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Pharmacognostical Standardization of Stem Bark of *Rhododendron arboreum* Sm.*(Pullas)*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the Pharmacognostical and Phytochemical characteristics of the stem bark of *Rhododendron arboreum* Sm.(*Pullas*)
Place and Duration of Study: Pharmacognostical and phytochemical laboratory, PG Department of Dravyaguna Vigyana, National Institute of Ayurveda, Jaipur-302002 and Ayushraj Laboratory, Jaipur. During the period of October 2017 to December 2017.
Methodology: *Rhododendron arboreum* Sm. was collected from Godawari Botanical Garden of Nepal, Ministry of forest, Govt. of Nepal, during the month of September 2017. The samples were Identified and authenticated by the same institution. Then the materials were subjected to evaluate the Macroscopic study, Microscopic study, Physicochemical study, Phytochemical screening, Heavy Metals Analysis, Determination of Aflatoxins and Pesticide residue analysis as per the standard available guideline.
Results: The microscopic feature of stem bark *Rhododendron arborieum* Sm. after treatment of different reagents (safranin, $fecl_3$, iodine etc.) showed various cellular and chemical structures like scaliform vessels, calcium oxalate, starch grain, fragment of cork cells and pitted vessel.

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Physicochemical analysis showed Moisture content-7.55%, Total ash-2.11%, Acid insoluble ash-3.47%, Water soluble ash-0.91%, Aqueous extractive value-3.45%, Ether extractive value-3.17%, Petroleum ether extractive value-1.83, Ph-4.6. Analysis of heavy metal and aflatoxin analysis showed parameters were the within limit as per API standard. HPTLC screening of ethanol extract of the test sample showed four unknown chemical constituent with R_f value 0.12, 0.25, 0.47, 0.71 ; where the mobile solution was used Toluene: Ethyl acetate: Formic acid (5: 4: 1).

Conclusion: Result of the study may act as future standard for authentication of the genuine plant, *Rhododendron arboreum* Sm.(*Pullas*) which is used as a source of famous *Ayurveda* plant *Rohitaka* (the plant used for liver and spleen disorders) in Nepal. There is a scope of further scientific research to explore this important medicinal in future.

Keywords: Rohitaka, pullas, Rhododendron arboretum, ayurveda, standard, liver.

1. INTRODUCTION

Rhododendron arboreum Sm. commonly known as *Pullas* in Sanskrit, *Burash* in Hindi and *Laligurash* in Nepali. It belongs to Ericaceae family which is found in Himalayan region of India and Nepal. This outstanding plant was designated the national flower of Nepal. It's even represented on the national coat of arms and it is also the state flower of Himachal [1]. *Laligurash* is the tallest of Nepal's Rhododendrons, reaching heights of more than 20m. It has the largest trunk and flowers, blossoming earlier and longer than the others. In its native land, huge trees of *R. arboreum* grow to a height of 25m or more. Trunk often much branched, bent or twisted, bark pinkish brown, somewhat rough, exfoliating in thin flakes. Young shoot of a plant is clothed with white scales [2]. Leaves are 7.5 to 15 cm in length and 3-5 cm in width which is crowded towards the ends of the branches, lanceolate or oblong, narrowed at both ends and glabrous and glossy green surface above and pale beneath due to film of small white scales, prominent mid ribs and nerves present beneath the surface of leaves. The petiole is stout, 1.3 to 2.5 cm in length and clothed with white scales at young age [3]. The flowers of *R. arboreum* range in colour from a deep scarlet, to red with white markings, to pink to white. Bearing up to twenty blossoms in a single truss this rhododendron is a spectacular sight when in full bloom. It is reported that the bright red forms of this Rhododendron are found at the lower elevations. Flowers are 2.5 to 5 cm long and deep red or pale pink in colour, which are crowded in large rounded corymbs. Pedicels are 0-7.5 mm in length; Corolla are campanulate, ovary mealy or rusty woolly. Fruit capsule oblong, curved, mealy, longitudinally ribbed, up to 3.8 cm X 1.25 cm sized; seeds are minute, dark brown and compressed oblong [1-4]. The bark of this important medicinal plant is used as an outstanding remedy for liver disorders. Among the traditional physicians, the plant *Rhododendron arboreum* Sm. is a famous drug for control and prevention of wide range of hepatic ailments. It is used in folklore medicine to treat many disorders and often used as a substitute to another famous plant drug *Rohitaka*. Pharmaceuticals companies and traditional practitioners of Nepal are using its bark, as a substitute of '*Rohitaka'* i.e. *Tecomella undulata* (Sm.) Seem. since 100 years. Ayurveda pharmaceutical companies in Nepal is formulating "*Rohitakyadi Churna*" where *Laligurash* i.e. *Rhododendron arboreum* Sm. is the active ingredient [3-5]. Also in folklore practices of Nepal *Rhododendron arboreum* Sm. is being largely and frequently used as a substitute of *Tecomella undulata* (Sm.) Seem. But until no scientific data are available regarding the Pharmacognostical and Phytochemical standards of this important medicinal plant. Hence the present study aimed to make a profile on Pharmacognostical and Phytochemical characteristics of the stem bark of *Rhododendron arboreum* Sm.(*Pullas*).

