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Pharmacognostical and chromatographic evaluation of market sample of rhododendron arboreum stem bark as a source plant for Rohitaka in Nepal

Dr. Puneshwar Keshari, Dr. Pradeep and Suchitra N Prabhu

Abstract
Rohitaka is one of the major drug mentioned in Ayurveda classics for spleenic and hepatic disorders. *Tecomella undulata* (Sm.) Seem. is considered as genuine source of Rohitaka. It is habitant of dry arid zone of India, Pakistan and Afghanistan. In Nepal *Rhododendron arboreum* Sm. is marketed and used by the name of Rohitaka. Traditionally it is also used in liver diseases. In this study preliminary pharmacognostic, phytochemical evaluation and HPTLC analysis of market samples of *Rhododendron arboreum* were done. The study revealed almost similar characteristics and phytoconstituents in both sample. Alkaloids, Tannins, Saponin, Cardenoloids, reducing sugars are present in both samples. These secondary metabolites are responsible for its pharmacological actions.

Keywords: Rohitaka, Ayurvedic classics. Hepatic disorders, *Tecomellla undulata*, *Rhododendron arboreum*, Pharmacognostic evaluation, HPTLC.

1. Introduction
Rhododendron arboreum Sm. family Ericaceae the national flower of Nepal and known as Laligurans and is the state tree of Uttarakhand [1]. It is evergreen, much branched tree, up to 14 m. in height and 2-4 m. in girth, found in the Himalayas from Kashmir to Bhutan and in the hills of Assam and Manipur at altitudes of 1200 to 4000 m. In Nepal it is abundantly found in Hilly region from east to west. Bark is reddish brown, soft, rough, exfoliating in thin flakes, 2.5 cm. thick; leaves lanceolate or oblong, 10- 20 cm. × 3.6 cm. crowded towards the end of branches, petiolate, covered with white scales when young; flowers showy, in shades of red, or nearly white, sometimes spotted, in many flowered, head like corymbs; fruit capsule oblong, curved, longitudinally ribbed, up to 3.8 cm. × 1.25 cm.; seeds minute, dark brown, compressed oblong [2].
Rhododendron arboreum Sm. to be tentatively accepted as Kurabaka described in Charak Samhita chikitsa sthana chapter 30, and it is kept in kashaya(astringent) group in Sushruta Samhita [3].
Rohitak is described as Yakritpleehgulmodarhar (useful in liver disorders, spleenic disorders, abdominal lumps and gastropathy) [4-5]. It is beneficial in liver and spleen disorders and has hepatoprotective action [6] and used in diseases like Kamala(Jaundice), Yakritroga(Liver disorders), Vibandha (Constipation), Gulma (abdominal lump), Raktavikara (haematological disorders) etc [7]. Rohitak soaked in Haritaki (*Terminalia chebula* Retz.) water or cow's urine for seven nights, orally administered is useful in Kamala [8].
Tecomella undulata (Sm.) Seem is considered as the original source of Rohitaka which is found in drier parts of Arabia, southern Pakistan, Afghanistan and North – West India. In India it occurs naturally in Maharashtra, Gujarat, Rajasthan, Punjab and Haryana [9].
In crude drug market of Nepal *Rhododendron arboreum* Sm. is sold by the name of Rohitaka and considered as substitute of *Tecomella undulata* (Sm.) Seem [10-12]. The Nepali name of Rohitaka is given as Guransa in Chandra Nighantu which is a hand written famous manuscript in Nepal and kept in Singha Darbar Vaidyakhana Vikas Samiti (SDVKVS) in Kathmandu. In this manuscript manually drawn picture of Rohitaka is given which is Guransa and is botanically identified as *Rhododendron arboretum* Sm. Traditional practitioners use this drug for liver disorders like Jaundice (Kamala), hepatitis, hepatomegaly etc [10, 11]. Leaves, flowers, bark are used for various purposes traditionally and in Ayurvedic practices. The dried flowers of *R. arboreum* are highly efficient in the treatment of diarrhea and blood dysentery and young leaves are used in alleviating headache when applied on forehead. Flowers are also used for the preparation of squash used in mental retardation [13, 14]. It has been reported that *R. arboreum* possess anti-inflammatory, hepatoprotective, antidiarrhoeal, antidiabetic,

antioxidant, adaptogenic and antimicrobial activities [14,15]. The different parts of *R. arboretum* contains different types of phytoconstituents for example; from bark isolated compounds are Taraxerol, Betulinic acid, Ursolic acid acetate; Arbutin, Hyperoside, Amyrin, Epifridilenol from leaves; while Quercetin, Rutin, Coumaric acid are isolated from flower [16].
Pharmacognosy is an important link between Pharmacology and Medicinal chemistry, means it is an important bridge between the pharmaceutical and basic sciences. It provides a system wherein the active principles of crude drugs derived from natural origin could be dispensed, formulated and manufactured in dosages forms acceptable to allopathic system of medicine. A systemic study of crude drug under pharmacognostic scheme involves its description on the following ways [17].

1. Official title, synonyms, or vernacular names,
2. Biological source and family
3. Geographical source or habitat
4. History and introduction of crude drug
5. Cultivation, collection, processing for market and commerce in crude drugs
6. Morphological or macroscopic characters
7. Microscopic or histo-pathological studies
8. Chemical constituents and qualitative chemical tests
9. Pharmacological actions, therapeutics and other pharmacological preparations or formulations
10. Commercial varieties, substitutes and adulterants.
11. Quality control of crude drugs and phytopharmaceuticals derived from them.

In this study an attempt is done to evaluate the two market samples of Rohitaka (*Rhododendron arboretum*-collected from two different areas of Kathmandu, Nepal) by basic steps of pharmacognosy i.e. macroscopic and organoleptic tests, powder microscopy, physical evaluation, preliminary phytochemical tests and High performance thin layer chromatography (HPTLC).

