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Hydrotropic Solvents Used For Extraction Techniques Of *Barleria Elegans L.* Leaves

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Abstract:
Plant species belonging to the family Acanthaceae are globally known to possess various medicinal properties and have cultural and economic importance in both traditional medicine and horticulture. They are important to both animals and humans and are used as food or for ornamental purposes worldwide. Barleria is the third largest genus in the family Acanthaceae. A few of the highly important and reported species of Barleria include B. prionitis, B. cristata, B. grandiflora, and B. lupulina. The flowers, leaves, stems, roots, and seed extracts of plants belonging to this genus are rich in bioactive compounds and have exhibited significant medicinal potential for the treatment of various ailments and infections.
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 *Barleriaelegans* collected from Shrinathji Institute of pharmacy, Nathdwara and the plant was authenticated by Dr. R.L. Bhardwaj. The leaves of *Barleriaelegans* plant was characterized by its morphological features like colour, shape, size and surface characteristics has been studies. The plant materials (500gm) 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 TLC, we found that the hydrotropic blends solution aurecoferindly and cost effective method for the extraction purpose. All the phytoconstitutents which was earlier reported in previous literature by the use of organic solvents which were also present for the use of hydrotropic solvents.

Key words: *Barleriaelegans*, Hydrotropy, Alkaloids, Tannins, Saponin, HPTLC.

Introduction

Traditional medicine is an ancient practice which is nearly as old as the existence of mankind. This declaration is backed by evidence obtained from studies of the older civilizations of human settlements where paleontologists discovered bunches of medicinal herbs among the fossilized remains of Neanderthal ancestors

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]

Barleriaelegans (Acanthaceae) is a pricky shrub commonly known as 'Pivalikoranti' native to India and SriLanka [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 *Barleriaelegans*. collected from Shrinathji Institute of pharmacy, Nathdwara and the plant was authenticated byDr. R.L. Bhardwaj Associate Professor (Horticulture) O. S. D. Botanist College of Agriculture, SumerpurDist: Pali (Raj) India. The autenification 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 *Barleriaelegans* plant was characterized by its morphological features like colour, shape, size and surface characteristics has been studies.

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 onDrying:

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 hydrotroicsolution blends with different composition were used for the extraction process as given in table.

Table 1: Compostion 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 *Barleriaelegans* 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].

Thin layer chromatography (TLC):

Chromatography is widely used for the separation, isolation, identification, and quantification of components in a mixture. Components of the mixture are carried through the stationary phase by the flow of a mobile phase. Separations are based on differences in migration rates among the sample components. TLC is chosen over other chromatography methods because it is a simple, quick and inexpensive procedure and very sensitive to even microgram amount of sample mixtures. TLC is a mode of liquid chromatography in which the sample is applied as a small spot or streak to the origin of a thin sorbent layer such as silica gel, alumina, cellulose powder, polyamides, ion exchangers or chemically bonded silica gel supported on a glass, plastic, or metal plate. This layer consists of finely divided particles and constitutes the stationary phase. The eluent or mobile phase is a solvent or a mixture of organic and/or aqueous solvents in which the spotted plate is placed. The mobile phase moves through the stationary phase by capillary action, sometimes assisted by gravity or pressure. The effectiveness of the separation depends on the mixture to be separated, the choice of the mobile phase and the adsorption layer and the term retention factor R_f, is commonly used to describe the chromatographic behavior of sample solutes [25]. The R_f value for each substance is the distance it has moved divided by the distance the solvent front has moved. The solvents, during this research for each crude extract, were chosen by trial and error. The selection was made on the basis of best resolution.

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 *Barleriaelegans* 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 *Barleriaelegans*

