towards the need for the standardization of the roots of *R. cordifolia*. Hence the dried samples sold in the market under the name of Majith were collected from various states of India and were compared with the botanically identified samples of *R. cordifolia*. In stead of root pieces, all the collected samples were found to contain the major amount of stem pieces. None of the samples were found to be identical to that of the authenticated roots of *R. cordifolia* but were found to be of *R. tinctorum.* Further, the samples collected from the number of reputed Ayurvedic Drug Manufactures and Practitioners, were found to be of *R. tinctorum*, instead of *R. cordifolia.* In fact under the name of Majith or Indian Maddar roots, nothing was mentioned in any text about the drug *R. tinctorum* (Anonymous, 1978; Banbadai, 1940; Pandey, 1969; Vaidya, 1972; Sharma, 1991) which is said to possess the carcinogenic property (Westendorf, 1988; Blomeke et al., 1990). Hence it was thought worth to study the morphological, microscopical and phytochemical comparison of the root and stem parts of *R. cordifolia* and *R. tinctorum*, so as to develop some parameters to differentiate between *R. cordifolia*, an official variety of Majith, from that of *R. tinctorum.*

Experimental

Collection and Identification:

Fresh and dried entire herbs and dried root and stem pieces of botanically identified plants of *R. cordifolia* and *R. tinctorum* were procured from Ooty, Tamilnadu and Bangalore, Karnataka. The market samples known under the name of Majith were also procured from various states of India, like

Gujarat, Maharashtra, Karanataka and Delhi. Further, the collection of the dried samples was also done from Nepal and few reputed Ayurvedic Industries, like Zandu Pharmacy (Bombay), Vishwamangal Pharmacy (Ahmedabad), etc. The percentage of stem and root parts present in these samples are mentioned in Table 1.

Materials and Methods:

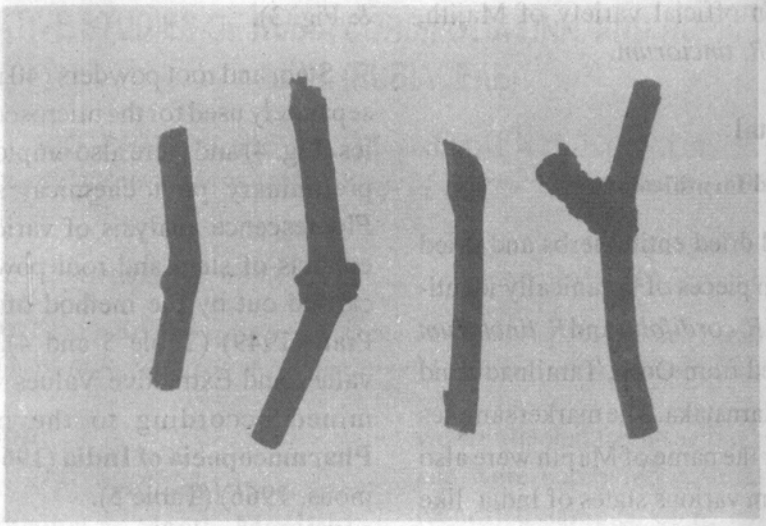
Morphological characters of dried pieces of stem and root of *R. cordifolia* and *R. tinctorum* (Fig.1) were studied and the characters are summerised in Table 2.

Free hand sections of roots and stems of both the species were taken from the fresh as well as dried samples. The sections, after clearing, were stained with various reagents and were drawn with the help of camera lucida (Evans et al., 1983), (Fig. 2 & Fig.3).