Material and Methods

- **Collection of Plant materials**- Market samples of Rohitaka (bark of *Rhododendron arboreum*) were collected from two different places of Kathmandu, Nepal. Sample no.1 was collected from Bhedasinga area while Sample no. 2 was collected from Teku area and both samples were identified and authenticated by Department of Dravya-Guna, SDM College of Ayurveda and Hospital, Hassan, Karnataka, India.
- **Macroscopic study/ organoleptic test-** The macroscopic study of two samples of bark of *R. arboretum* was done by the morphological description of the bark seen by naked eye and magnifying lens. Organoleptic evaluation was done by means of sense organs and shape, size, colour, odour, taste and fracture of stem bark were evaluated [17.18].
- **Microscopic study**- The pieces of bark of both sample no. 1 and sample no. 2 were soaked in water for 72 hrs and tried to take transverse sections and longitudinal sections. After taking sections temporary slides were prepared and observed under electrical compound microscope under 10 x and 25 x.
- **Powder microscopy**- Fine powder of both sample no.1 and sample no.2 were prepared in Khalwa yantra and powder microscopy were studied as per the standard procedures by capturing the images of different fragments of tissues [17, 18].
- **Physiochemical analysis-** The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values were carried out as per the standard procedures [17.18].
- **Preparation of water and alcohol extract-** Water extract and alcohol extract of both the sample were extracted by cold maceration method. For preparation of Water extract, 4gm of coarse powder of both samples were taken separately and kept in conical flask having 100 ml of water and frequently shaked for 8 hrs and then left for 16hrs then filtered and filtrate were vaporized on water- bath and extract was collected separately. Similar process was done for alcoholic extract but instead of 100 ml of water, 100ml of ethanol was used.
- **Phytochemical analysis [18] -** Phytochemical tests were carried with water and alcohol extract of each of sample of *R. arboretum* using standard procedures to identify the phytoconstituents.

1. Test for reducing sugars

a. Fehling's test- 1 ml Fehling's A and 1ml of Fehling's B solutions were mixed and boiled for 1 minute and equal amount of extract of both samples added separately and heated in boiling water-bath for 5-10 min. and color change was noted. This procedure was done for both water and alcoholic extract.
b. Benedict's test- 1 ml of each extract was mixed with 1 ml of Benedict's reagent separately in test tubes and heated in boiling water-bath for 5 min. and color change was observed.

2. Test for Proteins

a. Xanthoprotein test (for protein containing tyrosine or tryptophan) - 3 ml of both extracts were added with 1 ml conc. H_2SO_4 separately and white ppt. was observed, then the test material was boiled and yellow color changed was observed. At last NH_4OH was added and ultimately ppt. turns into orange.

3. Test for Steroid-(Salkowski reaction): 2 ml of each extract of each sample was added with 2 ml of chloroform and 2 ml of conc. H_2SO_4 were mixed in each test solutions separately and shaken well and color changes in chloroform and acid layers were observed.

4. Test for glycosides

a. Legal's test (Test for cardenoloids) – 1 ml pyridine and 1 ml of sodium nitrprusside were added in 1 ml of each extract of each sample and color changes were observed.
b. Test for deoxysugars (Keller-Killiani test) – 2 ml of each extract of each sample was taken separately and glacial acetic acid, one drop 5% $Fecl_3$ and conc. H_2SO_4 were added and color changes were observed.
c. Borntrager's test for anthraquinone glycosides- 3 ml of each extract of each sample was taken and diluted H_2SO_4 was added then boiled and filtered. After cooling of each filtrate, 3 ml of chloroform was added in each test solution and shaken well. After shaking organic solvent was separated and ammonia was added and color change in ammoniacal layer was observed.

5. Test for Saponin- (foam test): Dry powder of each sample was vigorously shaken with water and persistent foam was observed.

6. Test for flavonoids

a. Shinoda test- 1 ml of extract of each of sample was taken and 5 ml 95% ethanol, few drops of conc. HCl and 0.5 g magnesium turnings were added, then color change was observed.
b. Sulphuric acid test- Sulphuric acid (66% or 80%) was added in test solutions and color changes were observed.

7. Test for Alkaloids: Residue of each extract was dissolved with HCl and filtered, then following tests with different reagents were done with the filtrate:

a. Dragendorff's test- Few drop of Dragendorff's reagent was added in filtrate of extract. Orange brown ppt. was formed.
b. Mayer's test- Few drops of Mayer's reagent was added in 2 ml of filtrate, ppt. was observed.
c. Wagner's test- Few drops of Wagner's reagent was added in 2 ml of filtrate, reddish brown ppt. was observed.

8. Test for Tannins and Phenolic compounds

a. Few drops of 5% $FeCl_3$ solution was added in 2 ml of each extract of each sample, color change was observed.
b. Few drops of lead acetate solution was added in 2 ml of extract and ppt. was observed.
c. Few drops of Bromine water was added in 2 ml of extract and decolouration of Bromine water was observed.

HPTLC Analysis

One gram of Rhododendron arboreum sample 1 and sample 2 was dissolved in 10 ml ethanol and kept for cold percolation for 24h and filtered. 4µl and 8µl of each of the above samples were applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.0: 1.0). The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254nm, 366nm and 620nm following derivatisation. R_f, colour of the spots and densitometric scan were recorded.

Observation and Result

Macroscopic and organoleptic characteristics of bark of *Rhododendron arboretum*-

Fig 1: *R. arboreum* sample no.1

Sample1. Shape- flat or slightly curved
Size- 3-5 cm. in length and 0.3- 0.5mm in thickness.
Surface- Outer surface rough, inner surface slight smooth.
Colour – Light brown
Taste – bitter
Odour- odourless
Fracture – laminated fracture

Fig 2: *R.arboreum* sample no.2

Sample 2. Shape- flat or slightly curved
Size-5- 9 cm. in length and 0.5- 0.7 mm.in thickness.
Surface- Outer surface rough, inner surface slight smooth.
Colour – Light brown
Taste – bitter
Odour- odourless
Fracture – laminated fracture

Microscopic study of bark - The microscopic study is the anatomical study which was done by taking appropriate section of the both samples under microscope. Each distinguishing character was noted down. Some of the chemicals which are used in obtaining clear sections were glycerine, chloral hydrate and safranine. Both the samples were laminar in structure and didn't absorb water even though soaked for prolong period, so it was difficult to take transverse sections and longitudinal sections. At time of sectioning it was breaking in tiny pieces and becoming powder, so those parts were observed under electrical compound microscope. On observation, in transverse section, multilayer loosely arranged parenchyama cells were observed, separated by thick layer of cortical cells. Vascular bundles were seen. On longitudinal section, innermost layer of systematically arranged cells of endodermis are observed but clear cut differentiation of cells couldn't be observed.