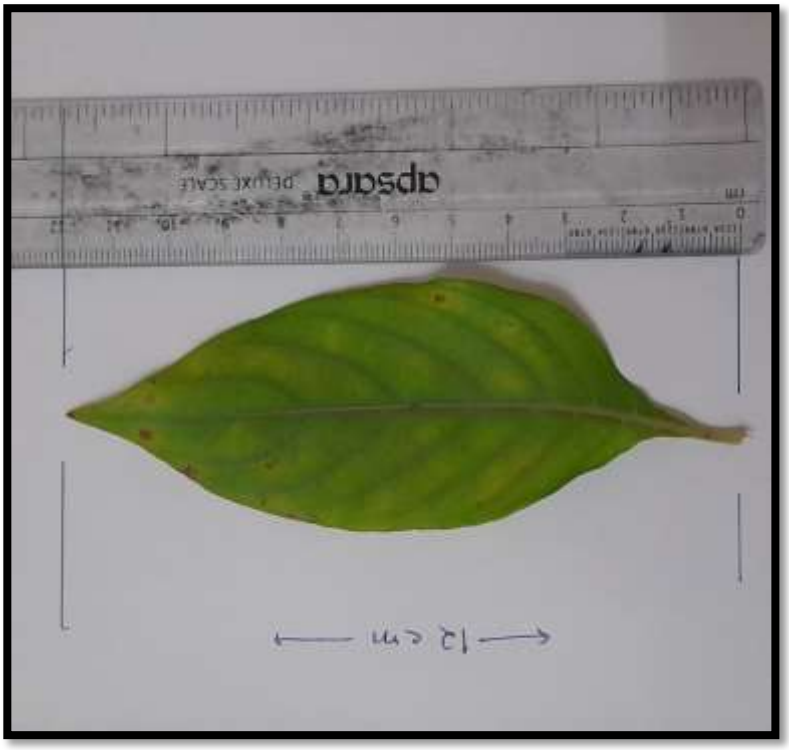

Fig 1: Length measurement for leaves of *Barleria elegans*

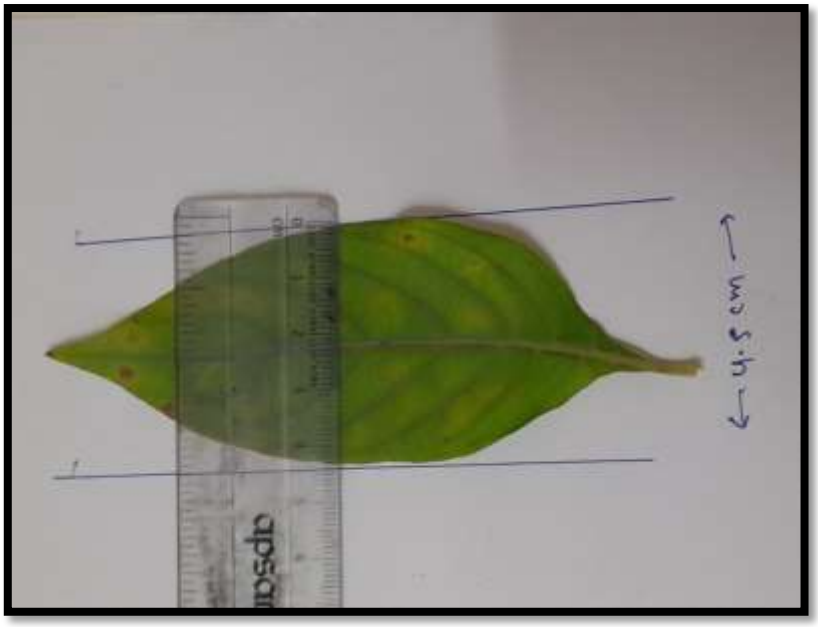

Fig. 2: Width measurement for leaves of *Barleria elegans*

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 lenth of flower were found 3.5 cm.

Fig. 3: Width measurement for Flower of *Barleria elegans*

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 7017 % w/w indicating the considerable presence of inorganic radicals. The acid insoluble ash was found to be 2.45 %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 3.15w/w.. and the moisture content was found 3.71%. 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	0.53	-
2	Moisture content	3.71	-
3	Total ash	7.17	8.31
4	Water soluble ash	3.15	2.10
5	Acid insoluble ash	2.45	2.48
6	Water soluble extractive value	11.25	11.94

Preliminary phytochemical study of the *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 *Barleria elegans*. 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 inTable-5

Table- 5: Phytochemical screening of extracts of *Barleria elegance*

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	+	+	+	+	+	+

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

ExtractiveValues:

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 *Barleria elegans*..

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

Thin layer chromatography Analysis of extracts:

The higestpercentage of yield of extractive value choose for further TLC analysis were given below.

	Biuret	-	-	-	-	-	-
Flavonoids	Shinoda	+	+	+	+	+	+
	Lead Acetate	+	+	+	+	+	+
Glycoside	Killer killani	_-	+	-	-	_	-
Carbohydrate	Molisch's	-	-	-	_	-	-
	Fehling's	_	_	_	_	_	_
Triterpenes	Vanillin- sulphuric acid test	-	-	-	-	-	-
Amino Acids	Ninhydrin	-	-	-	-	_	-
Sterols	Liebermann- Burchard's	-	-	_	_	_	_
	Salkowski's	_	_	_	_	-	_
Phenol		_	_	_	_	_	_