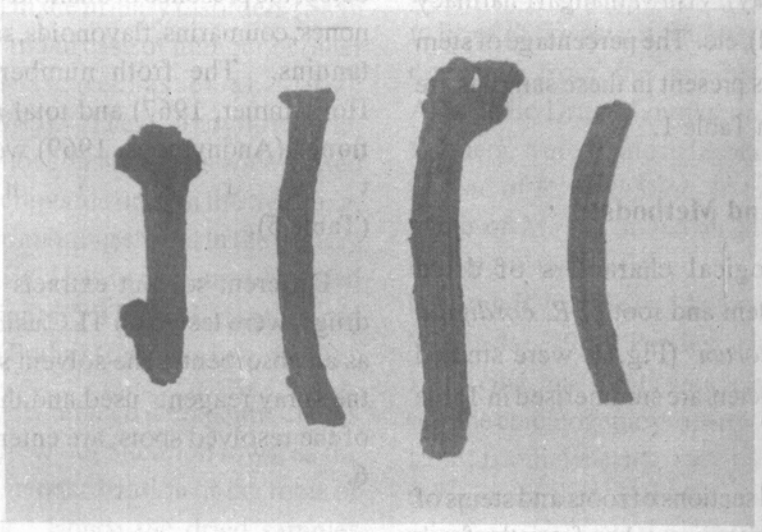
Stem and root powders (40 mesh) were separately used for the microscopical studies (Fig.4) and were also employed for the preliminary phytochemical screenings. Fluorescence analysis of various solvent extracts of stem and roots powders, were carried out by the method of Chase and Pratt (1949) (Table 3 and 4). The Ash values and Extractive Values were determined according to the method of **Pharmacopoeia of India (1966)** (Anonymous, 1966) (Table 5).

In the preliminary phytochemical screening, the stem and the root powders of *R. cordifolia* and *R. tinctorum* were subjected to various chemical testing, to detect the presence of alkaloids, anthraquinones, coumarins, flavonids, saponins and tannins. The froth number (List and Horhammer, 1967) and total **anthraquinones** (Anonymous, 1969) were also determined (Table 5).

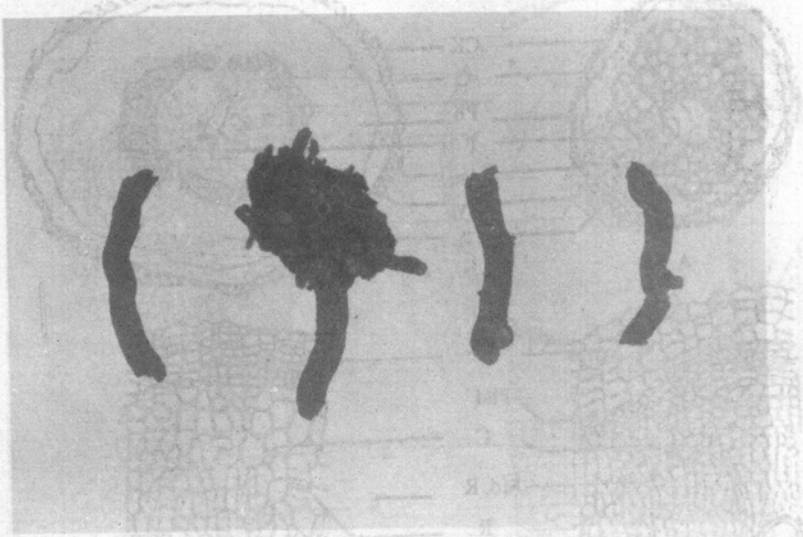
Different solvent extracts of both the drugs, were tested on TLC using silica gel as an absorbent. The solvent systems and the spray reagents used and the Rf values of the resolved spots, are entered in Table 6.



