

Research Article,

Phytochemical Screening of *Barleria Prionitis L* Leaves Using Hydrotropic Solvents

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Abstract:

Hydrotropic agents are stated as ionic organic salts which help to increase or decrease the solubility of solute in a given solvent via ‘salt in’ or ‘salt out’ effects, respectively. Salts which show ‘salt in’ of non-electrolytes are called “hydrotropic salts” and the phenomenon is known as “hydrotropism. There are about 45,000 plant species in India, with concentrated hotspots in the region of Eastern Himalayas. Entire plants of *Barleriaprimonitis L.* collected from Shrinathji Institute of pharmacy, Nathdwara and the plant was authenticated by Dr. R.L. Bhardwaj. The leaves of *Barleria Prionitis L* plant was characterized by its morphological features like colour, shape, size and surface characteristics has been studies. The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with blends RP-1 to RP-6 using a Soxhlet apparatus. The yield of the plant extracts measured about 20 g each after evaporating the solvent using water bath. As per result of HPTLC, we found that the hydrotropic blends solution aur ecoferindly and cost effective method for the extraction purpose. All the phytoconstitents which was earlier reported in previous literature by the use of organic solvents which were also present for the use of hydrotropic solvents.

Keywords: *Barleria Prionitis L*, Hydrotrophy, Alkaloids, Tannins, Saponin, HPTLC.

Introduction:

Herbs are defined in several ways depending on the context, which the world is used. In the field of medicine, they are most accurately defined as crude drugs of vegetable origin utilized for the treatment of disease states, often of a chronic nature, or to attain or maintain a condition of improved health. Pharmaceutical preparations made by extracting herbs with various solvents to yield tinctures, fluidextracts, extracts, or the like, are known as phytomedicinals. Herbs are used as medicine by about 80% of the world population, mainly in the developing countries, for primary healthcare because of better cultural acceptability, better compatibility with the human body and lesser side effects. India is one of the countries in the world today where ancient system of medicine, such as Ayurveda, Siddha, Unani, Tribal medicine and Naturopathy have been in practice for several years [1-2]

Medicinal plants are not only used for primary health care and not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used [3]. In western world also, the use of herbal medicines is steadily growing with approximately 40 percent of population reporting use of herb to treat medical illnesses in 2004 [4]. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions

and economic burden of the modern system of medicine [5-6]

Barleria prionitis L. (Acanthaceae) is a prickly shrub commonly known as 'Pivali koranti' native to India and Sri Lanka [7]. It is used for various medicinal purposes in ayurvedic medicine. The plant is used for the treatment of tooth ache, strengthening of gums, whooping cough. The juice of the leaf is used in cataract and fever.

Hydrotropy and hydrotropic agents:

In 1916, 'hydrotropy' term was coined by the scientist Carl A. Neuberg [8]. Hydrotropes with an amphiphilic molecular structure possess the ability to increase the solubility of sparingly soluble organic molecules in water [9]. It is a molecular phenomenon whereby adding a second solute (hydrotrope) helps to increase the aqueous solubility of poorly soluble solutes [10]. Simply the presence of a large quantity of one solute enhances the solubility of another solute [11]. They do not exhibit any colloidal properties but they improve solubility by forming weak interaction with solute molecules [12]. A hydrotropic molecule interacts with a less water-soluble molecule via weak van der Waals interactions such as π - π or attractive dipole-dipole interaction [13]. Hydrotropes contain both hydrophobic and hydrophilic fractions in them. In comparison to surfactant, they contain a very small hydrophobic fraction [14].

Materials and Methods:

Collection and identification:

Entire plants of *Barleriaprimonitis* L. collected from Shrinathji Institute of pharmacy, Nathdwara and the plant was authenticated by Dr. R.L. Bhardwaj Associate Professor (Horticulture) O. S. D. Botanist College of Agriculture, Sumerpur Dist: Pali (Raj) India. The authentication letter Ref No.: F./Estt/COA/SUM/2021/652).

Preparation of plant material:

The Leaves of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use.

Macroscopical evaluation of plant materials:

The leaves of *Barleria Prionitis* L plant was characterized by its morphological features like colour, shape, size and surface characteristics has been studied.

Preliminary test

The leaves powder was characterized by its morphological features like white colour, presence of specific odour and taste.

Analytical Parameter:

Ash Values:

The residues remaining after incineration is the ash content of the leaves powder. Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information regarding its adulteration with inorganic matter [15]. Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash, and water soluble ash.