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Sample

Rhododendron arboreum Sm. was collected from Godawari Botanical Garden of Nepal, during the month of September, 2017. The samples were Identified and authenticated (Ref. no-289 dated-22/04/2017) by same institution which is under Ministry of forest, Govt. of Nepal.

2.2 Macroscopic Study

The collected samples of *Rhododendron arboreum* Sm. studied organoleptically, with the

naked eye and magnifying lens and with the help of Pharmacognostical procedure. Appearance, size, shape, colour, odour and all other findings were recorded.

2.3 Microscopic Study

Thin hand sections of Bark of *Rhododendron arboreum* Sm. were done using blades, stained as the standard methodology and then examined under microscope. Photomicrographs of the microscopical sections were captured in Zeiss Axio Lab. A1 microscope (fitted with Ziess AxioCamERc5s) and images were analysed using the Zeiss Axio Vision software [6].

2.4 Physicochemical Study

Determination of moisture content, pH value, alcohol soluble extractive, water soluble extractive, total ash, water soluble ash and water insoluble ash was performed as per the standard guideline mentioned in API (Ayurvedic Pharmacopoeia of India) [7].

2.5 Phytochemical Screening

Freshly prepared extracts were tested for the presence of various active phyto-compounds like phenols, tannins, flavonoids, proteins, reducing sugar, carbohydrates, lipids, saponin and alkaloids [8].

2.6 Chromatographic Screening

4 gm powdered drug was extracted with 100 ml of ethanol (90 percent) in a Soxhlet apparatus consecutively three times. Extract was filtered and concentrated to 10 ml. This was used for HPTLC analysis. Mobile phase used-Toluene: Ethyl Acetate: Formic Acid (5 : 4 :1). Color of the spot with R_f value were noted before and after the derivatization. Whole the process was followed as per the standard available guideline [9].

2.7 Heavy Metals Analysis

Atomic absorption spectrophotometer was used for the determination of heavy metal elements. The assay of the element being examined was tested by determining the decreased degree of light intensity of radiation. Atomic absorption obeys the general rule for absorption spectrophotometry. The assay was carried out by comparing the absorbance of the test preparation with that of the reference preparation. Determination of lead and cadmium (Cd) was done by graphite oven method, Determination of arsenic (As) was done by Hybrid method and Determination of Mercury (Hg) was done by cold absorption method [10].

2.8 Determination of Aflatoxins

Study was done to detect the possible presence of aflatoxins B1, B2, G1 and G2 in the studied plant material by thin-layer chromatographic technique. Zinc Acetate – Aluminum Chloride Reagent was used and Test Solution was prepared by adding 50 gm of the powdered plant material in of methanol and water (17**:** 3) [11].

2.9 Pesticide Residue Analysis

The chromatographic procedure was carried out to analyze the pesticide residue. For Quantitative analysis of Organophosphorus insecticides, gas chromatography 2.28 was used and carbophenothion R as internal standard. The procedure was followed as per the standard method described in the Indian pharmacopoeia 1996 and Ayurvedic pharmacopoeia of India [12].

3. RESULTS AND OBSERVATION

Transverse section of bark *Rhododendron arborieum* Sm. is a key parameter for proper identification of species and establish authenticity of sample. Microscopic features in stem bark after observation in 40x in compound microscope was found multilayer loosely arranged parenchyma cells separated by thick layer of cortical cells and vascular bundles was observed in section. Microscopy feature of stem bark powder of *Rhododendron arborieum* Sm. was found after treatment of different reagents (safranin, $fecl_3$, iodine etc.) showed cellular and chemical structure like scaliform vessels, calcium oxalate, starch grain, fragment of cork cells and pitted vessel in 40 x visualization.