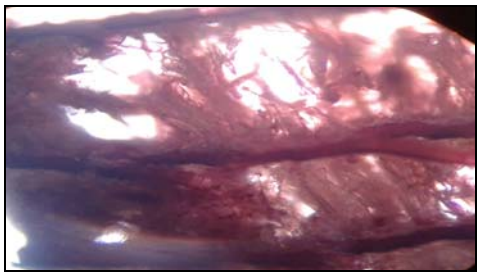


Fig 3-A: T.S. of bark of Rhododendron arboreum Sample no. 1

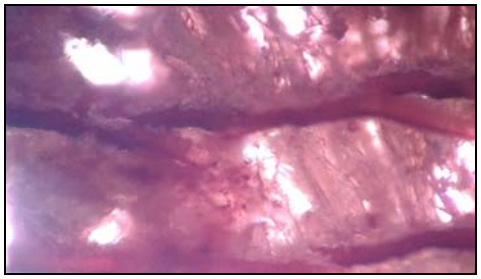


Fig 3 B: T.S. of bark of R.arboreum Sample no. 2

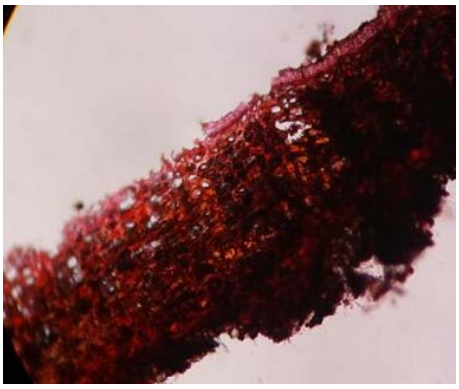


Fig 4 A: L.S. of bark of *R. arboreum*, sample no. 1

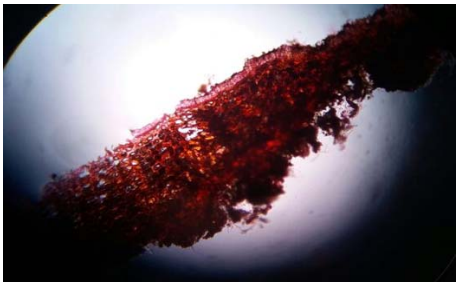
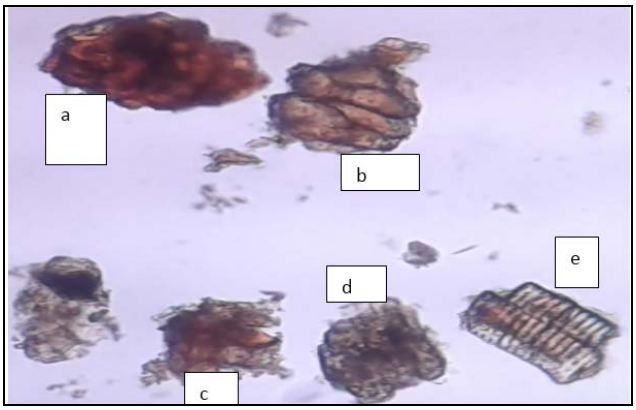


Fig 4 B: L.S. of bark of *R. arboreum*, sample no. 2

Powder Microscopy studies

Powder of bark is brown, non aromatic, astringent. The microscopic examination of the powder showed fragments of cork cells, Vessels and fibers of various shapes and thickness, tannin cells, stone cells and other contents (Fig. 3 and 4).



Powder microscopy of *R. arboreum* (bark), sample no. 1

Fig 5: A (a- brown materials, b- stone cells, c- fragments of cork cells, d- palisade fibres, e- scaliform vessels)

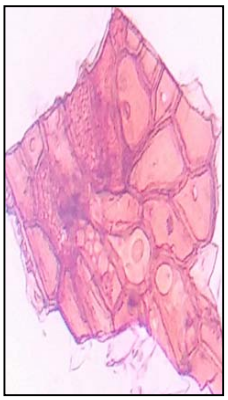


Fig 5: B- Cork cells

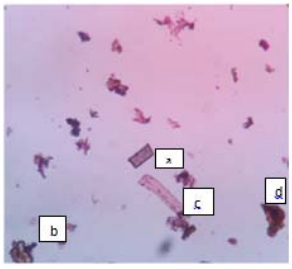


Figure 6 A

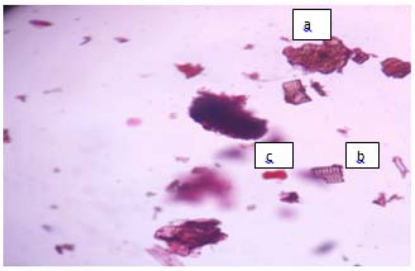


Figure 6 B

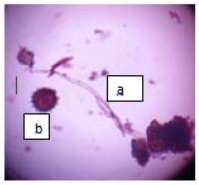


Figure 4C

Figure 6 D

Fig 6: Powder microscopy of *R. arboreum* (bark), sample no. 2. Fig. A (a- pitted vessel, b- glandular trichome, c- simple fibre, d- cell brown material). Fig. B- (a- cork cells, b- scaliform vessel, c- brown matter), Fig. C- (a- fibre, b- rosette crystal of calcium), Fig. D-simple fibre

Physiochemical analysis- The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values were carried out as per the standard procedures for both the samples of *R. arboreum* and results are given in table no. 1.