Determination of total ash value:

Accurately weighed about 3 gm of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

Determination of acid insoluble ash value:

The ash obtained as directed under total ash value was boiled with 25 ml of 2N HCl for 5 minutes. The

insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Determination of water soluble ash value:

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C [16]. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Extractive Values:

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Loss on Drying:

Loss on drying is the loss in weight in % w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Dessicator or hot air oven). If the sample in the form of large crystals, then reduce the size by quickly crushing to a powder.

Procedure:

About 1.5 gm, of powdered drug was weighed accurately in a porcelein dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated [17].

Extraction and fractionation:

The hydrotroic solution blends with different composition were used for the extraction process as given in table.

Table 1: Composition of Hydrotropic blends solution

Blends code	Hydrotropic blends	Concentration
RP-1	Urea solution	10% w/v
RP-2	Urea+Sodium benzoate	10% w/v +10% w/v
RP-3	Sodium acetate	10% w/v
RP-4	Trisodium citrate	10% w/v
RP-5	Thymol + Camphor Eutectic mixture	10% w/v +10% w/v
RP-6	Aqueous (Distilled water)	-

The extraction yield of the extracts from plant species is vastly depends on the solvent polarity, which find out both qualitatively and quantitatively the extracted compounds. Ethanol and water are the commonly used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios [18].The plant materials (500 gm) were initially defatted with petroleum ether and then extracted with blends RP-1 to RP-6 using a Soxhlet apparatus. The yield of the plant extracts measured about 20 g each after evaporating the solvent using water bath [19]. The standard extracts obtained from *Barleria prionitis L* were then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening [20].

Phytochemical screening:

Qualitative examination of phytoconstituents:

Qualitative examination of phytoconstituents were performed on the basis of test of phtoconstituents like alkaloids, saponin, flavonoids, tepenoids, carbohydrate etc [21-24].

Quantitative estimation of urine component by HPTLC

Table 2: Instrument Detail:

Instrument	CAMAG TLC Scanner “Scanner_170825” S/N 170825(2.01.02)
Executed by	Anchrom
Number of track	1
Position of first track X	15.0 mm
Distance between tracks	0.0 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	75.0 mm
Slit dimensions	4.00 × 0.30 mm, Micro
Optimize optical system	Light
Scanning speed	20 mm/s
Data resolution	100 µm/step

Table: 3 Measurement Criteria:

Wavelength	254
Lamp	D2 & W
Measurement type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	295 V

Method for HPTLC Analysis:

A densitometric HPTLC analysis was performed for the development of characteristic finger printing profile. HPTLC was performed on 5x10 cm silica gel 60 F254 aluminum TLC plate (E. MERCK, KGaA). The sample was diluted with water in the ratio 1:9. The diluted sample was applied to the TLC plates using Camag Linomat V Sample Applicator equipped with Hamilton syringe of 100µl capacity. 5 µl and 10 µl of the diluted sample were applied on two separate tracks of 8mm band width. For the separation of sample acetonitrile : water (6:4, 5:5, 4:6) was a recommended mobile phase. The same mobile phase was used for the analysis. The plates were developed in twin-trough chamber previously saturated with the mobile phase up to a migration distance of 90mm [25]. The developed plate was dried using hot air to evaporate solvents from the plate. The plate was kept in UV cabinet and observed at 254 nm and 366 nm. The plate was sprayed with vanillin sulphuric acid reagent and observed for colouration in visible light. Finally, the plate was fixed in scanner stage in Camag TLC Scanner and scanned at 254 nm. The Peak table, Peak display and Peak densitogram were identified.

Results and discussion:

The Ayurvedic system of medicine includes number of plants and materials which should be investigated to determine the hidden potential by using the modern methodology. The goal of Pharmacognosists should be searching for drugs of plant origin with minimum side effects and maximum benefits. The plant *Barleria prionitis L* commonly is an indigenous herb which was chosen for this study. Various part of this plant is being used for their medicinal properties.

Pharmacognostical studies:**Morphology of *Barleriaprionitis L***



Fig 1: Length measurement for leaves of Barleria prionitis L.



Fig. 2: Width measurement for leaves of Barleria prionitis L Flowers:

Flowers:

The yellow–orange tubular flowers are found bunched tightly together at the top of the plant, but they also occur singly at the base of leaves. The size length of flower were found 3.5 cm.