Studied bark sample of *Rhododendron arborieum* Sm. was free from any other plant material and other foreign matter. Water and alcohol soluble extractive values, total ash and acid insoluble ash values were determined as per the method described in the Indian pharmacopoeia 1996 and Ayurvedic pharmacopoeia of India.

Fig-1.1 *Rhododendron arborieum* Sm.(stem bark)

Fig-1.2 *Rhododendron arborieum* Sm. (powder)

Fig. 1. Macroscopic evaluation of *Rhododendron arborieum* Sm.

Table 1. Shows organoleptic examination of *Rhododendron arborieum* Sm.

S. No	Organoleptic	Bark
1	Surface	Outer surface rough, inner surface slight smooth
2	Colour	Light brown
3	Odour	Odorless
4	Taste	Bitter
5	Fracture	Laminated fracture

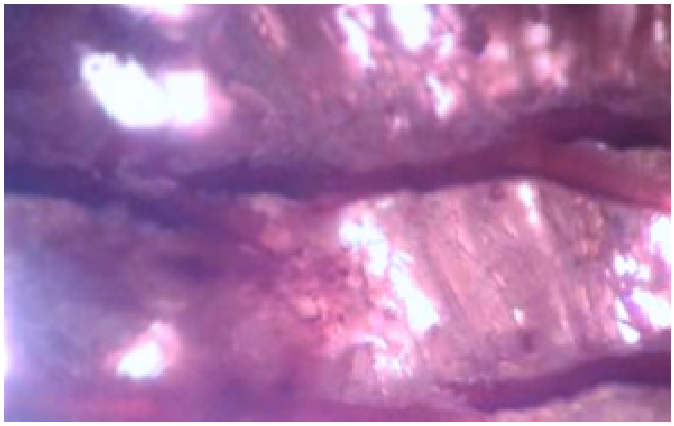


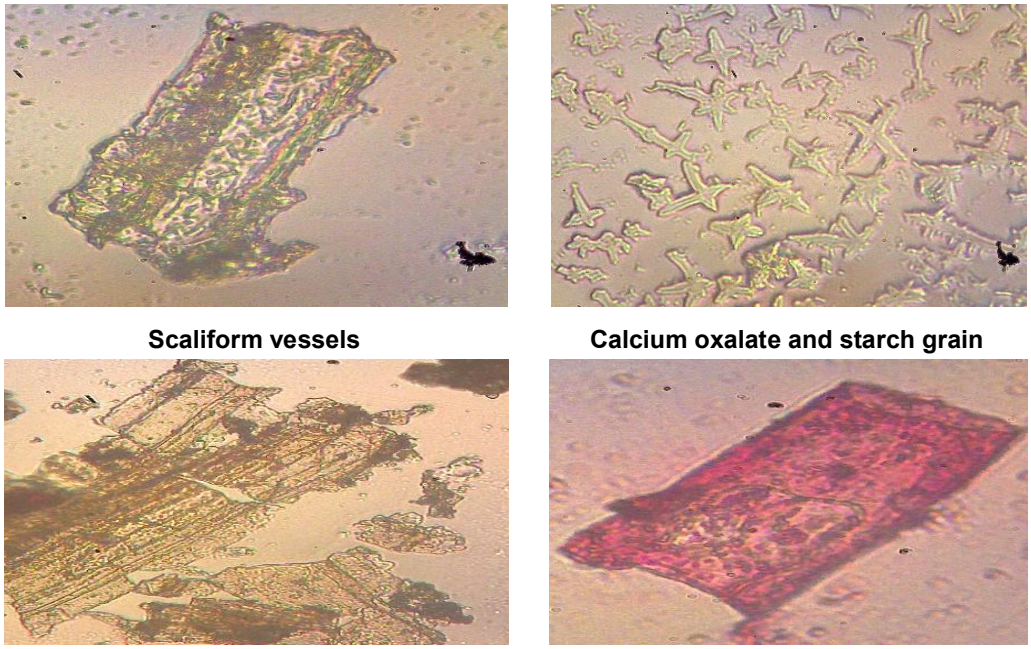
Fig. 2. Showing transverse section of bark of *Rhododendron arborieum* Sm.

Heavy metals and aflatoxins were found within the limit in stem bark of *Rhododendron arborieum* Sm. But there is no reference limit values are available for pesticide residues. As per the method described in the Indian pharmacopoeia 1996 and Ayurvedic pharmacopoeia.