Table 1: Physiochemical evaluation of bark of Rhododendron arboretum

S. No.	Parameters	Sample no.1	Sample no.2
1.	Loss on drying	No change	1.5%
2.	Total ash	2%	2%
3.	Acid insoluble ash	0.5%	0.5%
4.	Water soluble extractive	2%	1%
5.	Alcohol soluble extractive	5%	14%

Phytochemical analysis

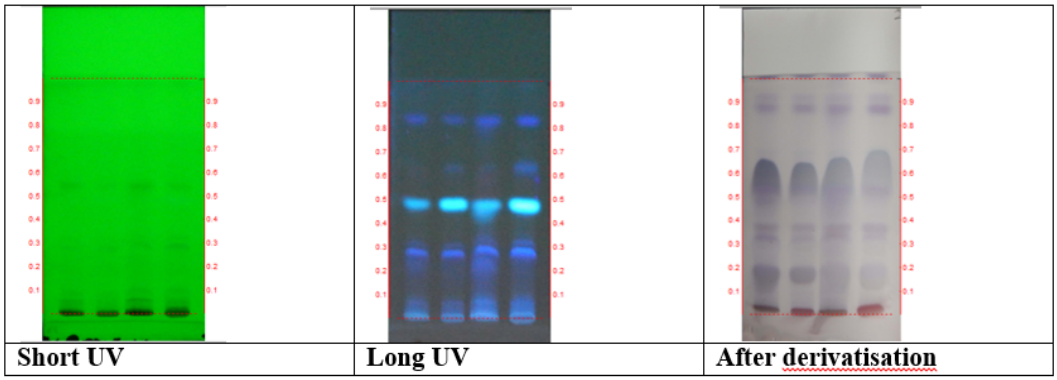
Table 2: Standard colour change and result obtained in phytochemical screening of both samples of Rhododendron arboretum

S. No.	Name of Test	Colour if Positive/ Other observation	Colour found / other observation		Inference	
			Sample no. 1	Sample no. 2	Sample no. 1	Sample no. 2
1.	Fehling's test	Brick red	Brick red	Brick red	++	++
2.	Benedict's test	Green to brick red	Green	Brick red	+	++
3.	Xanthoprotein test (for protein containing tyrosine or tryptophan	First white ppt. forms which changed into yellow and ultimately Orange after adding NH_4OH	No colour change		-	-
4.	Test for Steroid-(Salkowski reaction)	Red coloured in Chlorofrm layer and acid layer greenish yellow	No colour change	No colour change	-	-
5.	Legal's test (Test for cardenoloids)	Pink colour	Pink colour	Pink colour	++	++
6.	Test for deoxysugars (Keller-Killiani test)	Reddish brown	No clour change	No clour change	-	-
7	Borntrager's test for anthraquinone glycosides	Pink or red	No clour change	No clour change	-	-
8.	Test for Saponin- (foam test)	Persisent foam	Persisent foam	Persisent foam	++	+
9.	Test for flavonoids a. Shinoda test	Orange, Pink, red to purple	Brown colour	Brown colour	-	-
	b. Sulphuric acid test	Deep yellow	No colour change	No colour change	-	-
10.	Dragendorff's test	Orange precipitate	Orange precipitate	Orange precipitate	++	++
11.	Mayer's test	White precipitate	White precipitate	White precipitate	++	++
12.	Wagner's test	Reddish brown ppt	Reddish brown ppt	Reddish brown ppt	++	++
13	Test for Tannins and Phenolic compounds a. With 5% $FeCl_3$ solution	deep blue-black	deep blue-black	deep blue-black	++	++
	b. With lead acetate solution	White ppt.	White ppt.	White ppt.	++	++

Table 3: Presence of Phytoconstituents in bark of *Rhododendron arboreum*

S. No.	Phytoconstituent	Sample no. 1	Sample no. 2	Remarks
1.	Reducing sugars	Present	Present	Strong positive in alcoholic extract of sample no. 2
2.	Protiens	Absent	Absent	
3.	Steroid	Absent	Absent	-
4.	Glycosides- a. Cardenoloids b. Deoxysugars c. Anthraquinone	 Present Absent Absent	 Present Absent Absent	-
5.	Saponin	Present	Present	Weak in sample no.1
6.	Flavonoids	Absent	Absent	
7.	Alkaloids	Present	Present	Weak in water extracts.
8.	Tannins	Present	Present	Absent in water extracts.

HPTLC Analysis-



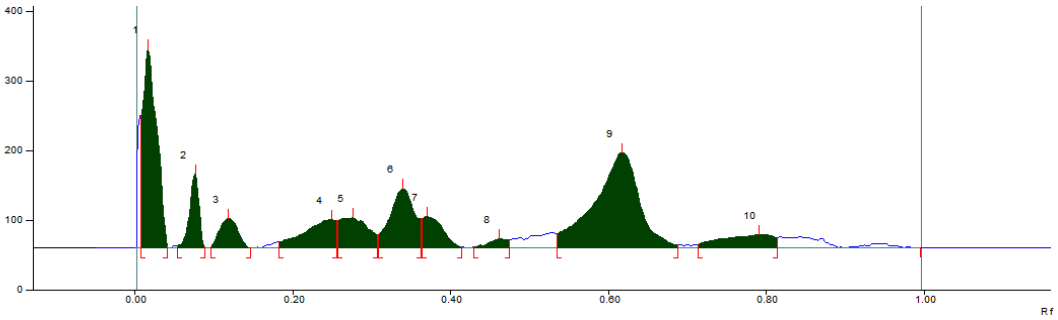
track 1- *Rhododendron arboreum* – 4 µl
track 2- *Rhododendron arboreum* – 4 µl
track 3- *Rhododendron arboreum* – 8 µl
track 4- *Rhododendron arboreum* – 8 µl
Solvent system – Toluene: Ethyl Acetate (9.0: 1.0)

Fig 7: HPTLC photo documentation of ethanolic extract of *Rhododendron arboretum*

Table 4: Rf values of samples

At short UV		At Long UV		After derivatisation	
Rhododendron arboreum (sample 1)	*Rhododendron arboreum* (sample 2)	*Rhododendron arboreum* (sample 1)	*Rhododendron arboreum* (sample 2)	*Rhododendron arboreum* (sample 1)	*Rhododendron arboreum* (sample 2)
0.06 (D. green)	0.06 (L. green)	0.06 (F. blue)	0.07(F. blue)	-	-
0.10 (L. green)	0.10 (L. green)	-	-	-	-
-	-	-	-	0.18 (D. purple)	0.18 (D. purple)
0.29 (L. green)	0.30 (D. green)	0.29 (F. blue)	0.29 (F. blue)	-	-
-	0.33 (L. green)	0.32 (F. blue)	0.32 (F. blue)	0.34 (D. purple)	0.34 (D. purple)
-	-	-	-	0.38 (D. purple)	0.38 (D. purple)
-	-	0.48 (F. blue)	0.47 (F. blue)	-	-
0.55 (D. green)	0.55 (D. green)	-	-	0.54 (D. purple)	0.54 (D. purple)
-	-	0.64 (F. blue)	0.65 (F. blue)	-	-
-	-	0.84 (F. blue)	0.85 (F. blue)	-	-
-	-	-	-	0.88 (D. purple)	0.88 (D. purple)
-	-	-	-	0.93 (D. purple)	0.93 (D. purple)