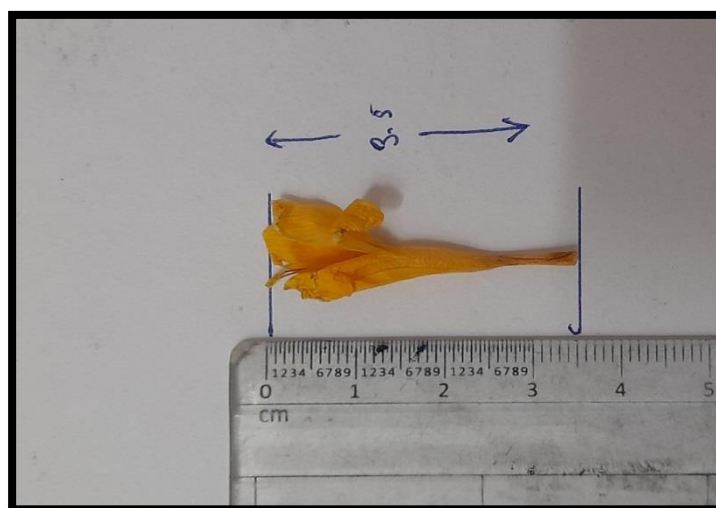


Fig. 3: Width measurement for Flower of Barleria prionitis L

flowers are 3.5 cm long and tubular, with several long protruding stalks (stamens). The bracts and calyx are green and oblong-lanceolate, with the outer bract usually foliaceous. The corolla is about 4 centimeters long. Corolla 1.5 cm across, pubescent to glabrous outside, tube 2-2.5 cm long; limb nearly as long as tube, lobes oblong-ovate, obtuse.

Physiochemical analysis of crude drug:

The Physiochemical analysis of leaves powder was carried out. In this study ash values (moisture content, total ash, acid insoluble ash, water soluble ash and water soluble ash) were determined. The total ash value was found to be 8.27% w/w indicating the considerable presence of inorganic radicals. The acid insoluble ash was found to be 2.43 %w/w. the difference between the total ash and acid insoluble ash indicates that the ash of leaf powder contains considerable amount of inorganic radicals like calcium oxalate which are acid insoluble. The water soluble ash value was found to be 11.21w/w. The water soluble ash value was found to be 2.17% w/w. and the moisture content was found 4.72%. All these values are expressed in table no 4.

Table 4: Physiochemical analysis of crude drug

Sr. No.	Physiochemical Parameter	Leaves (% w/w)	Reported (% w/w)
1	Foreign organic matter	0.43	-
2	Moisture content	4.72	-
3	Total ash	8.27	8.31
4	Water soluble ash	2.17	2.10
5	Acid insoluble ash	2.43	2.48
6	Water soluble extractive value	11.21	11.94

Preliminary phytochemical study of the *Barleria Prionitis* and *Barleria Elegance* extracts:

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. The preliminary phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts and fractions from *Barleriaprionitis* L. by using precipitation and coloration reaction to identify the major natural chemical groups. General reactions in this analysis revealed the presence or absence of these compounds in the crude extracts and fractions tested. Summary of preliminary phytochemical screening of different extracts and fractions is depicted in Table-5.

Table- 5: Phytochemical screening of extracts of *Barleriaprionitis* L.

Chemical Constituents	Chemical Test	Extracts					
		Urea solution	Urea+Sodium benzoate	Sodium acetate	Trisodium citrate	Thymol + Camphor	Aqueous
Alkaloids	Mayer's	-	-	-	-	-	-
	Dragendorff's	-	-	-	-	-	-
Saponin	Foam forming test	+	+	+	+	+	+
Tannins	Ferric Chloride	-	-	-	-	-	-
	Dilute nitric acid	+	+	+	+	+	+

	Biuret	-	-	-	-	-	-
Flavonoids	Shinoda	+	+	+	+	+	+
	Lead Acetate	+	+	+	+	+	+
Glycoside	Killer killani	-	+	-	-	-	-
Carbohydrate	Molisch's	-	-	-	-	-	-
	Fehling's	-	-	-	-	-	-
Triterpenes	Vanillin-sulphuric acid test	-	-	-	-	-	-

Rakesh pal singh1.et al./ Phytochemical Screening of Barleria Prionitis L Leaves Using Hydrotropic Solvents

Amino Acids	Ninhydrin	-	-	-	-	-	-
Sterols	Liebermann-Burchard's	-	-	-	-	-	-
	Salkowski's	-	-	-	-	-	-
Phenol		-	-	-	-	-	-

Key (+) = Presence, (-) = Absent

Extractive Values:

The phytoconstituents were extracted by using different solvent of increasing polarity like Urea solution, Urea+Sodium benzoate, Sodium acetate, Trisodium citrate and water. The extractive values of various extract are expressed in table and figure No. 6.