Phytochemical screening of bark of *Rhododendron arborieum* Sm. Was found both primary and secondary metabolites in aqueous, alcoholic and ether extract. The test sample contain carbohydrate, alkaloids, protein, glycosides, phenolic compound, tannins and

saponin. Steroids and Amino acids was absent in extract samples.

HPTLC screening of ethanol extract of *Rhododendron arborieum* (stem bark) in mobile solution Toluene: Ethyl acetate: Formic acid (5: 4: 1) and observation in UV light and p-anisaldehyde-sulphuric acid reagent found that four unknown chemical constituent visualized in UV (254, 366 nm) have R_f value 0.12, 0.25, 0.47, 0.71 and after spraying p-anisaldehyde- sulphuric acid regent on TLC plate found 4 chemical constituent in day light (0.24, 0.42, 0.44, 0.79) and 3 chemical constituent in UV 254 (0.24, 0.72, 0.80) presence of blue, green, violet, red, grey colour of spot in TLC plate after derivatization showed phenols, sugars, steroids, and terpenes chemical constituent separate in this mobile solution.



Scaliform vessels **Calcium oxalate and starch grain**

Fragment of cork cells **Pitted vessel**

Fig. 3. Showing powder microscopy of *Rhododendron arborieum* Sm.

Table 2. Physio-chemical analysis of *Rhododendron arborieum* Sm.

S.n	Test name	Value
1	Foreign matter	0%
2	Moisture content	7.55%
3	Total ash	2.11%
4	Acid insoluble ash	3.47%
5	Water soluble ash	0.91%
6	Aq ext value	3.45%
7	Eth extract	3.17%
8	Pet ether extract	1.83
9	Ph	4.6

4. DISCUSSION AND CONCLUSION

Rhodendron arboreum Sm. belongs to the Ericaceae family. The genus *Rhododendron* consists of more than 1000 species which are distributed throughout the world mostly concentrated in India, China, Malaysia and Nepal. The genus has been reported to be effective as astringent, diuretic, choleretic, antispasmodic, antidiarrheal, antidysentery, antiirritable bowel syndrome and a good hepatoprotective therapy [13,14]. The flowers

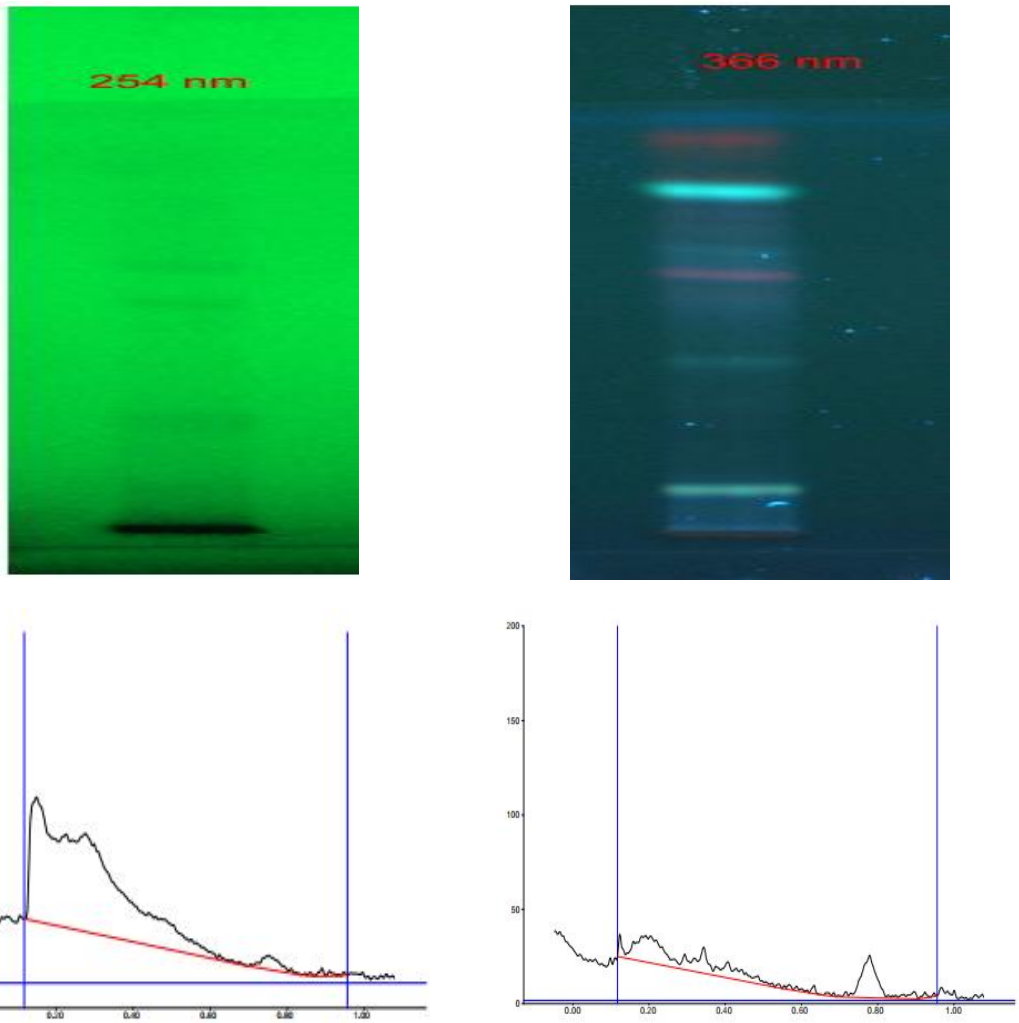
Table 3. Heavy metals, aflatoxins and pesticide residue analysis of *Rhododendron arborieum* Sm. Bark

