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Pharmacognostic and phytochemical evaluation of leaf galls of *Kakadshringi* used in Indian system of medicine

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Kakadshringi, a well-known crude drug used as antidiarrheal in Indian system of traditional medicine, is leaf gall of *Pistacia integrrima* Stew. ex Brindis., *Terminalia chebula* Retz. and *Garuga pinnata* Roxb. This study presents pharmacognostic and phytochemical evaluation on galls of three botanically different plant species to establish identification markers. Phytochemical analysis revealed that gallic acid and phenol content were higher in *P. integrrima* than other two samples. Occurrence of gallic acid was: *P. integerrima* (15.41 ± 0.016 mg/g) > *T. chebula* (8.65 ± 0.024 mg/g) > *G. pinnata* (6.80 ± 0.0113 mg/g). Thus, *P. integrrima*, which contains higher amounts of tannins than *T. chebula* and *G. pinnata*, is recommended as potential for antidiarrheal activity.

Keywords: *Garuga pinnata,* HPTLC profile, *Kakadshringi*, Pharmacognosy, *Pistacia integerrima, Terminalia chebula*

Introduction

Kakadshringi, leaf galls of botanically different plant species, are used as antidiarrheal in Indian System of traditional medicine. It is one of the main components in *Bal-Guti*, *Karkatadi churna*, *Brihattalishadi churna*, and *Balachatur bhadra*[1-3]. As *Kakadshringi*, galls on *Pistacia integerrima* Stew. ex. Brandis. (*Anacardiaceae*) are commonly marketed in North India, whereas leaf galls on *Terminalia chebula* Retz. (*Combretaceae*) are used in commerce in South Indian market. Galls on *Rhus succedanea* L. (*Anacardiaceae*) are also reported as adulterant[4]. Galls on *Garuga pinnata* Roxb. (*Burseraceae*) are being used by local people in Western Ghats of Maharashtra and observed as admixture with others in market samples[5].

This paper presents pharmacognostic and phytochemical studies of leaf galls of *P. integrrima*, *T. chebula* and *G. pinnata* using macroscopy, microscopy, physicochemical and phytochemical analysis to establish identification and authentication of *Kakadshringi*.

Experimental Section

Materials and Methods

Galls on *T. chebula* and *G. pinnata* were collected from Mahabaleshwar, Satara and Vazeghar, Purandhar, Pune, Maharashtra during winter season 2007-2008 respectively. Galls on *P. integerrima* were purchased from Pune crude drug market. Samples were authenticated and deposited in crude drug repository of Agharkar Research Institute, Pune 411 004, vide voucher specimen numbers *P. integrrima* Stew. ex Brindis. - AO 013, *T. chebula* Retz. -AO 018 and *G. pinnata* Roxb -AO 020.

All samples were powdered, sieved (80-mesh) and stored in an airtight container at 25°C. Macroscopic characters were studied as per standard methods[6]. Powder analyzed as per standard procedure[7]. A small quantity of powdered material was washed with water to remove sugar and cleared by heating gently with saturated choral hydrate solution, cooled and mounted in glycerin for microscopic observation. Phloroglucinol stain and dilute iodine solution were used to observe lignified stone cell and starch grains respectively.

Physiochemical and HPTLC Studies

Physicochemical values of total ash, acid insoluble and water-soluble ash and petroleum ether, alcohol, and water soluble extractives were calculated as per Indian Pharmacopoeia[6,8] and tannin[9], phenol[10] content were determined. Phytoconstituents (triterpenoids, steroids, alkaloids, sugar, tannins, glycosides and flavanoids, etc.) were detected by usual prescribed methods[11]. For HPTLC study, each sample (1 g) was boiled in 2 N hydrochloric

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Table 1—Comparative distinguishing characters of three samples of Kakadshringi