Table 6: The extractive values of various extract of leaves of Barleriapronitis L

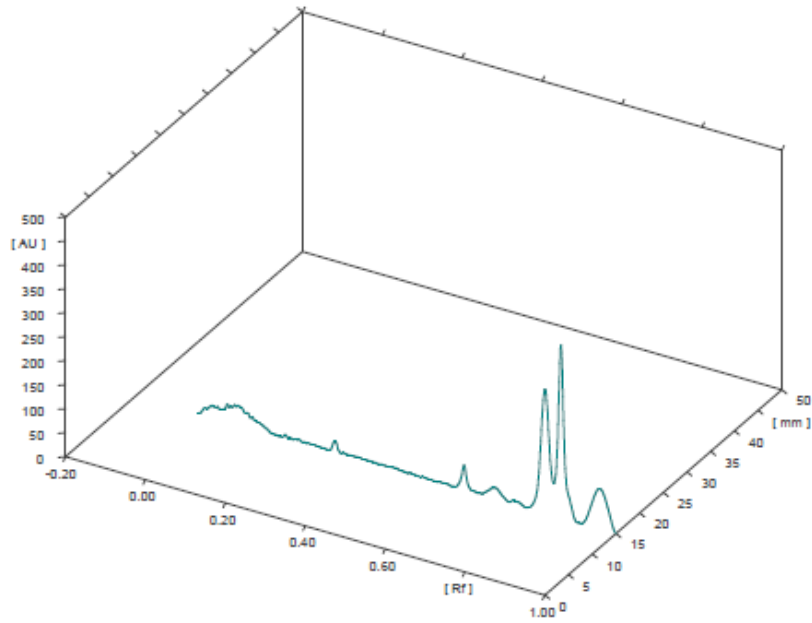
Sr. No.	Extracts/ Fractions	Estimated percentage	Colour of extract
1.	Urea solution	9.45 % w/w	Dark green
2.	Urea+Sodium benzoate	11.21% w/w	Dark Brown
3.	Sodium acetate	3.73 % w/w	Greenish brown
4.	Trisodium citrate	4.52 % w/w	Dark green
5.	Thymol + Camphor	8.02 % w/w	Light Brown
6.	Aqueous	7.08 % w/w	Light green

HPTLC Analysis of extracts:

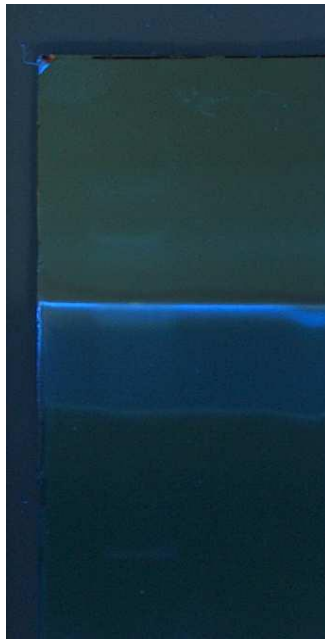
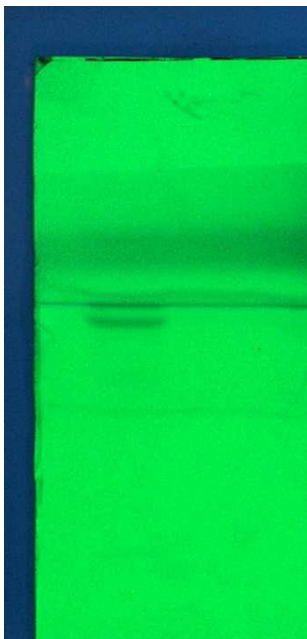
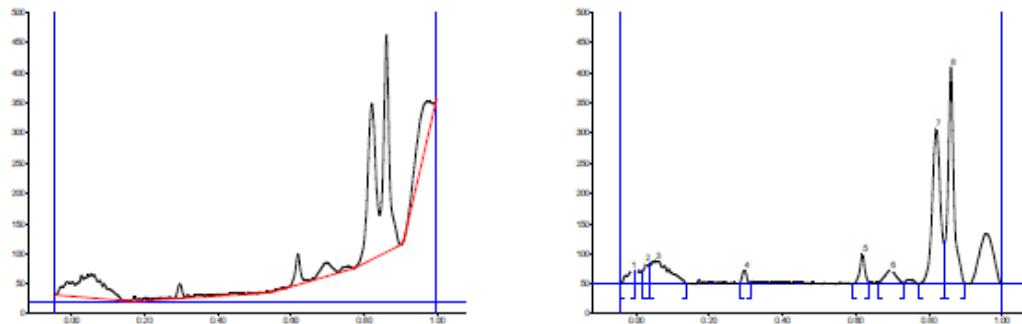
The highest percentage of yield of extractive value choose for further HPTLC analysis were Urea solution, Urea+Sodium benzoate and Thymol + Camphor.