S.no	Parameters	Valve	Ref. Value
I	**Heavy metals**		
1.	Lead	0.694 ppm	Nmt 10 ppm
2.	Cadmium	0.073 ppm	Nmt 0.3 ppm
3.	Arsenic	0.197 ppm	Nmt 3 ppm
4.	Mercury	0.147 ppm	Nmt 1 ppm
II	**Aflatoxins**		
1.	Aflatoxin b1	Not detected	0.5 ppb
2.	Aflatoxin b2	Not detected	0.1 ppb
3.	Aflatoxin g1	Not detected	0.5 ppb
4.	Aflatoxin g2	Not detected	0.1 ppb
III	**Pesticide residue analysis**		
1.	Sulfotep	75.401 ppb	-
2.	Phorate	74.001 ppb	-
3.	Alfa-hch	74.580 ppb	-
4.	Dimethoate	77.571 ppb	-
5.	Beta hch	75.593 ppb	-
6.	Gamma hch	74.638 ppb	-
7.	Disulfoton	74.491 ppb	-
8.	Delta hch	76.253 ppb	-
9.	Parathion methyl	72.416 ppb	-
10.	Heptachlor	75.029 ppb	-
11.	Aldrin	76.178 ppb	-
12.	Parathion	72.502 ppb	-
13.	Heptachlor epoxide	75.781 ppb	-
14.	Trans-chlordane	76.440 ppb	-
15.	Cis-chlordane	77.322 ppb	-
16.	Alpha endosulfan	78.060 ppb	-
17.	P.p-dde	76.701 ppb	-
18.	Dieldrin	75.220 ppb	-
19.	Endrin	72.567 ppb	-
20.	Beta endosulfan	77.794 ppb	-
21.	P.p-ddd	73.153 ppb	-
22.	Endrin aldehyde	79.856 ppb	-
23.	Famphur	73.118 ppb	-
24.	Endosulphan sulphate	80.161 ppb	-
25.	P.p-ddt	73.819 ppb	-
26.	Endrin ketone	75.042 ppb	-
27.	Methoxychlor	71.698 ppb	-

and barks of *R. arboreum* showed antidiabetic, anti-hyperlipidimic, anti-inflammatory and anti-nociceptive activities [15-20]. In present study an attempt was taken to standardize the bark of this plant. The standardization of a crude drug is an integral part for establishing its correct identity [21]. 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 or orgaonoleptic and microscopic studies of a

medicinal plant is the first step towards establishing its identity and purity.[6-10].

Organoleptic evaluation is a qualitative assessment technique 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 a 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[6,7]. 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.