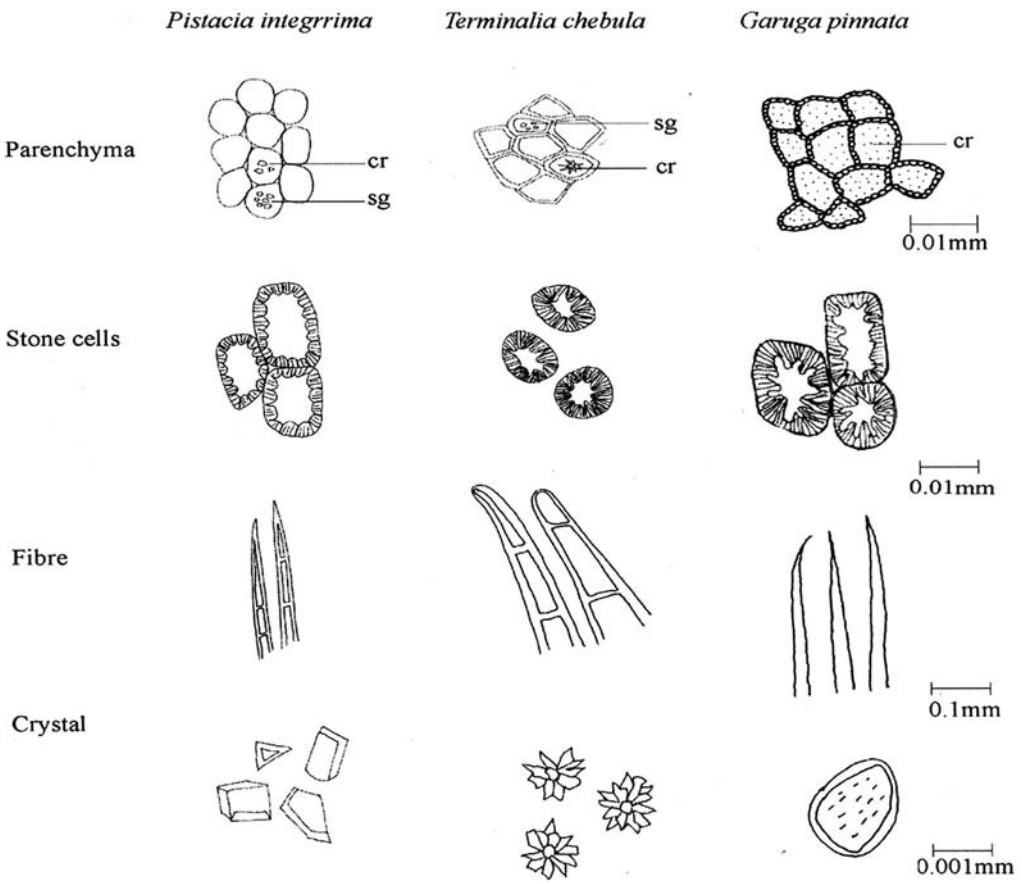
No.	Character	*Pistacia integrrima* Stew. ex Brindis.	*Terminalia chebula* Retz.	*Garuga pinnata* Roxb.
1	*Macroscopy*			
	Size	3-9 cm long, 2-3 cm diam	2-3 cm long, 1.6-4 cm wide	1.5-2.5 cm long, 0.8-1.2 cm wide
	Shape	Curved elongated like horn, hollow, twisted, broader at one end and tapering to other end	Fan shaped, some are oval oblong	Oval, some times beaked
	External markings	Longitudinal ridges	Faint granular markings	Faint granular markings
	Fracture	Easy to break	Vary hard	Hard
	Opening	Round	Slit like oval	Not well marked
	Colour	External and internal reddish brown	External yellowish- brown, internal brown	External reddish-yellow, internal reddish-brown
	Odour	Aromatic	Not characteristic	Not characteristic
	Taste	Astringent	Not characteristic	Not characteristic
2	*Microscopy*			
	Powder analysis	Thick walled parenchyma cells with prismatic crystals and starch grains; rectangular highly thick stone cells; some stone cells with narrow boundaries and broad lumen; fragments of septated thick walled fibers with oblique ends.	Thick walled, polygonal lignified parenchyma cells with rosette crystals; small starch grains; stone cells radiating pitted with narrow lumen; some stone cells are thin walled oval to spherical with wide lumen; fragments of fiber very long, broad lumen, septated with blunt end.	Beaded phloem parenchyma; thin walled parenchyma with dark yellow content, microsphenoidal crystals, starch grains abundant and round; stone cells squarish to oblong with lumen; thick walled, fragments of non septated fibers with pointed end.
3	*HPTLC profile* of processed extract under 254 nm	Nine bands at Rf 0.09, 0.13, 0.24, 0.33, 0.51, 0.60, 0.73, 0.88, 0.91.	Eight bands at Rf 0.15, 0.34, 0.51, 0.65, 0.87, 0.91, 0.95.	Five bands at Rf 0.13, 0.30, 0.50, 0.61, 0.90.

