Authentication of Kampillaka (Mallotus philippinensis): An Important Drug of Ayurveda (Indian Traditional Medicine)

Shradha, V Joshi, S Maurya, U Singh, G Nath, A Singh

Citation

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Abstract

Kampillaka (Mallotus philippinensis Muell. Arg.), a promising drug used since 1000 B.C. as anthelmintic, in dermal problems and infectious wounds is commonly found adulterated in the crude drug market. An attempt was made to authenticate the crude drug samples collected from different places of India by subjecting them to HPLC analysis with particular reference to phenolic acids. The sample from Dehradun was found to have high amount of phenolic acids than other samples. Pharmacological activity of the phenolic acids was seen to correspond with the medicinal property of the drug.

INTRODUCTION

Kampillaka, one of the Audbhida dravya (vegetable drugs) is well described in Charaka Samhita[1] and Sushruta samhita[2] (Ayurvedic classics of ancient traditional medicine of India). Glands and hairs of the fruits (phalarajah) and seed oil (beejataila) are administrated in various disease conditions. The glands and hairs of the fruits are used to remove intestinal worms and also as a purgative. Its oil is indicated in dermal problems and non-healing wounds[1,2]. It is stated to be undoubtfully effective in intestinal worms when administered with jaggery[3].

Mallotus philippinensis Muell. Arg. belongs to the family Euphorbiaceae. The tree grows throughout tropical India and particularly along the foot of Himalaya from Kashmir Eastwards upto a height of 1500 m[4,5]. During the month of February-March its fruits ripen becoming brick-red in colour[6], which is synonymous to Raktangi that is during fruiting season the tree is covered with red fruits[7]. The mature fruits are collected and the hairs and glands are gently separated from them.

Due to increase in demand of crude drugs by pharmaceutical industries and others, adulteration and/or substitution is the common practice by drug traders. This kind of business is accelerating very rapidly because of increased demand of plant-based drugs throughout the world. With a greater realization of tremendous potential of the therapeutic uses and economic values of herbal therapy, the marketed drugs are frequently adulterated as collection of glandular hairs is quite laborious and the material may not be enough to meet the demand. The powder is adulterated with Annatto dye (Bixa orellana Linn), ferric oxide, brick dust, ferruginous sand, Casearia tomentosa stem bark powder and fruit hairs of Flemingia macrophylla [8]. The pharmaceutical industries, traditional Ayurvedic physicians and research institutions are dependent on drug traders for their need. In Indian crude drug market of different regions, the drug is supplied in the name of Kamila/Kabila/Rohini.

The World Health Organization (W.H.O) has given guidelines to the member states to ensure about genuine use of plants and their parts before their use for human health[9]. In view of the above direction by W.H.O. it was thought to have a survey of crude drug market.

The samples were collected from different places of India and subjected to chemical analysis with particular reference to phenolic acids through High Performance Liquid Chromatography (HPLC).

MATERIALS AND METHODS

COLLECTION OF SAMPLES AND EXTRACTION OF PHENOLIC COMPOUNDS

The phenolic acids were extracted as per the method of Singh et al. [10]. Ten samples of Mallotus philippinensis were collected from different places of India. One gram of each

sample was macerated and suspended in 5 ml ethanol-water (80:20; v/v). The collected samples were subjected to ultrasonication (Branson Sonifier, Danbury, CT, USA) for 15 min at 4°C followed by centrifugation at 12 500 x g for 15 min. The clear yellowish supernatant was subjected to charcoal treatment. The residue was re-extracted twice with the same extracting solution and the supernatant was pooled prior to evaporation under vacuum (Buchi Rotavapor Re Type, Labco, India; Ambala Cantt. India). Dried samples were resuspended in 1.0 ml high-performance liquid chromatography (HPLC)-grade methanol by vortexing and filtered through ultra membrane filter (pore size 0.45 µm: Millipore) before HPLC analysis.