HPTLC sample 1 (Urea solution):

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance	Area %
1	-0.04	0.1	-0.01	23.5	2.88	-0.00	22.1	385.2	unknown	3.03
2	0.02	23.4	0.03	35.7	4.38	0.04	33.2	362.9	unknown	2.86
3	0.04	33.5	0.06	39.7	4.87	0.14	0.9	1549.7	unknown	12.19
4	0.28	0.6	0.30	23.6	2.90	0.31	1.2	224.7	unknown	1.77
5	0.59	0.7	0.62	50.6	6.21	0.64	3.3	541.8	unknown	4.26
6	0.66	2.2	0.70	22.9	2.81	0.74	1.3	577.2	unknown	4.54
7	0.78	0.3	0.82	258.9	31.76	0.84	66.8	4586.6	unknown	36.08
8	0.85	69.9	0.86	360.1	44.19	0.90	0.2	4483.8	unknown	35.27



Track 1, ID:

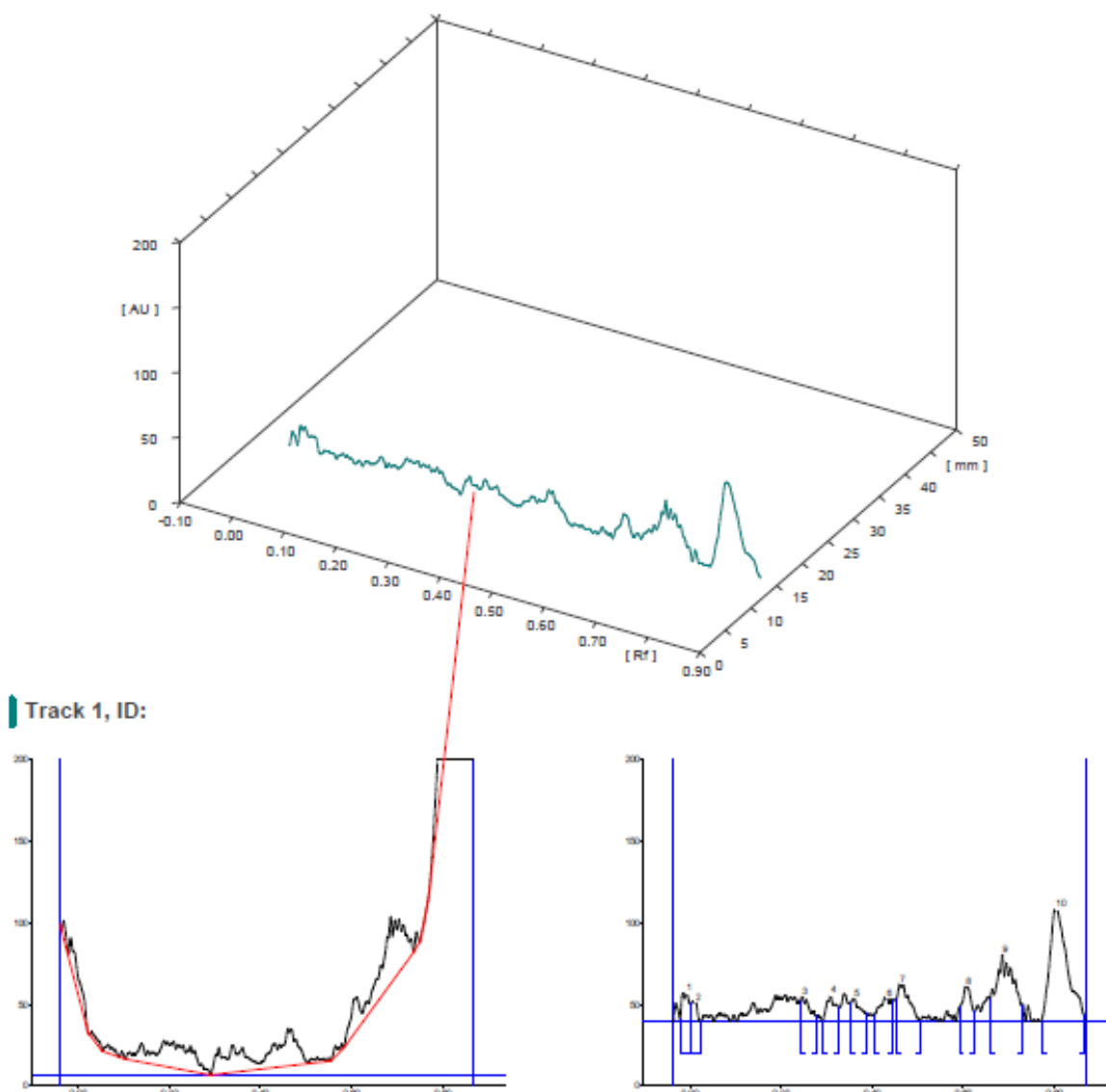


Exposure mode: Automatic, digital level80 % Band

Exposure mode: Automatic, digital level85 % Band

Exposure mode: Automatic, digital level85 % Area

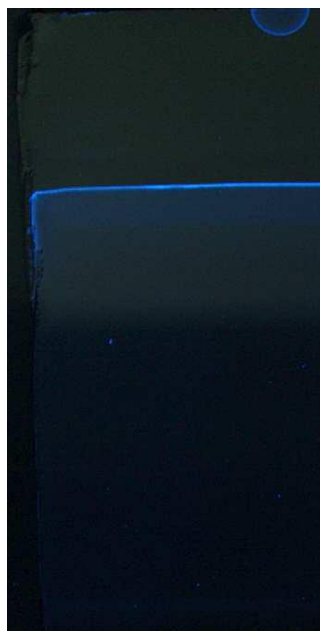
HPTLC sample 2 (Urea+Sodium benzoate)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance	Area %
1	-0.02	1.6	-0.01	17.4	7.14	0.00	10.4	236.2	unknown	3.87
2	0.00	10.5	0.01	12.0	4.93	0.02	0.0	139.4	unknown	2.28
3	0.24	11.8	0.25	15.5	6.35	0.28	3.2	255.7	unknown	4.19
4	0.29	0.3	0.31	16.1	6.60	0.33	8.2	272.3	unknown	4.46
5	0.35	10.7	0.36	14.4	5.91	0.39	5.0	256.7	unknown	4.21
6	0.41	4.1	0.43	14.4	5.92	0.45	12.5	349.6	unknown	5.73
7	0.45	13.0	0.46	22.9	9.41	0.51	0.4	532.6	unknown	8.73
8	0.59	8.5	0.61	21.2	8.71	0.62	5.7	365.0	unknown	5.98
9	0.66	13.4	0.69	40.9	16.82	0.73	9.8	1332.8	unknown	21.84
10	0.77	0.4	0.81	68.6	28.20	0.87	3.5	2363.1	unknown	38.72



Exposure mode: Automatic, digital
level80 % Band



Exposure mode: Automatic, digital