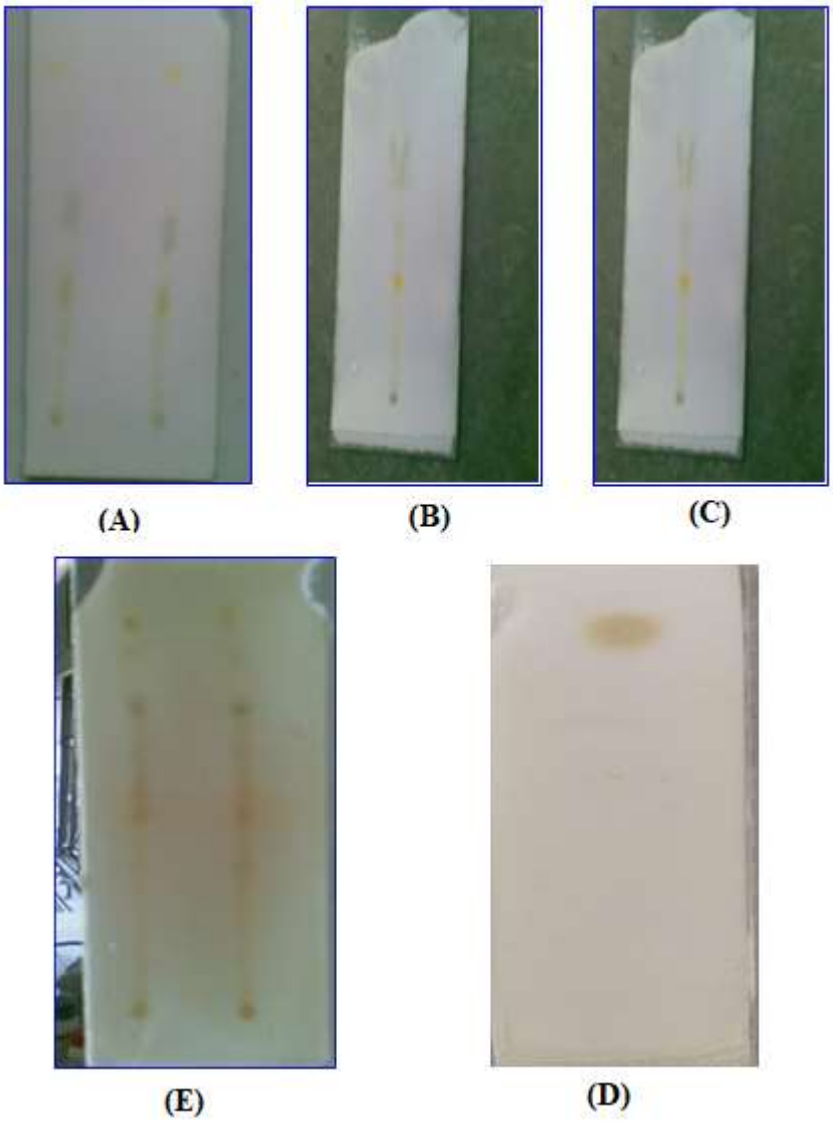

Fig 5.8: TLC studies of Urea extract [A], water extract [B],Urea+ sodium benzoate [C], sodium benzoate [D], Thymol+Camphor extract[E], Trisodium citrate

Extract	**Solvent system**	**No of spots**	**TLC profile**	
			R_f value	**Color**
Urea	Toluene:Ethyl acetate:Metenol: Water(7:6:5:2)	3	0.74;0.69;0.61;	Dark green, green, green, faint green
Aqueous	Ethyl acetate: methanol: toluene: water (5:4:6:5)	2	0.90;0.86;	Dark green, green,

sodium benzoate	Hexane: methanol (7:3)	3	0.57; 0.40;0.38	Light brown , light yellow , light brown
Urea+ sodium benzoate	Hexane: Ethyl acetate: methanol (10:3:7)	5	0.70;0.64;0.58; 0.47;0.39	Light green, Very light green, Light brown , light yellow , light brown
Camphor+ thymol	Hexane:DCM: Ethyl acetate: Methanol (10:5:2:3)	6	0.76;047;0.41 0.29;0.27;0.22	Dark yellow, Dark brown, Dark brown, Very light green. Light yellow, light brown
Trisodium citrate	Toluene:Ethyl acetate:Metenol: Water(7:6:5:2)	1	0.94	Dark brown

Conclusion:

Entire plants of ***Barleriaelegans*. collected from Shrinathji Institute of pharmacy, Nathdwara** and the plant was authenticated byDr. R.L. Bhardwaj Associate Professor (Horticulture) O. S. D. Botanist College of Agriculture, SumerpurDist: Pali (Raj) India. The autenification 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 7.17% w/w indicating the considerable presence of inorganic radicals. The acid insoluble ash was found to be 2.45 %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 3.15w/w. The water soluble ash value was found to be 2.17%w/w. and the moisture content was found 3.71%. 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 TLC, we found that the hydrotropic blends solution aurecoferindly and cost effective method for the extraction purpose. All the phytoconstitutents which was 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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