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Comparative Studies of *Rubia cordifolia* L. and its Commercial Samples

S. Pathania, R. Daman, S. Bhandari, B. Singh and Brij Lal*

Institute of Himalayan Bioresource Technology (CSIR),
Palampur-176 061(H.P.), India
*Corresponding author: brijihbt@yahoo.co.in

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Abstract

Rubia cordifolia L. (Family - Rubiaceae), is a common medicinal plant used in the preparation of different formulations in Ayurveda. The root of the plant is commonly known as Manjistha and its dried samples are sold in the market under the name Manjith. The present study was carried out to compare the authentic sample from its commercial samples keeping in mind the pharmacopoeial standards of Ayurveda. The quantitative phytochemical studies of the drug samples were carried out by studying the percentage of ash, extractive values and qualitative screening was carried out by Thin Layer Chromatography and different biochemical tests. Thus, the present work aims in forming certain parameters for identification of drug with the help of various phytochemical observations.

Keywords: Rubia cordifolia, commercial samples, roots.

Introduction

"Manjistha" *Rubia cordifolia*, L. (family-Rubiaceae), is an important herbal drug used in Indian system of medicine. The root of the plant is commonly known as Manjistha and sold in the market under the commercial name Manjith. Plant drug has number of vernacular names like Aruna, Bhandi, Bhandiralatik in Sanskrit, Mandar, Majathi in Assamme, Manjith, Manjistha in Bengali, Indian Madder in English, Manjithi in Malayalum, Manjestha in Marathi and Majit, Manjit in Hindi (Sharma, 1969). The roots of the plant are sweet, bitter, acrid and used as anti-inflammatory (Antarkar, *et al*. 1984) haemostatic (Kosuge, *et al*. 1982), antidysentric, antipyretic, analgesic, anthelmentic, improves the voice, the complexion and cures the Kapha, the inflammation diseases of the uterus, the vagina, the eye, the ear and the blood. It is also used in the cure of leucoderma, ulcers, urinary discharges, jaundice, and piles (Sivarajan and Balachandran, 1994).

The Ruberythric acid is one of the major constituents of the root and is widely used as phytotherapeutic drug in the treatment of calcium containing stones in the urinary tract (Laszlo, et al. 1992).

Rubia cordifolia is used in Ayurveda as an ingredient of popular formulations like Candana Sava, Asvagandhady Arista, Jatyadi Ghrta, Phala Ghrta, Brhanmajisthadi Kvatha Curna, Pinda Taila,

Manjisthadi Taila (Anonymous, 1978). The drug according to Ayurveda is Guru and Ruksa in Guna, Kasaya, Tikta and Madhura in Rasa, Katu in Vipaka and Usna in Veerya. Thus, due to its high medicinal value, the present work was carried out to study the root of authentic sample with the commercial samples from Gwalior, Coimbatore, Thiruvananthapurum, Palampur and Lucknow.

Materials and Methods

Genuine material of *Rubia cordifolia* (root-part), was collected from Himachal Pradesh and market samples were procured from the markets of Gwalior, Coimbatore, Thiruvananthapurum, Palampur and Lucknow. Voucher specimen of the plant is deposited in the Herbarium of Biodiversity Division of Institute of Himalayan Bioresource Technology, Palampur (H.P.).

Quantitative studies were carried out according to the methods described in Ayurvedic Pharmacopoeia of India -Part-I (Anonymous, 1989).

Thin layer chromatography technique was used for detection of number of major constituents in the samples. Diethyl ether, acetone, benzene, alcohol and methanol extracts of all the samples were run on the TLC plates pre-coated and manually prepared with silica gel-G. The resolution of plates was tried in different solvent system and best resolving system was chosen for running the plates, which were then exposed, to U.V., Iodine and Libberman-Buchard reagent (Wagner and Bladt, 1996). The R_f values were calculated as (Stahl, 1969; Ukanl, 1998).

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

Qualitative phytochemical evaluation was also carried out to test the presence of alkaloids, carbohydrates, glycosides, sterols, phenolics, saponins, resins, flavonoids, proteins and volatile oils in the drug and its commercial samples (Daniel, 1991).