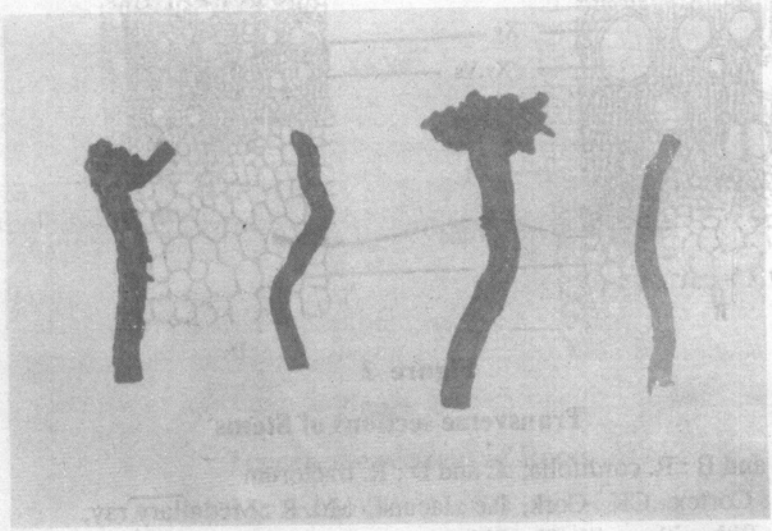
STEMS OF R. CORDIFOLIA



STEMS OF R. TINCTORUM



ROOTS OF R. CORDIFOLIA



ROOTS OF R. TINCTORUM

FIG. 1 : STEMS AND ROOTS OF R. CORDIFOLIA AND R. TINCTORUM

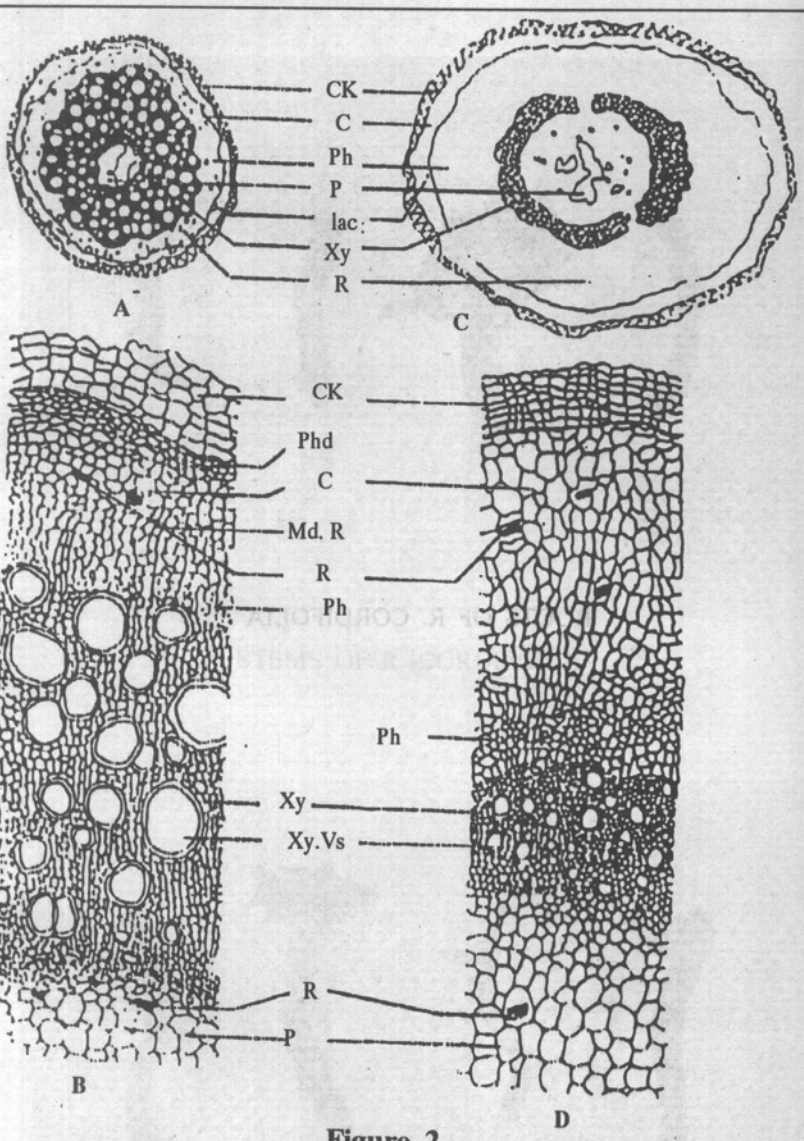


Figure 2

Transverse sections of Stems

A and B : R. cordifolia; C and D : R. tinctorum
C : Cortex, CK : Cork, lac : lacuna, Md. R : Medullary ray,
P : Pith with cavity, Ph : Phloem,
R : phidas of calcium oxalate, Xy : Xylem,
Xy. Vs : Xylem Vessel