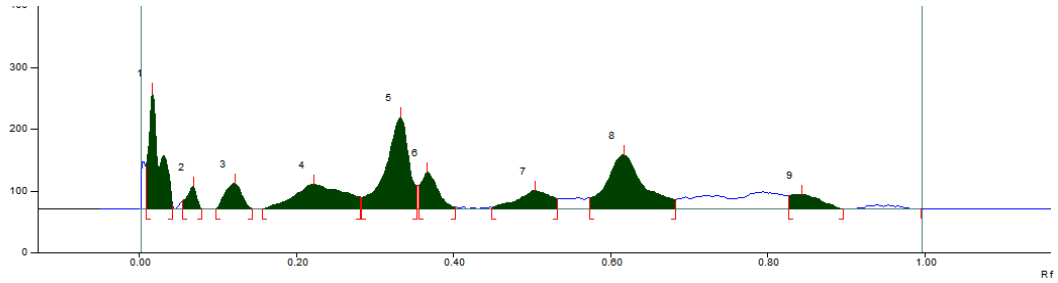
*D-dark; L-light; F-fluorescent



Track 3, ID: Rhododendron arboreum bark sample 1

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	188.8 AU	0.02 Rf	284.7 AU	34.97 %	0.04 Rf	2.5 AU	3693.0 AU	21.81 %
2	0.05 Rf	3.6 AU	0.08 Rf	105.5 AU	12.95 %	0.09 Rf	1.3 AU	895.3 AU	5.29 %
3	0.10 Rf	0.8 AU	0.12 Rf	42.3 AU	5.20 %	0.15 Rf	0.1 AU	702.5 AU	4.15 %
4	0.18 Rf	8.4 AU	0.25 Rf	40.4 AU	4.96 %	0.26 Rf	38.7 AU	1168.7 AU	6.90 %
5	0.26 Rf	39.0 AU	0.28 Rf	43.0 AU	5.29 %	0.31 Rf	18.5 AU	1127.1 AU	6.66 %
6	0.31 Rf	19.4 AU	0.34 Rf	84.8 AU	10.41 %	0.36 Rf	41.3 AU	1860.1 AU	10.99 %
7	0.36 Rf	41.8 AU	0.37 Rf	44.8 AU	5.50 %	0.41 Rf	0.8 AU	838.4 AU	4.95 %
8	0.43 Rf	1.5 AU	0.46 Rf	12.7 AU	1.56 %	0.48 Rf	11.1 AU	226.3 AU	1.34 %
9	0.54 Rf	20.6 AU	0.62 Rf	136.5 AU	16.77 %	0.69 Rf	3.9 AU	5523.5 AU	32.62 %
10	0.71 Rf	4.8 AU	0.79 Rf	19.5 AU	2.39 %	0.81 Rf	14.6 AU	897.2 AU	5.30 %

Fig 8a. *Rhododendron arboretum* - sample 1 (8µl)

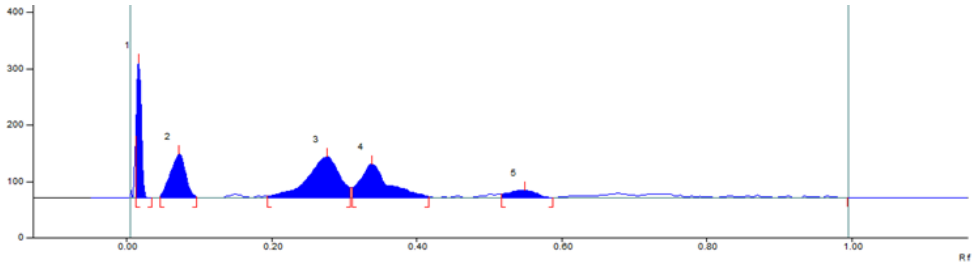


Track 4, ID: Rhododendron arboreum bark sample 2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	68.4 AU	0.02 Rf	188.9 AU	28.86 %	0.04 Rf	5.9 AU	1980.8 AU	14.98 %
2	0.06 Rf	13.9 AU	0.07 Rf	35.7 AU	5.45 %	0.08 Rf	0.0 AU	310.3 AU	2.35 %
3	0.10 Rf	0.5 AU	0.12 Rf	41.6 AU	6.36 %	0.15 Rf	0.4 AU	627.9 AU	4.75 %
4	0.16 Rf	0.1 AU	0.22 Rf	40.1 AU	6.12 %	0.28 Rf	19.1 AU	1809.6 AU	13.68 %
5	0.28 Rf	18.8 AU	0.33 Rf	148.6 AU	22.70 %	0.35 Rf	37.2 AU	3098.1 AU	23.43 %
6	0.36 Rf	37.4 AU	0.37 Rf	59.2 AU	9.05 %	0.40 Rf	3.5 AU	921.7 AU	6.97 %
7	0.45 Rf	2.4 AU	0.50 Rf	30.0 AU	4.58 %	0.53 Rf	15.9 AU	904.2 AU	6.84 %
8	0.57 Rf	18.1 AU	0.62 Rf	87.1 AU	13.31 %	0.68 Rf	15.0 AU	2921.8 AU	22.09 %
9	0.83 Rf	21.8 AU	0.85 Rf	23.4 AU	3.58 %	0.90 Rf	0.0 AU	650.8 AU	4.92 %

Fig 8b. *Rhododendron arboreum* - sample 2 (8µl)