Results

1. Distribution

Rubia cordifolia is common throughout the hilly tracts of India from the Northern western Himalayas, eastward ascending to 2500m. Also reported from Greece, Africa and other Asiatic countries like China, Japan, Afghanistan, Vietnam and Malaysia(Sivarajan and Balachandran, 1994; Chatterjee and Pakrashi, 1997).

2. Morphology

Rubia cordifolia is a perennial, herbaceous climber, roots long, cylindrical, flexuose, with a thin red bark. The stems are often many yards long, rough, grooved, becoming slightly woody at the base, bark white, petioles quadrangular, sometimes prickly on the angles, glabrous and shining. Fruits 4-6 mm, didymous or globose, smooth, shining and purplish black when ripe (Kirtikar and

Basu, 1935).

3. Quantitative Phytochemical Examination

On comparing commercial samples with authentic sample of *Rubia cordifolia* it was found that instead of root pieces all the collected samples were containing stem pieces. Further, the samples collected from Coimbatore and Thiruvananthapuram market were found to be of *Rubia tinctorum* as depicted in Fig. 1. Comparative morphological, microscopical and phytochemical studies of *Rubia cordifolia*, L. and *Rubia tinctorum*, L. have been reported (Dengre, *et al.* 1993).

Foreign matter percentage (excluding stem part) and the average percentage of total ash, acid insoluble ash, water soluble ash, alcohol, water and ether soluble extracts were calculated according to the standards described in Ayurvedic Pharmacopoeia of India and the results are depicted in Fig. 2, 3 and 4.[7-14]