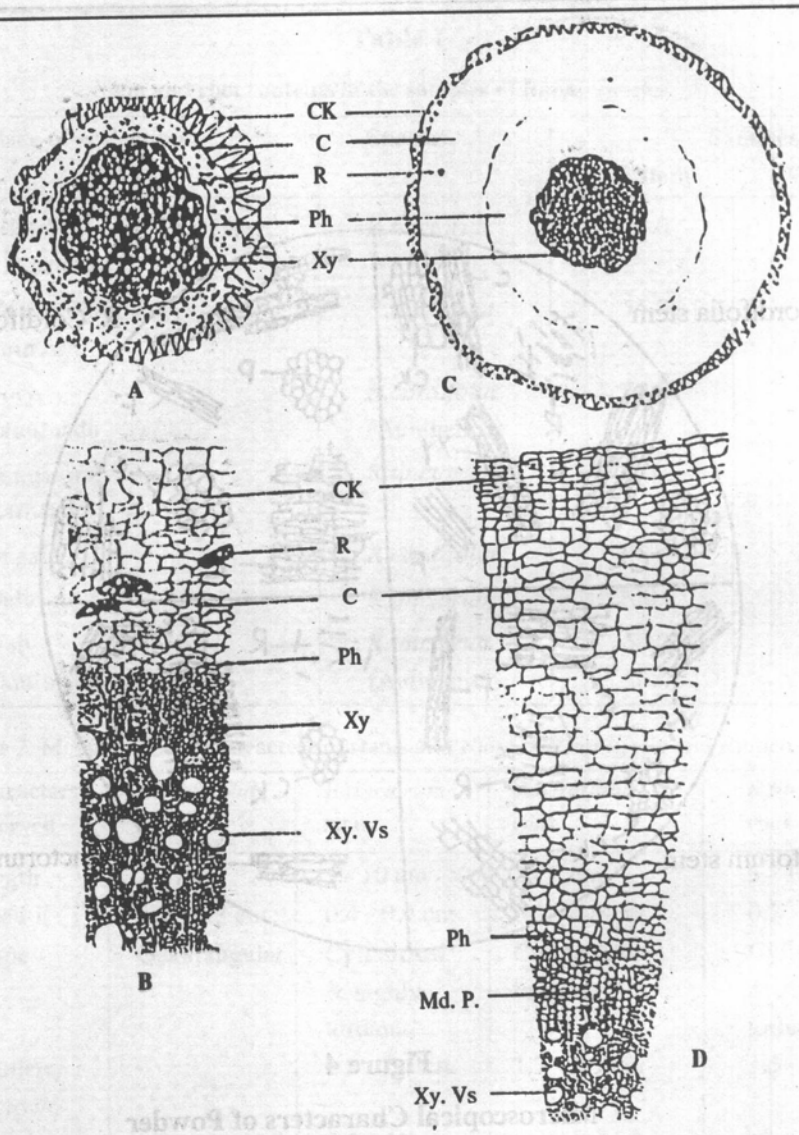


Figure 3

Transverse sections of Roots

A and B : R. cordifolia; C and D : R. tinctorum
C : Cortex, CK : Cork, Md. R : Medullary ray,
Ph : Phloem, R : Raphides of calcium oxalate,
Xy : Xylem, Xy. Vs : Xylem Vessel