level85 % Band



Exposure mode: Automatic, digital
level85 % Area

Conclusion:

Entire plants of *Barleriapronitis* L. collected from Shrinathji Institute of pharmacy, Nathdwara and the plant was authenticated by Dr. R.L. Bhardwaj Associate Professor (Horticulture) O. S. D. Botanist College of Agriculture, Sumerpur Dist: Pali (Raj) India. The authentication letter Ref No.: F./Estt/COA/SUM/2021/652). The Leaves of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use.

The total ash value was found to be 8.27% w/w indicating the considerable presence of inorganic radicals. The acid insoluble ash was found to be 2.43 % w/w. the difference between the total ash and acid insoluble ash indicates that the ash of leaf powder contains considerable amount of inorganic radicals like calcium oxalate which are acid insoluble. The water soluble ash value was found to be 11.21w/w. The water soluble ash value was found to be 2.17%w/w. and the moisture content was found 4.72%. The phytoconstituents were extracted by using different solvent of increasing polarity like Urea solution, Urea+Sodium benzoate, Sodium acetate, Trisodium citrate and water were found as 3.73 to 11.21%w/w observed. As per result of HPTLC, we found that the hydrotropic blends solution are ecofriendly and cost effective method for the extraction purpose. All the phytoconstituents which were earlier reported in previous literature by the use of organic solvents which were also present for the use of hydrotropic solvents.

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