acid (5 ml) for 30 min, cooled, filtered and extracted with diethyl ether (10 ml, three times). Diethyl ether extract was concentrated and dried. Known quantity of extract was dissolved in methanol and used as test solution for HPTLC. Gallic acid (Fluka 48630) was used as reference solution. Calculated quantities of methanolic extracts along gallic acid were applied on HPTLC (pre-coated silica gel G 60 F_{254} Merck) aluminum plate (20 cm x 10 cm) by using CAMAG Linomat applicator IV. Plate was eluted to a distance of 8.5 cm at room temperature (25°C) in a solvent system [chloroform: ethyl acetate: formic acid (46: 44: 1, *v*/*v*/*v*)] in previously saturated twin through chamber (CAMAG). Dried plate was scanned initially at 254 nm for quantitative analysis and then at 280 nm (with λ_{max} of gallic acid) using a Camag Scanner 3 (CAMAG) with software CATS 4. Photo-documentation was done under UV 254 nm (CAMAG) using Olympus- CAMEDIA c-7070. Results of all experiments were reported as mean ± S.E.M of three replicates.

Results and Discussion

Galls on *P. integerrima*, *T. chebula* and *G. pinnata* attributed to *Kakadshringi*, can be differentiated on the basis of macro-microscopic characters (Table 1, Fig. 1). Shape of galls on *T. chebula* is fan like, *P. integerrima* as horn like and *G. pinnata* as oval like. Gall opening in *P. integerrima* is round and slit like oval in *T. chebula.* Presence of prismatic crystals in *P. integerrima*, rosette in *T. chebula* and microsphenoidal in *G. pinnata* identify three species. Tannin and phenol content in *P. integerrima* were higher than other two samples (Fig. 2). Presence and absence of different phytoconstituents were detected (Table 2).

Comparative HPTLC finger print of galls on *P. integerrima*, *T. chebula* and *G. pinnata* shows significant



cr-Crystal; sg- Starch grain

Fig. 1–Comparative microscopy –powder analysis of *Kakadshringi* samples

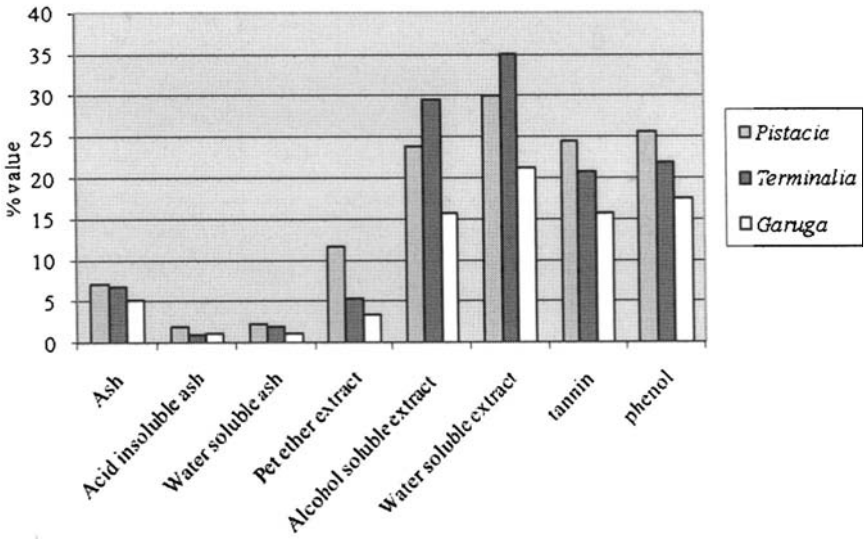
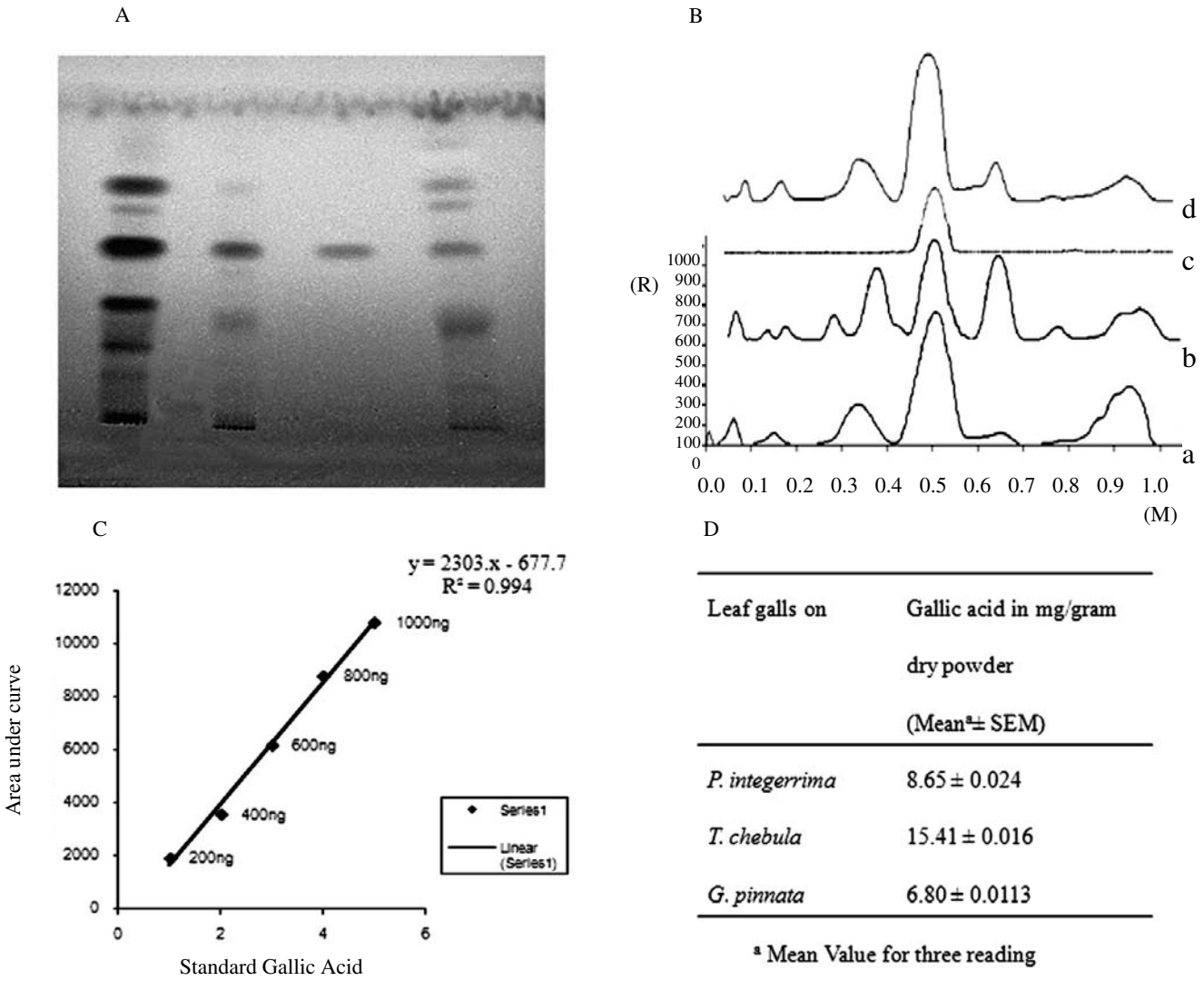