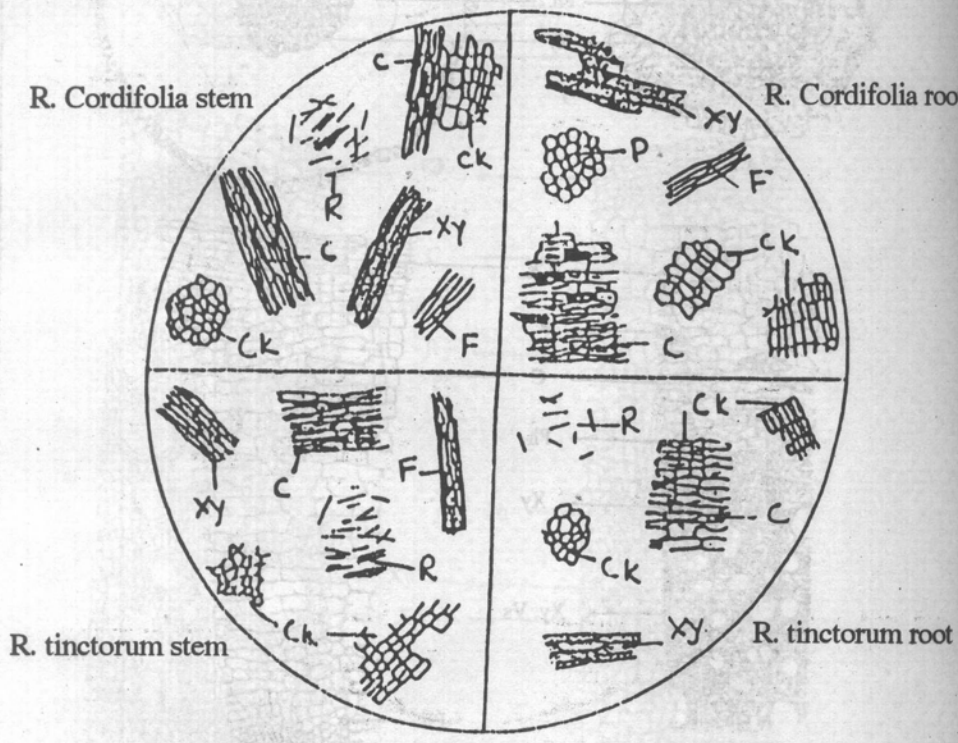


Figure 4

Microscopical Characters of Powder

Abbr. C : Cortex, Ck : Cork, F : Fibre, P : Pith

R : Raphides of Calcium Oxalate,

Xy : Xylem elements

Table 1
Stem and root contents in the samples of *Rubia* species

S. No.	Place of collection	Species	Samples	
			% stem	% root
1	Vishwamangal Pharmacy, Ahmedabad	*R. tinctorum*	70.0	30.0
2	Zandu Pharmacy, Bombay	*R. tinctorum*	55.0	45.0
3	Ooty, Tamilnadu	*R. cordifolia* (Aunthentic)	50.0	50.0
4	Bangalore, Karnataka	*R. tinctorum*	100.0	--
5	Nepal	*R. cordifolia*	40.0	60.0
6	Delhi	*R. tinctorum*	100.0	--
7	Ooty, Tamilnadu	*R. tinctorum* (Authentic)	50.0 50.0	50.0 50.0

Table 2
Morphological Characters of stems and roots of *R. cordifolia* and *R. tinctorum*

S. No.	Characters observed	*R. cordifolia* stem	*R. tinctorum* stem	*R. cordifolia* root	*R. tinctorum* root
1	Length	2 – 7 cm	3 – 10 cm	2 – 9 cm	3 – 10 cm
2	Breadth	0.3 – 0.8 cm	0.4 – 0.9 cm	0.2 – 0.6 cm	0.2 – 0.9 cm
3	Shape	Quadrangular	Cylindrical & highly tortuous	Cylindrical tortuous	Cylindrical & highly tortuous
4	Diameter of crown	--	1.2 – 2.6 cm	1.7 – 3.9 cm	1.3 – 2.8 cm
5	Diameter Xylem	39.3 – 91.52 Microns	4.0 – 5.0 Microns	30.0 – 166.2 Microns	15.0 – 45.0 Microns
6	Surface smooth	Smooth	Rough	Comparatively	Rough
7	Colour	Dark reddish brown	Light brown	Dark reddish black	Light brown
8	Length of internode	2.1 – 5.3 cm	3.4 – 7.1 cm*	--	--
9	Taste	Sweet then acrid	Acrid & disagreeable	Sweet then acrid & disagreeable	Acrid & disagreeable

Table 3

The Flourescence Analysis of the stems of *R. cordifolia* and *R. tinctorum*

S. No.	Type of Extract	*R. cordifolia*			*R. tinctorum*		
		Day light	**Short wave length**	**Long wave length**	**Day light**	**Short wave length**	**Long wave length**
1	Ether	Light orange	Yellow	Orange	Yellow brown	---	Orange
2	Chloroform	Light red	Yellow brown	Orange red	Light brown	Light brown	Yellow green
3	Acetone	Orange	Green Yellow	Dark Orange	Light brown	Green Yellow	Reddish orange
4	Benzene	Light Orange	Yellow green	Orange red	Light orange	Light brown	Orange
5	Petrol	Light Brown	---	Light orange	---	---	---
6	Methonal	Dark red	Light brown	Orange pink	Yellow brown	Yellow	---
7	Water	Dark red	Green brown	Green yellow	Red brown	Green yellow	Yellow green
8	Hexane	Light red	--	Orange	---	---	---

Table 4

The Flourescence Analysis of the roots of *R. cordifolia* and *R. tinctorum*

S. No.	Type of Extract	*R. cordifolia*			*R. tinctorum*		
		Day light	**Short wave length**	**Long wave length**	**Day light**	**Short wave length**	**Long wave length**
1	Ether	Light orange	Yellow green	Orange red	Light Yellow	---	Green Yellow
2	Chloroform	Red	Brown	Dark Orange	Light brown	Light brown	Green Yellow
3	Acetone	Light Orange	Yellow Green	Dark Orange	Light orange	Yellow Green	Orange green
4	Benzene	Light Orange	Yellow green	Orange red	Brown green	Light green	Yellow green
5	Petrol	Light Brown	---	Light orange	Light brown	---	---
6	Methonal	Red	---	Dark Orange	Light Red	Yellow green	Orange green
7	Water	Dark red	Green brown	Yellow green	Dark Red	Yellow Green	Yellow green
8	Hexane	Light red	--	Orange pink	---	---	---