HPLC ANALYSIS

Quantitative analysis of the samples was performed according to the method of Singh et al[10]. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable Shimadzu SPD-10 AVP UV-VIS detector and a Rheodyne Model 7725 injector with a loop size of 20 µl. The peak area was calculated with a Winchrom integrator. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250 x 4.6 mm i.d., particle size 5 µm, Luna 5µ C-18(2); phenomenex, Torrance, CA, USA) at 25°C. Running conditions included: injection volume, 5µl; mobile phase, methanol: 0.4% acetic acid (80: 20 v/v); flow rate, 1 ml/min; and detection at 290 mm. Samples were filtered through an ultra membrane filter (pore size 0.45 µm; E-Merck, Darmstadt, Germany) prior to injection in the sample loop. Tannic, gallic, vanillic, caffeic, ferulic, chlorogenic and cinnamic acids were used as internal and external standards. Phenolic acids present in each sample were identified by comparing chromatographic peaks with the retention time (R_t) of individual standards and further confirmed by co-injection with isolated standards. The amount of each phenolic acid is expressed as micrograms per gram of fresh weight unless otherwise stated.

RESULTS AND DISCUSSION

Recent researches indicate that phytophenols, being chief secondary metabolites, are present in rich amount in several plants. Many of them posses antioxidant, antiinflammatory and several other therapeutic properties. (Table 1.). Fig. 1,a shows the peaks of reference phenolic compounds. The analysis of different samples of M. philippinensis showed that, Dehradun sample had six phenolic acids. (Fig. 1,b) i.e., vanillic, ferulic, chlorogenic, cinnamic, oxalic and salicylic acids, in which oxalic acid (262.567 µg/g) was maximum followed by ferulic (368.302 µg/g), chlorogenic (153.85 µg/g), cinnamic (33.359 µg/g) and salicylic acids (3.102 µg/g) but vanillic acid (0.0376 µg/g) was in trace. Dehradun sample mixed with jaggery revealed ferulic acid maximal (127.649µg/g) followed by chlorogenic acid (55.351µg/g), gallic (4.605 µg/g) vanillic (2.154 µg/g) and caffeic acids (1.944 µg/g) (Fig. 1,c). Samples from Udupi (Fig. 1,e) and Bangalore (Fig.1,f) had two phenolic acids (caffeic and oxalic acid) but in Varanasi sample (Fig. 1,d) only caffeic acid was observed. Sample from

Figure 1

Table 1: Therapeutic uses of phenolic acids

Phenolic acids	Therapeutic uses
Phenolic compounds	Antioxidant[11]
Tannic acid	Astringent, haemostatic, in solution for burns, internally as an astringent. Heavy metal antidote[12]
	Antioxidant[13]
Gallic acid	Antinflammatory[14]
	Antibacterial against Gram negative and Gram positive bacteria[15]
Caffeic acid	Probably account for the clinically established antiviral activity[16]
	Anti-inflammatory[17]
	Antibacterial and Antifungal[18]
	Antioxidant[7,19,20,21]
Vanillic acid	Antisickling property in sickle cell anaemia[22]
Ferulic acid	Anti-inflammatory[17]
	Antifungal[23,27]
	Antioxidant[24]
Chlorogenic acid	Antioxidant[23]
Cinnamic acid	Anti-viral activity[25]
	Antioxidant[26]
	Antifungal[27]
	Natural protection against infections by pathogenic micro-organism[28]
	Anthelmintic[12]
P.Coumaric	Antifungal[27]
Oxalic acid	Haemostatic agent (vet)[12]
Salicylic acid	Antipyretic and antiflammatory[29]
	Topical keratolytic, Externally as antiseptic, antifungal agent and for various skin conditions[12]