R_f value	Area %	R_f value	Area %
0.12	35.07	0.12	35.07
0.25	53.81	0.25	53.81
0.47	7.45	0.47	7.45
0.71	3.70	0.71	3.70

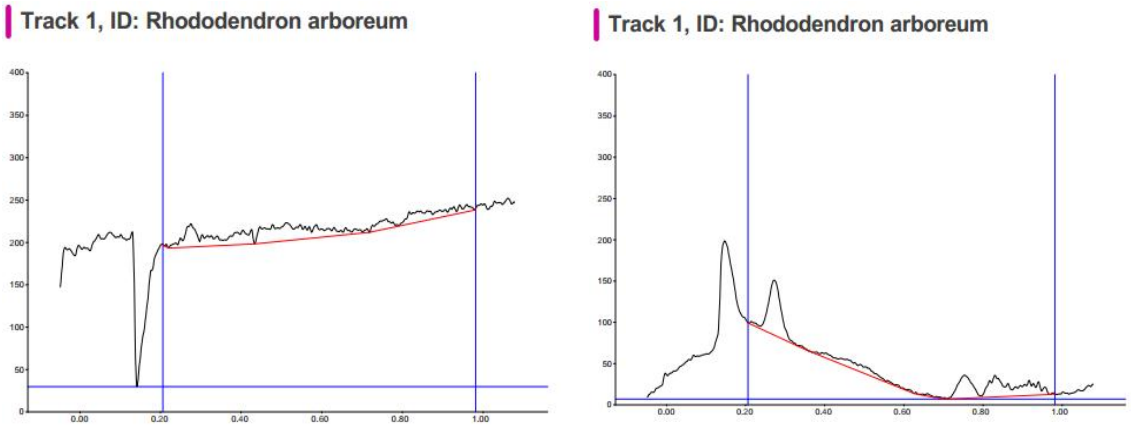
Fig. 4. HPTLC screening of ethanol extracts of *Rhododendron arborieum* (stem bark) before derivatization

Table 4. Qualitative phytochemical tests of extracts of *Rhododendron arborieum* stem bark

Name of test	Aquas extract	Alcohol extract	Petroleum ether extract
Carbohydrate			
Molish test	-ve	+ve	+ve
Benedict test	-ve	-ve	-ve
Fehling test	+ve	+ve	-ve
Barfoad test	-ve	-ve	-ve
Alkaloids			
Dragendorff test	+ve	+ve	+ve
Wagner's test	-ve	-ve	-ve
Hager's test	-ve	+ve	-ve
Amino acids			
Ninhydrine	-ve	-ve	-ve
Protein			
Biuret test	-ve	-ve	-ve
Xenthoprotic test	+ve	+ve	-ve
Millon test	-ve	+ve	-ve
Saponin			
Foam test	+ve	-ve	-ve
Glycosides			
Borntrager's test	+ve	+ve	+ve
Phenolic compound			
Phenolic test	-ve	+ve	+ve
Steroids			
Salkowaski	-ve	-ve	-ve
Tannins			
$Fecl_3$	-ve	-ve	-ve
Lead acetate	+ve	+ve	+ve
Pot. Dichromate	+ve	+ve	+ve

254 nm

366 nm



R_f value	Area %	R_f value	Area %
0.24	49.93	0.24	52.81
0.42	9.98	0.72	26.32
0.44	24.2	0.80	20.87
0.79	15.9	-	-

Fig. 5. HPTLC screening of ethanol extracts of *Rhododendron arborieum* (stem bark) after derivatization

Microscopy of *Rhododendron arboreum* Sm. have multilayer loosely arranged parenchyama cells separated by thick layer of cortical cells and Vascular bundles. Powder have scaliform vessels, Calcium Oxalate and Starch Grain, Fragment of Cork cells, pitted vessel. It contains Phloem fibre, Calcium oxalate crystals, Tanninferous cells, Cork cells, Cutin and Cellulose in 40 X visualization. Physiochemical parameters of stem bark was found as Physiochemical property is essential for evaluate nature and quality of sample and each test is responsible for following property. Moisture content is a water holding capacity of sample, higher moisture content in sample show that it may decrease stability. Total Ash is a quantity analysis technique for determine Siliceous Material and Inorganic Substance in sample. Acid Insoluble Ash shows Siliceous Material and Heavy Metals. Water Soluble Ash shows Quantity of water Inorganic Substance. Extractive Value is a property of sample which denote water, alcoholic and ether soluble chemical constituent present in sample. pH gives acidic and basic nature of sample. Phytochemical analysis showed that sample of stem bark have primary and secondary metabolites (Carbohydrate, Alkaloids, Protein, Phenolic Compound, Tannins and Saponins.

Result of present study in addition to the special findings of HPTLC analysis, Aflatoxin, Pesticides residue and heavy metal analysis may also act as future standard for authentication of the genuine species *Rhodendron arboreum* Sm. Present work acclaims for further multidisciplinary studies to explore this important medicinal plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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