Table 5

Some differentiating properties of the stems and the roots of *R.cordifolia* and *R. tinctorum*

S. No.	Properties	R. cord stem.	R.tinct stem	R. cord root	R. tinct root
1.	Petrol soluble extractive value	0.4%	0.8%	0.6%	0.7%
2.	Chloroform soluble extractive value	0.7%	1.8%	0.9%	1.6%
3.	Alcohol soluble extractive value	9.0%	20.0%	4.0%	38.0%
4.	Water soluble extractive value	32.6%	22.9%	37.8%	63.5%
5.	Total ash value	9.8%	8.2%	10.6%	7.95%
6.	Froth number	166.0	166.0	200.0	154.0
7.	Free anthraquinones	0.64%	0.28%	0.34%	0.38%
8.	Combined anthraquinones	0.98%	1.02%	0.62%	1.26%

Table 6

***Rubia* species : TLC : Rf Values : of different spots**

S. No.	Type of extract	Solvent systems	Visualizing agents	*R. cord.* stem	*R. tinct.* stem	*R. cord.* root	*R. tinct.* root
1	Ether	B:EF:FA 75:24:1	Ammonia vapours	0.11 0.22 0.48 0.53 0.66 0.79 --	0.14 0.24 0.48 0.71 0.81 -- --	0.10 0.19 0.27 0.51 0.70 -- --	0.06 0.19 0.45 0.51 0.56 0.69 0.80
2	Benzene	B:EF:FA 75:24:1	Methanolic KOH (10%)	-- --	0.63 --	-- --	0.66 0.80
3	Acetone	B:EA 9:1	KI + Iodine Soln. (10%)	0.35 0.55	0.33 --	0.32 0.52	0.33 --
4	Water	EA:M:W 77:13:10	Methanolic KOH (10%)	0.22 0.43 0.56 --	0.23 0.31 -- --	0.17 0.25 0.41 0.53	0.18 0.26 0.48 --
5	Methanol	B:EF:FA 75:24:1	Methonalic KOH (10%)	0.07 0.31 0.49 --	0.08 0.32 0.55 0.62	0.08 0.16 0.55 0.61	0.08 0.35 0.51 --

B: Benzene, EA : Ethyl Acetate, EF : Ethyl Formate, FA : Formic Acid, M : Methanol, W : Water

Observations

Table 1 to 6 summerise the comparative studies of the stems and roots of *R. cordifolia* and *R. tinctoru.* Morpholigically, the roots of both these species differ particularly in (i) size of the crown, being much larger in *R. cordifolia,* (ii) colour, dark reddish black in *R. cordifolia* and (iii) surface, more rough in *R. tinctorum.* Similarly the stems can be differentiated by (i) colour, being much darker in *R. cordifolia,* (ii) surface, very smooth in *R. cordifolia* and (iii) length of the internode, longer in *R. tinctorum.*

Histologically, the stems of the two species differ in (i) the size of the central pith, very narrow in *R. cordifolia,* (ii) the size of the xylem zone, very wide in *R. cordifolia,* (iii) size of the phloem, very wide in *R. tinctorum* and (iv) size of the xylem vessels, very large of *R. cordifolia.* Similarly, the roots of the two species, differ in (i) size of the central wood, very wide in *R. cordifolia,* (ii) size of the bark, very wide in *R. tinctorum* and (iii) size of the xylem vessel, bigger in *R. cordifolia.*

In physico-chemical characters, practically all the parameters were found to be different in the two species but the difference was more noticeable in the extractive values of alcohol and water. Phytochemically, the two species differ in the present of (i) **flavonoids** in *R. tinctorum* only and (ii) **coumarins** in *R. cordifolia* only. The TLC studies of different extracts of the drugs showed (i) the absence of 1,8-dihydroxy anthraquinone, in the ether extracts of *R. cordifolia* root only, (ii) no spots in benzene extracts of *R. cordifolia* stem and roots, (iii) only one spot, in stems and roots of *R. tinctorum* and two spots, in stems and roots of *R. cordifolia,* in the acetone extracts.