Figure 2

Figure 1: HPLC of various market samples of (a) Standard phenolic acids, (b) (Dehradun), (c) (Dehradun + Jaggery), (d) (Varanasi), (e) (Udupi), (f) (Bangalore), (g) (Lucknow), (h) (B.H.U. Garden, Immature), (i) (B.H.U. Garden, Mature), (j) (Mumbai), (k) (Jaipur). Peak Nos. 1, tannic acid; 2, gallic acid; 3, oxalic acid; 4, caffeic acid; 5, vanillic acid; 6, ferulic acid; 7, chlorogenic acid; 8, o-coumaric acid; 9, cinnamic acid; 10, salicylic acid

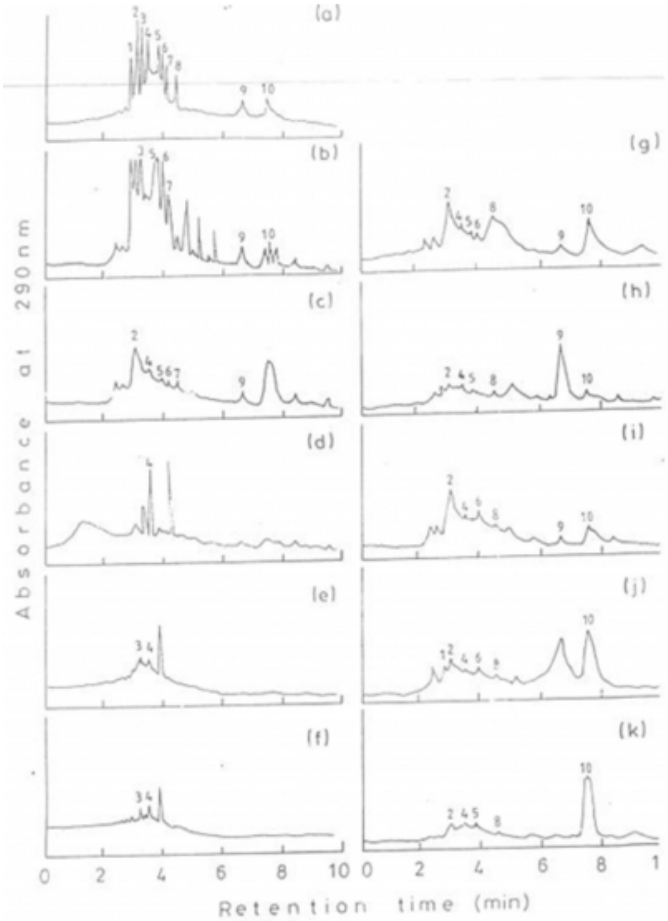

Figure 3

Table 2: Phenolic acids in the fruits of glands and hairs of

Plant	Place of Collection	Phenolic acid (µg/g dry wt)									
		TA	GA	Caff A	VA	FA	Chl A	CA	O Coum	OA	SA
Mallotus philippinensis	**Dehra Dun**				0.036	368.302	153.853	33.359		262.5673	3.102
M. philippinensis +Jaggery	Dehradun		4.605	1.944	2.154	127.649	55.351	13.744			
M. philippinensis	**Varanasi**			2.829							
M. philippinensis	Udupi			0.662						3.781	
M. philippinensis	Bangalore			0.378						5.514	
M. philippinensis	Lucknow		2.443	0.358	0.361	0.352		2.336	0.063		1.458
M. philippinensis (Immature)	B.H.U. Garden		0.869	0.609	1.098			0.849	0.374		0.697
M. philippinensis (Mature)	B.H.U. Garden		2.069	0.453		0.417		2.412	0.448		0.371
M. philippinensis	Mumbai	0.383	3.298	0.725		0.882			1.114		6.859
M. philippinensis	Jaipur		1.289	1.458	1.452				0.502		3.914

TA = Tannic acid, GA = Gallic acid, Caff-A = Caffeic acid, VA = Vanillic acid, FA = Ferulic acid, Chl. A = Chlorogenic acid, CA = Cinnamic acid, O-Coum = O-coumaric acid, OA = Oxalic acid, SA = Salicylic acid, O-Gentisic = O-Gentisic acid.