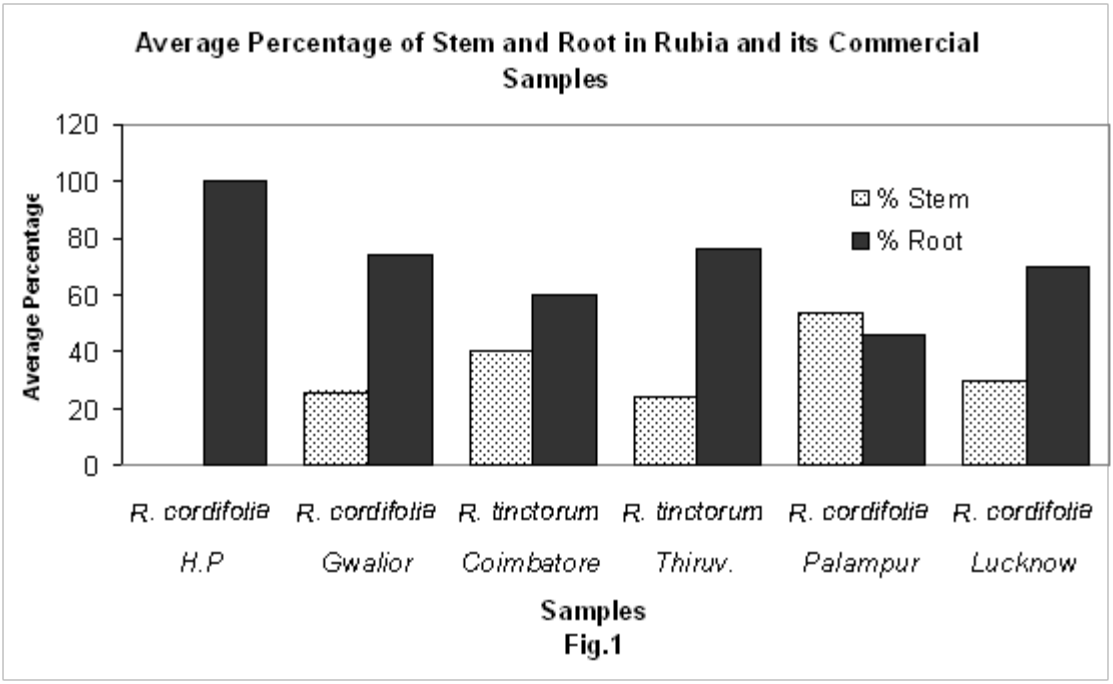

Fig.1

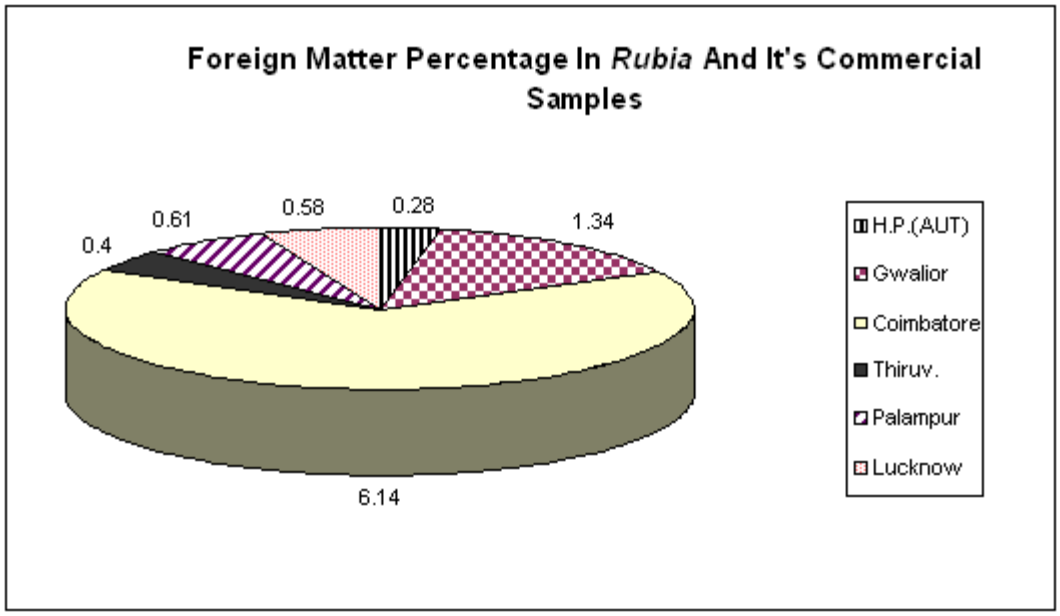
Foreign Matter Percentage In *Rubia* And It's Commercial Samples
0.4
0.61
0.58
0.28
1.34
6.14
H.P.(AUT)
Gwalior
Coimbatore
Thiruv.
Palampur
Lucknow

Fig.2

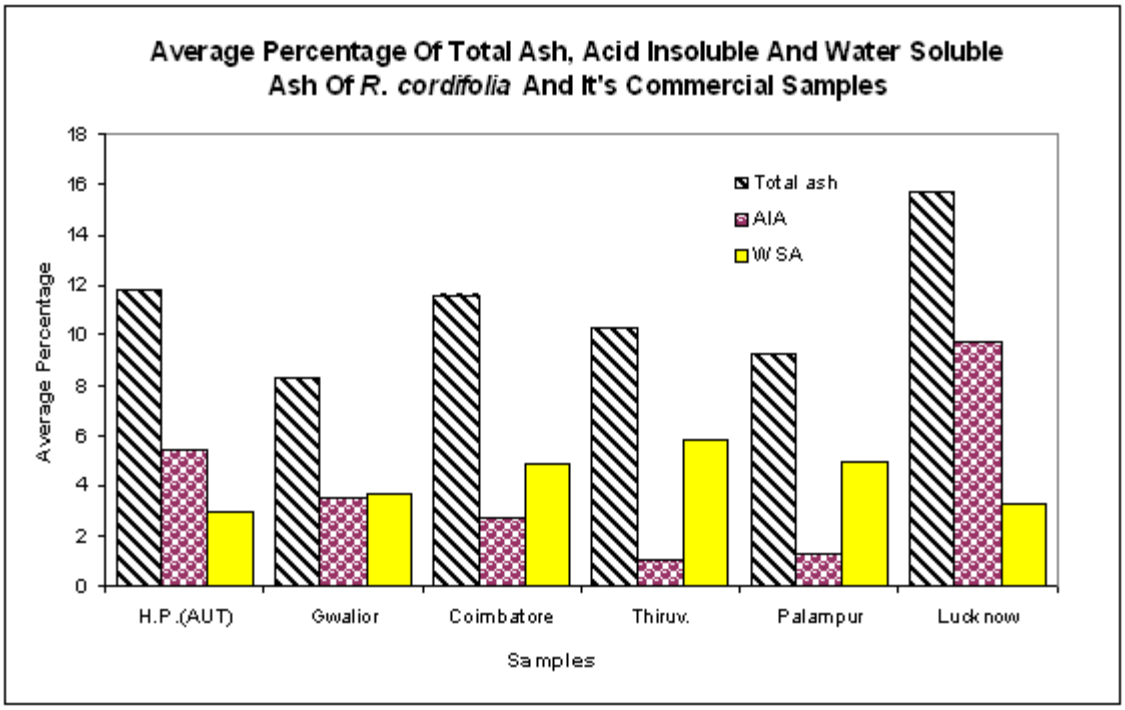
Average Percentage Of Total Ash, Acid Insoluble And Water Soluble Ash Of *R. cordifolia* And It's Commercial Samples
Average Percentage
0
2
4
6
8
10
12
14
16
18
Total ash
AIA
WSA
H.P.(AUT)
Gwalior
Coimbatore
Thiruv.
Palampur
Lucknow
Samples