DISCUSSION AND CONCLUSION

Under the name of **Majith** or **Manjishtha,** even though, all the Ayurvedic Texts, mention *R. cordifolia L. (Rubiaceae),* as an official drug, commercial samples collected from all over India, were found to be comprising of *R. tinctorum L. (Rubiaceae),* which is usually sold in the Indian market under the name of **Irani Majith.**

R. cordifolia roots, which are commonly named as **Deshi Majith**, probably are not available in sufficient quantity, to meet the commercial demand of the Indian market. This may be one of the reasons for the substitution of *R. cordifolia* with that of *R. tinctorum* and hence to differentiate *R. tinctorum* from *R. cordifolia,* some morphological, microscopical and phytochemical distinguishing characters are discussed in the present paper.

The most striking morphological characters of *R. cordifolia* which distinguishes it from *R. tinctorum*, is its enlarged crown, dark reddish colour and the comparatively smooth surface of the root and stem pieces. Similarly, the bigger size of the xylem vessels and the greater area occupied by the xylem region, forms an important histological characters to distinguish *R. cordifolia* from *R. tinctorum.* Coumarins, which have been reported here for the first time in roots and stems of *R. cordifolia*, are absent in *R. tinctorum*. **Flavonoids** which have been reported in *R. tinctorum*, were found to be absent in *R. cordifolia* and hence presence or absence of these two species.

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REFERENCES

1. ANTARKAR, D.S., BHATT, M., CHINWALLA, T. and VAIDYA, A.B., *IInd. Jour. of Pharmacs.*, 15, 3, 185, 1984.

2. KOSUGE, T., OCHI, A., YOKOTA, M and YOSHIDA, M., Med. and Aro. *Plant Abs.*, Vol. 4, I, 25, 0169, 1982.

3. SHAH, S.C and GUPTA, L.K., *Indian Drgus*, Vol. 13, 47, 1976.

4. MASCARENHAS, C.D. D'SILVA, A. and ALI, M., Med. and *Aro. Plant Abs.* Vol.2, 156, 0698, 1980.

5. AGARWAL, S., Med. and *Aro. Plant Abs.*, Vol.7, 5, 412, 2599, 1985.

6. LASZLO, M., GYURJAN, I., DANOS, B., HOLLOSY, F., LISZT, K., KRETOVICS, J., SZOKAN, GY. and TOTH, Z., *Planta Medica*, Vol. 56,7, A613, 1992.

7. ANONYMOUS, *The Ayurvedic Formulary of India*, Ist ed., Manager of Publication, Delhi, 1978.

8. BAMBDAI, G.K., *Vanaspatisrushti*, Ist ed., Part II, Navjeevan Press, Ahmedabad, 158, 1951.

9. PANDEY, G.S.and CHUNKKAR, K.C., *Indian Materia Medica*, The Vidya-Bhavan Ayurvedic Granthmala, Varanashi, 28, 110, 1969.

10. VAIDYA, B.G., *Materia Medica*, 1st ed., University Grantha Nirman Board, Gujarat, 407, 1972.

11. SHARMA, P.V., *Dravyagunavignana*, Vol. II, 12th ed., Chaukhambha Bharati Academy, Varanasi, 800, 1991.

12. WESTENDORF, J., POGINSKY, B., MARQUAROT, H. and KRAUS, LJ., *Planta Medica*, Vol. 54,6,562,1988.

13. BLOMEKE, B., DOMINIAK, M., MARQUARDH, H., POGINSKY, B. and WESTENDORF, T. *Planta Medica*, Vol. 56, 6, 509, 1990.

14. EVANS, W.C. and TREASE, G.E., *Pharmacognosy*, 12th ed., Bailliere, Tindall, London, 716, 1983.

15. CHASE, C.R. and PRATT, R.J., J. *Amer. Pharm. Assoc.* (Sci. Ed.,), 38, 324, 1949.

16. ANONYMOUS, *The Pharmacopoeia of India*, 2nd ed., Government of India, Delhi, 947, 1966.

17. LIST, P.H. and HORHAMMER, L. *Hagera Handbuch der Pharmazeutischen Praxis*, Vol. I, Springer-Verlag Publication, Berling, 454, 1967.

18. ANONYMOUS, *The European Pharmacopoeia*, Vol.I, Council of Europe, Maisonneuve S.A., Publication, France, 361, 1969.