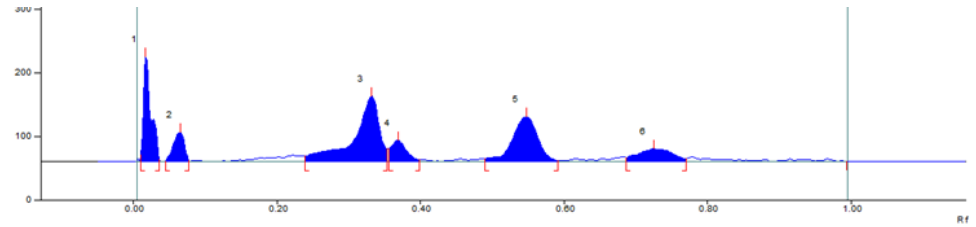
Fig 8: Densitometric scan at 254nm



Track 3, ID: Rhododendron arboreum bark sample 1

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	108.5 AU	0.02 Rf	239.1 AU	51.62 %	0.04 Rf	0.0 AU	1204.0 AU	17.95 %
2	0.05 Rf	1.5 AU	0.07 Rf	77.5 AU	16.74 %	0.10 Rf	1.1 AU	1156.0 AU	17.23 %
3	0.20 Rf	3.0 AU	0.28 Rf	73.3 AU	15.82 %	0.31 Rf	17.5 AU	2321.2 AU	34.60 %
4	0.31 Rf	17.7 AU	0.34 Rf	59.6 AU	12.87 %	0.42 Rf	2.2 AU	1664.9 AU	24.82 %
5	0.52 Rf	6.0 AU	0.55 Rf	13.7 AU	2.95 %	0.59 Rf	0.1 AU	362.2 AU	5.40 %

Fig 9a. *Rhododendron arboreum* - sample 1 (8µl)

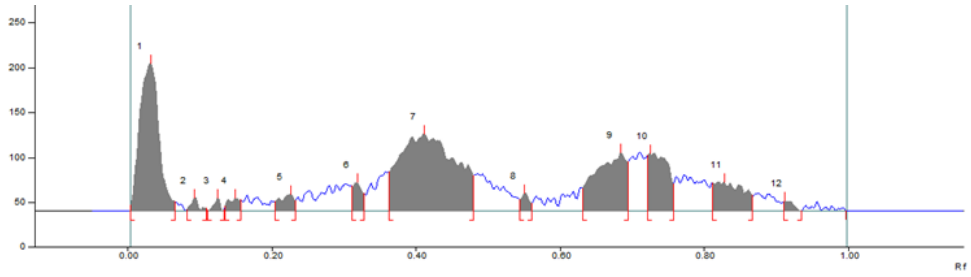


Track 4, ID: Rhododendron arboreum bark sample 2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	10.7 AU	0.02 Rf	164.9 AU	37.73 %	0.04 Rf	3.5 AU	1252.6 AU	17.16 %
2	0.05 Rf	0.4 AU	0.07 Rf	46.0 AU	10.52 %	0.08 Rf	1.9 AU	493.5 AU	6.76 %
3	0.24 Rf	8.4 AU	0.33 Rf	102.7 AU	23.50 %	0.35 Rf	19.8 AU	2574.4 AU	35.27 %
4	0.36 Rf	20.0 AU	0.37 Rf	33.3 AU	7.62 %	0.40 Rf	2.2 AU	517.1 AU	7.08 %
5	0.49 Rf	4.5 AU	0.55 Rf	70.2 AU	16.06 %	0.59 Rf	1.8 AU	1766.8 AU	24.21 %
6	0.69 Rf	6.2 AU	0.73 Rf	20.0 AU	4.58 %	0.77 Rf	4.2 AU	694.1 AU	9.51 %

Fig 9b. *Rhododendron arboreum* - sample 2 (8µl)

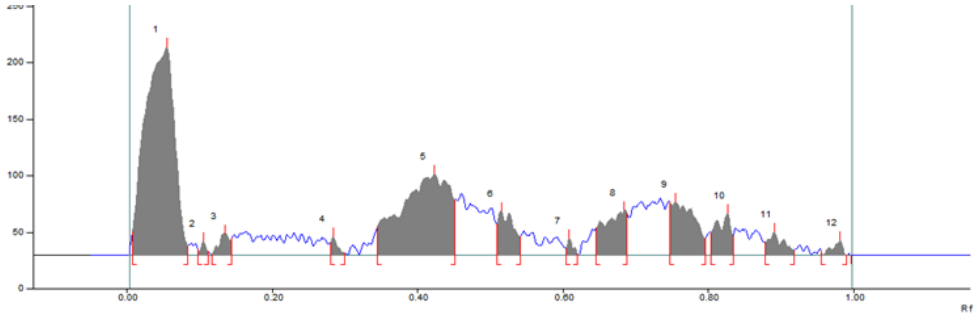
Fig 9: Densitometric scan at 366nm



Track 3, ID: Rhododendron arboreum bark sample 1

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	6.5 AU	0.03 Rf	163.9 AU	30.54 %	0.07 Rf	10.4 AU	3149.7 AU	23.62 %
2	0.08 Rf	0.9 AU	0.09 Rf	14.8 AU	2.76 %	0.11 Rf	3.2 AU	109.2 AU	0.82 %
3	0.11 Rf	1.2 AU	0.13 Rf	14.7 AU	2.74 %	0.13 Rf	1.1 AU	81.1 AU	0.61 %
4	0.14 Rf	5.4 AU	0.15 Rf	14.4 AU	2.68 %	0.16 Rf	11.9 AU	170.2 AU	1.28 %
5	0.20 Rf	10.1 AU	0.23 Rf	19.1 AU	3.55 %	0.23 Rf	11.5 AU	269.0 AU	2.02 %
6	0.31 Rf	27.9 AU	0.32 Rf	31.8 AU	5.93 %	0.33 Rf	19.2 AU	316.2 AU	2.37 %
7	0.36 Rf	42.9 AU	0.41 Rf	85.6 AU	15.94 %	0.48 Rf	39.4 AU	4793.4 AU	35.95 %
8	0.55 Rf	11.9 AU	0.55 Rf	19.9 AU	3.71 %	0.56 Rf	8.5 AU	159.5 AU	1.20 %
9	0.63 Rf	25.2 AU	0.69 Rf	64.7 AU	12.05 %	0.70 Rf	54.5 AU	1950.8 AU	14.63 %
10	0.72 Rf	61.8 AU	0.73 Rf	63.9 AU	11.90 %	0.76 Rf	30.3 AU	1284.2 AU	9.63 %
11	0.81 Rf	31.1 AU	0.83 Rf	32.3 AU	6.02 %	0.87 Rf	17.8 AU	941.4 AU	7.06 %
12	0.91 Rf	10.6 AU	0.91 Rf	11.6 AU	2.16 %	0.94 Rf	0.4 AU	107.6 AU	0.81 %