Fig. 3

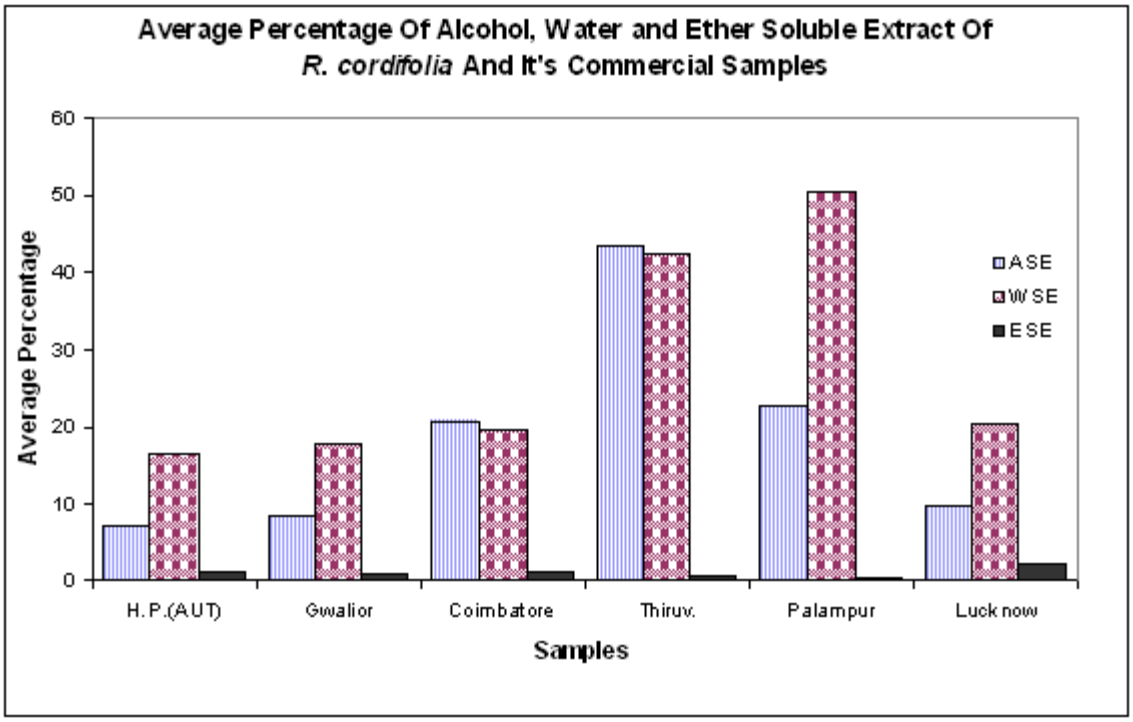

Fig. 4

4. Thin Layer Chromatography

TLC of *Rubia cordifolia* and its commercial samples was carried out, the resolution of TLC plates was tried in different solvent system and best resolving system was chosen for running the plates. The plates were then exposed to-

i) UV-light (Table-1)

ii) I_2-vapour (Table-2)

iii) Libberman -Buchard reagent (Table-3)

Table 1. - R_f values of different samples under UV.