Fig. 2–Physicochemical parameters of *Kakadshringi*



Leaf galls on	Gallic acid in mg/gram dry powder (Mean[a] ± SEM)
P. integerrima	8.65 ± 0.024
T. chebula	15.41 ± 0.016
G. pinnata	6.80 ± 0.0113

[a] Mean Value for three reading

(A) HPTLC profile under 254 nm 1. *Pistacia integerrima,* 2. *Terminalia chebula,* 3. gallic acid, 4. *Garuga pinnata* (B) TLC densitometry scanning at 280 nm a. *Pistacia integerrima,* b. *Terminalia chebula,* c. gallic acid, d. *Garuga pinnata* (C) Linear regression of gallic acid (D) Quantification of gallic acid

Fig. 3–HPTLC profile of three samples of *Kakadshringi*

Table 2—Preliminary phytochemical screening of three samples of Kakadshringi

Chemical constituents	*Pistacia integerrima*	*Terminalia chebula*	*Garuga pinnata*
Triterpenoids	+	+	-
Steroids	-	-	-
Alkaloids	-	-	-
Flavonoids	+	+	+
Reducing sugars	+	+	+
Glycosides	-	+	+
Tannins	+	+	+
Saponins	-	-	-

+ Present, - Absence

disparity. One major band (dark brown at R_f 0.51) was comparable with standard gallic acid observed at identical R_f 0.51. Calibration curve of marker gallic acid was in the range of 200-1000 ng. Amount of gallic acid in galls were; *P. integerrima*, 15.41 ± 0.016; *T. chebula*, 8.65 ± 0.024; *G. pinnata*, 6.80 ± 0.0113 mg/g (Fig. 3).

Conclusions

Presence of higher amount of phenol and gallic acid in *P. integerrima* is recommended as potential for antidiarrheal activity. Pharmacognostic, phytochemical, physicochemical parameters and HPTLC profiles developed for each source will be helpful to

pharmaceutical industries for quality control, ensuring batch to batch consistency of raw drug and in the field of medical and pharmacological evaluation.

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