Fig 10a. *Rhododendron arboretum* - sample 1 (8µl)



Track 4, ID: Rhododendron arboreum bark sample 2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	20.5 AU	0.06 Rf	183.9 AU	35.69 %	0.08 Rf	8.5 AU	5349.9 AU	41.79 %
2	0.10 Rf	5.0 AU	0.11 Rf	12.0 AU	2.34 %	0.11 Rf	3.6 AU	69.8 AU	0.54 %
3	0.12 Rf	0.0 AU	0.14 Rf	19.1 AU	3.72 %	0.15 Rf	14.8 AU	212.5 AU	1.66 %
4	0.28 Rf	10.5 AU	0.29 Rf	16.1 AU	3.13 %	0.30 Rf	1.9 AU	109.5 AU	0.86 %
5	0.35 Rf	24.4 AU	0.42 Rf	72.1 AU	13.99 %	0.45 Rf	49.0 AU	3420.4 AU	26.72 %
6	0.51 Rf	27.3 AU	0.52 Rf	39.1 AU	7.58 %	0.54 Rf	16.2 AU	608.4 AU	4.75 %
7	0.60 Rf	5.5 AU	0.61 Rf	14.8 AU	2.88 %	0.62 Rf	0.5 AU	83.1 AU	0.65 %
8	0.65 Rf	24.3 AU	0.69 Rf	39.9 AU	7.74 %	0.69 Rf	37.6 AU	857.7 AU	6.70 %
9	0.75 Rf	46.9 AU	0.76 Rf	47.2 AU	9.16 %	0.80 Rf	14.9 AU	1130.5 AU	8.83 %
10	0.80 Rf	20.1 AU	0.83 Rf	37.5 AU	7.27 %	0.84 Rf	16.9 AU	531.6 AU	4.15 %
11	0.88 Rf	11.2 AU	0.89 Rf	20.6 AU	4.00 %	0.92 Rf	3.9 AU	303.0 AU	2.37 %
12	0.96 Rf	3.8 AU	0.98 Rf	12.9 AU	2.51 %	0.99 Rf	0.3 AU	126.5 AU	0.99 %

Fig 10b. *Rhododendron arboretum* - sample 2 (8µl)

Fig 10. Densitometric scan at 620nm

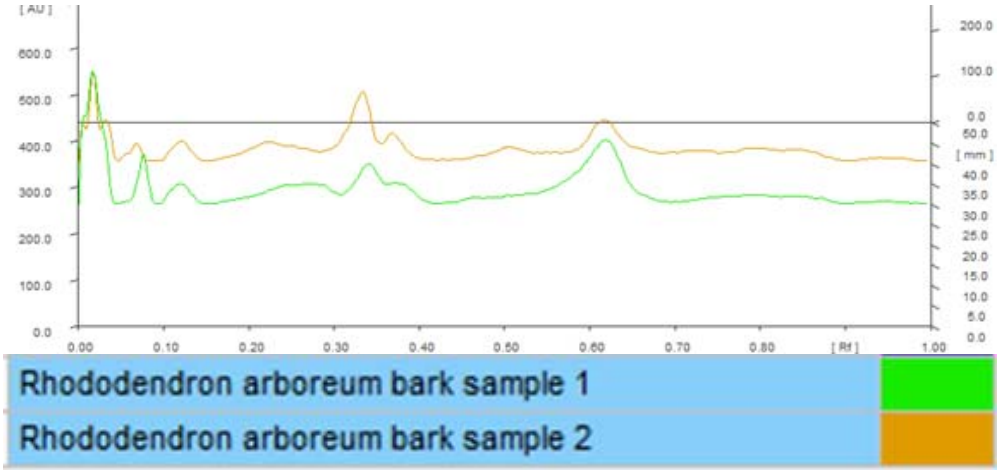


Fig 11: a. At 254nm

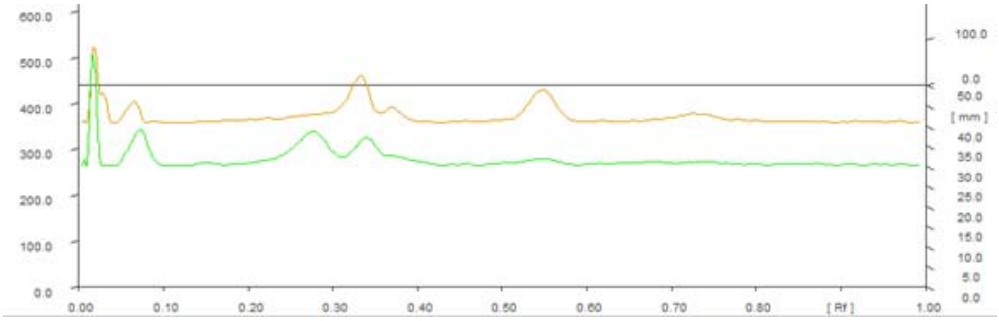


Fig 11: b. At 366nm

Fig 11: 3-D chromatogram

Discussion

The standardization of a crude drug is an integral part for establishing its correct identity. Before any crude drug can be included in an Ayurvedic pharmacopoeia, pharmacognostic parameters and standards must be established. Despite the modern techniques, identifications evaluation of herbal drugs by Pharmacognostic studies is still more reliable, accurate and inexpensive means. The macroscopic / orgaonoleptic and microscopic studies of a medicinal plant is the first step towards establishing its identity and purity. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of crude drugs and these are the useful diagnostic criteria. Physical parameters such as, foreign matters, ash values and extractive values can be used as reliable aid for detecting adulteration. Ash values of drug give an idea of inorganic composition of crude drugs and adulteration of earthy materials and other inorganic impurities. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. It is also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents.