S.No	Extract	TLC system	R_f values in UV
1.	Diethyl ether	Benzene: Ethyl acetate (9.9:0.1) ml	A. 0.085, 0.134, 0.488, 0.671, 0.720. B. 0.085, 0.134, 0.183, 0.317, 0.720. C. 0.243, 0.829. D. 0.243, 0.720. E. 0.085, 0.183, 0.256. F. 0.085, 0.134, 0.159, 0.317, 0.585, 0.720.

S.No	Extract	TLC system	R_f values in UV
2.	Acetone	Benzene: Ethyl	A. 0.145, 0.723.

		acetate (5:0.2) ml	B. 0.096, 0.145, 0.193, 0.313, 0.566. C. 0.193, 0.566. D. 0.096, 0.193, 0.723. E. 0.145. F. 0.096, 0.145, 0.265, 0.313, 0.422, 0.590, 0.711, 0.819.
3.	Benzene	Benzene: Ethyl acetate: Acetic acid (9.9:0.3:0.2) ml	A. 0.110, 0.207, 0.293, 0.573, 0.768. B. 0.293, 0.768. C. 0.207, 0.695. D. 0.217, 0.695. E. 0.293, 0.695. F. 0.159, 0.207, 0.695, 0.854.
4.	Alcohol	Benzene: Chloroform: Methanol (1:2:0.1) ml	A. 0.183, 0.354, 0.841. B. 0.354, 0.427, 0.683. C. 0.048, 0.305, 0.427. D. 0.134, 0.354, 0.427. E. 0.183, 0.305. F. 0.183, 0.256, 0.354, 0.402, 0.427, 0.616, 0.841, 0.890.
5.	Methanol	Toluene: Diethyl ether: Acetic acid (25:25:25) ml	A. 0.575, 0.80, 0.95. B. 0.575, 0.763, 0.80, 0.913. C. 0.70, 0.763. D. 0.213, 0.363, 0.70, 0.734, 0.80, 0.091. E. 0.575, 0.70, 0.763. F. 0.183, 0.256, 0.354, 0.402, 0.427, 0.616, 0.841, 0.890.

A. Himachal Pradesh (Authentic Sample)

B. Gwalior (Market Sample)

C. Coimbatore (Market Sample)

D. Thiruvananthapuram (Market Sample)

E. Palampur (Market Sample)

F. Lucknow (Market Sample)

(As in Table-1, 2, 3)

Table 2. - R_f values of different samples in Iodine.

S.No	Extract	TLC system	R_f values in Iodine
1.	Diethyl ether	Benzene: Ethyl acetate (9.9:0.1) ml	A. 0.549, 0.951. B. 0.085, 0.183, 0.951. C. 0.085, 0.183, 0.549, 0.951. D. 0.085. E. 0.085, 0.951. F. 0.085, 0.134, 0.427, 0.598, 0.829, 0.951.
2.	Acetone	Benzene: Ethyl acetate (5ml:0.2) ml	A. 0.096. B. 0.096, 0.313. C. 0.193. D. 0.096, 0.193. E. 0.193. F. 0.096, 0.193, 0.422, 0.590, 0.819, 0.928.
3.	Benzene	Benzene: Ethyl acetate: Acetic acid (9.9:0.3:0.2) ml	A. 0.073, 0.293, 0.695, 0.939. B. 0.695 C. 0.293. D. 0.207. E. 0.329. F. 0.293, 0.695.
4.	Alcohol	Benzene: Chloroform: Methanol (1:2:0.1) ml	A. 0.183. B. 0.354. C. 0.122, 0.305, 0.427. D. 0.305, 0.314. E. 0.354. F. 0.244, 0.427, 0.841, 0.898.
5.	Methanol	Toluene: Diethyl ether: Acetic acid (25:25:25) ml	A. 0.575, 0.65, 0.80. B. 0.163, 0.575, 0.70, 0.763, 0.80. C. 0.75, 0.86. D. 0.143, 0.80, 0.86. E. 0.163, 0.575, 0.70, 0.763. F. 0.163, 0.5.75, 0.70, 0.80, 0.913.

Table 3. - R_f values of different samples with LBR.