Lucknow (Fig. 1,g) had seven phenolic acids eg. gallic, caffeic, vanillic, ferulic, cinnamic, o-coumaric, salicylic acids. Sample of immature fruits from Ayurvedic garden of Banaras Hindu University (Fig. 1,h) had six phenolic acids in which vanillic acid was maximum. Mature hairs and glands from fruits from the garden of Banaras Hindu University (Fig. 1,i) revealed cinnamic acid (2.412 µg/g) in maximal amount followed by gallic (2.069 µg/g), ferulic (0.417 µg/g) Caffeic (0.453 µg/g) and O-coumaric (0.448 µg/g). Salicylic acid was maximum in the sample of Mumbai (Fig. 1,,j) followed by gallic, o-coumaric, tannic, caffeic, ferulic acids. That of Jaipur (Fig. 1,k) had salicylic (3.914 µg/g), gallic (1.289 µg/g), vanillic (1.452 µg/g), caffeic (1.458 µg/g) (Table 2.).

M. philippinensis, a plant with very high medicinal value, has been in use since 1000 B.C. It is stated to be effective in non-healing or infectious wounds, dermal problems, intestinal worms etc. Therapeutic useful parts of the plant are glands and hairs of mature fruit which is commonly adulterated on account of less yield and laborious collection. The adulteration reduces clinical efficacy. With a view to see the quality of the drug, several samples were collected from different places of India and subjected to HPLC analysis with special reference to phenolic acids which are secondary metabolites and play a major role in human health.

Diverse pharmacological activities have been accredited to phenolic acids for instance, gallic acid has antiinflammatory[14], antibacterial[15], caffeic acid with anti inflammatory[17], antibacterial, antifungal[18]; ferulic acid with anti-inflammatory[17], antifungal[27]; cinnamic acid with antifungal[27], anthelmintic[12], natural protection against infections by pathogenic microorganisms[28]; salicylic acid with antipyretic and antiinflammatory[29], externally used as antiseptic, antifungal and for various skin conditions[12].

HPLC analysis of the samples revealed wide-variability in their phenolic acid content. Maximal amount of phenolic acids was detected in Dehradun sample. A comparative analysis between immature and mature samples showed increase in cinnamic acid and gallic acid content in the later. M. philippinensis is a well known anthelmintic drug. Cinnamic acid has anthelmintic property. Cinnamic acid was maximal in Dehradun sample followed by the sample mixed with jaggery. Rest of the samples had either minimal or absence of cinnamic acid indicating genuinity of the sample of Dehradun. Ferulic and salicylic acids are also found in Dehradun sample which may contribute to the anthelmintic action of M. philippinensis in infectious or nonhealing wounds. This is the first report of phenolic acid estimation in M. philippinensis.

CONCLUSION

Mature glands and hairs of Mallotus philippinensis are commonly used as anthelmintic. Samples of these materials obtained from different places of India showed adulteration which reduced clinical efficacy in curing the disease caused by helminths in human beings. High performance liquid chromotographic (HPLC) analysis of different phenolic acids indicated that Dehradun sample was best as it contained maximum phenolic acids as compared to other samples. Cinnamic acid was maximum in Dehradun sample. Based on these results only Dehradun sample is recommended for use against worm infestation in human beings.

CORRESPONDENCE TO

Tel: 91-542-2318388; (M): 919415354325 E-mail: upneem@sify.com; upneem@gmail.com

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Author Information

Shradha, B.S., MD (Ay.), Ph.D. Scholar
Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University

V.K. Joshi, MD (Ay.), Ph.D.
Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University

S. Maurya, MSc (Ag), Ph.D Scholar
Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University

U.P. Singh, MSc (Ag), Ph.D
Director, Institute of Bioengineering and Biological Sciences

G. Nath, M.D.
Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University

Amitabh Singh, M.Sc. (Ag), Ph.D
Plant Pathologist, Institute of Bioengineering and Biological Sciences