The macroscopic and organoleptic characteristics of both the samples of *Rhododendron arboreum* are almost similar only size difference is there. Similarly both samples resemble to each other on powder microscopic studies. Though the samples of bark of *Rhododendron arboreum* are laminar and not absorbing the water, and difficult to take transverse section so that detailed microscopic studies aren't done. Physiochemical reference values aren't mentioned in Ayurvedic pharmacopoeia of India and any other s standard text books. Phytochemical evaluation revealed the similarities in both samples. Alkaloids, tannins, saponins, cardenoloids and reducing sugars were found in both samples which is also supported by previous studies.Previous studies have also shown the presence of alkaloids, tannins, saponins, cardenoloids and reducing sugars and the specific phytoconstituents present in different extracts of bark of R. arboreum contains triterpenoid substance taraxerol (C30H50O) & ursolic acid acetate (C32H50O4), betulinic acid (C30H48O3 leuco-pelargonidin (C15H14O6) [14, 15].

In previous study of preliminary phytochemical analysis of genuine source of Rohitaka (*Tecomella undulata*) has shown that there are presence of alkaloids, phenols/tannins, flavanoids, saponins, steroids & glycosides. Betulinic acid is one among biomarker compound found in bark extract of *Tecomella undulata*, which is partially responsible for hepatoprotective activity [19.20]. These findings support for the use of *R. arboreum* as substitute of *T. undullata*

The HPTLC fingerprinting of market samples of *Rhododendron arboreum* bark extracts revealed the presence of several peaks. Bark extract of sample no.1 showed the presence of 4 peaks where as sample 2 showed 5 peaks when observed under short UV with different intensities of light and dark green. When observed under long UV sample 1 and sample 2 both showed 6 peaks which were all fluorescent blue in color. After derivatisation with vanillin sulphuric acid, when the samples observed under white light, sample 1 and sample 2 showed 6 peaks each (all dark purple) in color mentioned in Table (Table no 3).

Densitometric scan at 254nm, sample 1 showed 10 peaks ,among which three are major peaks with R_f of 0.62 (32.62%), 0.02 (21.81%), 0.34 (10.99%), sample 2 showed total of 9 peaks of which R_f. 0.33 (23.43%), 0.62 (22.09%), 0.02 (14.98%) are prominent (Fig 8a, Fig 8b).

Densitometric scan at 366nm sample 1 showed a total of 5

peaks among which Rf 0.28(34.60%), 0.34(24.82%), followed by 0.02 and 0.07 each with 17.95% and 17.23% area, sample 2 showed 6 peaks of which peak with Rf 0.33 (35.27%), 0.55 (24.21%) are major showed in figures (Fig 9a, Fig 9b).
Densitometric scan at 620nm following derivatisation, sample 1 showed 12 peaks amongst these Rf of 0.41(35.95%), 0.03(23.62%) are major peaks, sample 2 showed a total of 12 peaks amongst them 0.06 (41.79%), 0.42(26.72%) are the major peaks as shown in figures (Fig 10a, Fig 10b).

Table 4: Rf values by densitmetric scan

At 254nm		At 366nm		After derivatisation (at 620nm)	
Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
0.02	0.02	0.02	0.02		
				0.03	
					0.06
	0.07	0.07	0.07		
0.08					
				0.09	
					0.11
0.12	0.12				
				0.13	
					0.14
				0.15	
	0.22				
				0.23	
0.25					
0.28		0.28			
					0.29
				0.32	
	0.33		0.33		
0.34		0.34			
0.37	0.37		0.37		
				0.41	
					0.42
0.48					
	0.50				
					0.52
		0.55	0.55	0.55	
					0.61
0.62	0.62				
				0.69	0.69
			0.73	0.73	
					0.76
0.79					
				0.83	0.83
	0.85				
					0.89
				0.91	
					0.98

There are 23 active phytoconstituents in each sample *Rhododendron arboreum* barks. On densometric scan, in sample no. 1, following phytoconstituents of Rf of 0.02, 0.03, 0.07, 0.08, 0.09, 0.12, 0.13, 0.15, 0.23, 0.25, 0.28, 0.32, 0.34, 0.37, 0.41, 0.48, 0.55, 0.62, 0.69, 0.73, 0.79, 0.83 and 0.91. Similarly in sample no.2 Rf of following constituents are detected; 0.02, 0.06, 0.07, 0.11, 0.12, 0.14, 0.22, 0.29, 0.33, 0.37, 0.42, 0.50, 0.52, 0.55, 0.61, 0.62, 0.69, 0.73, 0.76, 0.83, 0.85, 0.89 and 0.98. Among all the active phytoconstituents found on both sample 1 and 2, 9 phytoconstituents are, having similar Rf value. The common active constituents having similar Rf on densometric scan are; 0.02, 0.07, 0.12, 0.37, 0.55, 0.62, 0.69, 0.73 and 0.85. Among the common phytoconstituents Rf of 0.02, 0.07, 0, 55 and 0.62 have occupied maximum area. This indicates that both sample 1 and sample 2 are mostly similar in phytoconstituents.

Conclusion

Genuine botanical source of Rohitaka is *Tecomella undulate* but in Nepal *Rhododendron arboreum* is marketed and used by name of Rohitaka due to non- availability of the genuine source of Rohitaka. This study is centered towards preliminary pharmacognostic and phytochemical evaluation of market samples of *Rhododendron arboreum* (soure plant of Rohitaka in Nepal) and it's chromatographic study. The study revealed that both the samples collected from market of Nepal by the name of Rohitaka are barks of *Rhododendron arboreum* and both the samples have almost similar pharmacognostic characteristics and phytocosnstituents are also almost same. Preliminary phytochemical analysis revealed that both the samples contain reducing sugars, Cardenoloids, Saponins, Alkaloids and Tannins. 23 active phytoconstituents are identified chromatographically, among them 9 phytoconstituents are common in both samples. The specific phytoconstituents or biomarker components are not analyzed. Further research should be taken up for analyzing the biomarker components. The results of HPTLC analysis of bark of *Rhododendron arboreum* can be taken as standard value for further studies.

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