S.No	Extract	TLC system	R_f values in LBR
1.	Diethyl ether	Benzene: Ethyl acetate (9.9:0.1) ml	A. 0.103, 0.351, 0.422, 0.506, 0.70. B. 0.103, 0.156, 0.351. C. 0.103, 0.351, 0.831. D. 0.103, 0.357, 0.831. E. 0.103, 0.156, 0.357. F. 0.103, 0.357, 0.585.
2.	Acetone	Benzene: Ethyl acetate (5:0.2) ml	A. 0.113. B. 0.113, 0.163. C. 0.123, 0.183. D. 0.123, 0.175. E. 0.113, 0.163. F. 0.113, 0.513, 0.813.
3.	Benzene	Benzene: Ethyl acetate: Acetic acid	A. 0.074, 0.185, 0.679. B. 0.074, 0.185.

		(9.9:0.3:0.2) ml	C. 0.054, 0.185, 0.247. D. 0.044, 0.185, 0.247. E. 0.074, 0.185. F. 0.074, 0.185, 0.679, 0.877.
4.	Alcohol	Benzene: Chloroform: Methanol (1:2:0.1) ml	A. 0.146, 0.195, 0.280, 0.939. B. 0.146, 0.195, 0.280, 0.939. C. 0.073, 0.146, 0.195, 0.341, 0.378. D. 0.195, 0.341. E. 0.341, 0.378, 0.524. F. 0.232, 0.280, 0.378, 0.524, 0.622, 0.939.
5.	Methanol	Toluene: Diethyl ether: Acetic acid (25:25:25) ml	A. 0.692. B. 0.692, 0.756. C. 0.167, 0.577, 0.692. D. 0.167, 0.577. E. 0.692, 0.756. F. 0.692, 0.949.

5. Qualitative Phytochemical Evaluation

Preliminary phytochemical screening was performed to determine the class of phytoconstituents present in the drugs. The alkaloids were evaluated with Dragendroff's, Wagner and Mayer's test, carbohydrates with Molish and Fehling's test, glycosides with Keller Killani and Born Trage test, sterols with Libberman-Buchard and Salkovski reaction, phenolics with Ferric chloride, Lead acetate and Potassium ferricyanide solutions, saponins with Sodium bicarbonate solution, resins with distilled water and Acetone solution, flavonoids with Ammonia test, proteins with Nitric acid and Biuret test, volatile oils with spot and Bromine water test (Wagner and Bladt, 1996; Stahl, 1969; Ukanl, 1998; Daniel, 1991). The observations are presented in Table-4.

Table 4. - Preliminary Phytochemical Tests.

Chemical Groups	H.P. (Auth.)	Gwalior	Coimbatore	Thiruvananthapuram	Palampur	Lucknow
Alkaloids	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Sterols	+	+	+	+	+	+
Phenolics	-	-	-	-	-	-
Saponins	+	+	+	+	+	+
Flavonoids	-	-	+	+	-	-
Proteins	+	+	+	+	+	+
Volatile Oils	-	-	-	-	-	-

Discussion and Conclusion

The commercial samples of Manjith particularly from Coimbatore, Thiruvananthapuram and Palampur markets showed a high %age of variation in their Quantitative analysis (Fig. 3 - 4). This may be attributed to the presence of high %age of stem part in the commercial samples. The samples of Coimbatore and Thiruvananthapuram were quite dissimilar as compared to the other samples and were identified to be *Rubia tinctorum* on the basis of morphological, anatomical and bio-chemical analysis. These samples showed the presence of flavonoids which have been reported in *Rubia tinctorum* and found to be absent in *R.. cordifolia* (Dengre, *et al.* 1993). Hence to meet market demand *Rubia tinctorum* is sold under the name Manjith.

Although the uniformity in presence of different groups of secondary metabolites (Table-4) was observed in all the extracts spotted on TLC plates but number of spots varied, as Lucknow sample is having higher number of U.V. sensitive compounds (Table-1). Variability was also observed in I_2 exposed plates (Table-2) as well as in LBR sprayed plates (Table-3) followed by heating at 120^0 C. Therefore it can be concluded that although all the samples have similar type of secondary metabolites their quantity differs as evident from quantitative values and TLC spots. This is due to variation in the time of harvesting of sample, location of collection, storage conditions, drying conditions, and adulteration with stem parts and contamination with other species to meet commercial demand. Thus the study showes that there is adulteration in the market sample which requires to be identified and standardized to prove the claims of traditional system of medicine and to rekindle the faith of the masses in this age old Indian system of medicine.

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