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Research Article

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Comparative Studies on Different Solvents Used for the Extraction of Phytochemicals from the Plant Parts of *Arnebia benthamii*. (Wall Ex. G. Don) Johnston

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ABSTRACT

Phytochemical screening was carried out using different solvent extracts of inflorescence, root and leaf of Arnebia benthamii to compare their efficacy. Qualitative analysis of ten phytochemical compounds (alkaloids, saponins, tannins, phenolics, glycosides, proteins and amino acids, sterols, terpenoids, flavonoids and carbohydrates) was made. Out of the selected plant parts, inflorescence gave positive test for all ten compounds in all selected solvents except for sterols and terpenoids. The phytochemicals of root part showed positive test for seven compounds in methanolic extract, ten in ethanolic extract, and four each in acetone and chloroform extracts. Saponins, tannins and phenolics were present in least number of solvent extracts as they showed their presence in ethanol extract only. The methanol and ethanol extracts of leaf showed positive results for all ten compounds. Acetone and chloroform leaf extracts showed presence of eight and six phytochemicals respectively. Sterols and terpenoids were found absent in acetone and chloroform extracts. The presence of various phytochemicals further established the previous findings that parts of Arnebia benthamii are good sources of therapeutical compounds.

Keywords: *Arnebia benthamii*; Herbal drug; Plant extracts; Solvents

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INTRODUCTION

Phytochemicals are bioactive compounds of plant origin and regarded as secondary metabolites because even the plants that manufacture them may not require them. Any part of the plant body may contain phytochemicals, however the quantity and quality of phytochemicals may vary in different parts [1]. Such naturally occurring bioactive substances of medicinal plants provide protection from several diseases. More than 4,000 phytochemicals have been catalogued till 2000 and about 150 phytochemicals have been studied in detail [2]. They are classified by protective, physical and chemical characteristics [3]. Some are responsible for color and other organoleptic properties, e.g. the smell of garlic. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients [4]. Pharmaceutically extraction methods involve the separation of bioactive compounds from the inactive components by using certain selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [5].

Arnebia benthamii (Syn.- *Macrotomia benthamii*) belongs to family Boraginaceae (Fig. 1). It occurs in the alpine and subalpine Himalayas, distributed in the Hindukush Himalayan range across Afghanistan, Pakistan, India and Nepal at an altitude range of 3000-4300 m above sea level. From India it has been reported from Jammu & Kashmir, Himachal Pradesh and Uttaranchal. It is locally called "*Kahzaban, Gaozaban, Laljari, Ratanjot"*. It is an erect, robust, herbaceous perennial plant, 30–90 cm in height with stout woody, deep red roots, monocarpic with hermaphrodite flowers and reaches reproductive maturity in 3–4 years [6,7].

Figure 1: *Arnebia benthamii* Plant

Different parts (root, stem, leaves, flowers and seeds) of *Arnebia benthamii* are traditionally used to cure cardiac disorders. The flowers are particularly reported to have soothing effect on patients with heart ailments. The plant also possesses stimulative, tonic, diuretic and expectorant properties; the syrup and jam from the flowering shoots are used for the treatment of tongue and throat disorders [8]. In Unani system of medicine, it is one of the ingredients of most of the composite medicines, used for the treatment of high fevers [9]. *Arnebia benthamii* is a major ingredient of the commercial herbal drug available under the name Gaozaban, which has antibacterial, antifungal, anti-inflammatory and wound-healing properties [10]. The plant is also supposed to possess antioxidant properties [11]. The tribals in the Himalayas collect huge quantity of this species, dried and boiled to prepare a special type of tea (without milk), locally known as 'Kahwa' which is used to cure the chest infections especially among children [9]. The root, which forms the actual drug, is considered to be anthelmintic, antipyretic, antiseptic and claimed to be useful in the treatment of diseases of the eye, bronchitis, abdominal pain, itches and many more [12]. A very little amount of work has been carried out on the phytochemistry and antimicrobial activity of *Arnebia benthamii* plant extracts. The main objective of the present work was to compare the efficiency of various solvents used for the extraction of phytochemicals and qualitative screening for phytochemicals using standard tests.

EXPERIMENTAL SECTION

Materials and methods

Fresh plant parts of *Arnebia benthamii* were collected from the hills of Kokernag Tehsil of Anantnag District in Jammu and Kashmir, India in the months of June and July 2012. Plants were identified with the help of published literature at Institute of Ethnobiology, Jiwaji University, Gwalior. The voucher specimens have been submitted in the herbarium of the institute. The fresh plant parts were properly cleaned to remove any dirt or filthy particles present on the surface with tap water and then rinsed with distilled water to avoid any contamination. The pieces of plant materials were air dried for 15 days. The dried plant materials were taken separately and crushed using mechanical grinder to obtain a fine powder and sieve to obtain finer particles. The powdered samples were stored in clean plastic containers until needed for analysis. Methanol, Ethanol, Acetone and Chloroform were used as extraction solvents. The powdered material (approx. 200 gm.) was put in the Soxhlet apparatus and extracted successively with methanol, ethanol, acetone and chloroform (40-60) (Merck, India). After completion, the extracted powder was discarded and the different extracts so obtained were further processed. The excess solvent in the extracts were removed by distillation and the concentrated extracts so obtained were further dried at a temperature (not exceeding 100°C) in water bath. The extracts were then collected and kept in petridish and stored in a desiccator at room temperature and the concentrates were used for the phytochemicals screening using standard procedures.

Test for alkaloids

Wagner's test: A portion of extract was treated with 3-5drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodine in 100ml of water) and observed for the formation of reddish brown precipitate.

Mayer's Test: Few drops of ammonium hydroxide were added to sample. Then Mayer's reagent (1.35g of mercuric chloride in 60 ml of water and 5g of potassium iodide in 10 ml of water) was added to it and observed for cream colored precipitate which confirmed the presence of alkaloids.

Dragendorff's Test: To a few ml of filtrate, 1 or 2ml of Dragendorff's reagent was added by the side of the test tube. A prominent yellow ppt. indicated test as positive.

Test for Saponins (Foam test): To 2ml of extract was added to 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for tannins
Alkaline reagent test: Test solution after treatment with sodium hydroxide solution exhibited yellow to red precipitate within short time.

Test for phenols (Ferric chloride test)
A portion of the extract was treated with aqueous 5% ferric and observed for formation of deep blue or black color.

Test for glycosides:
Raymond's test: Test solution when treated with dinitro- benzene in hot methanolic alkali gave violet color.

Legal's test: When extract was treated with pyridine and sodium nitroprusside, a blood red color appeared showing the presence of glycosides.

Test for proteins and amino acids:
Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicated the presence of proteins.

Millon's test: Test solution after treatment with 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) showed white precipitate, which turned red upon gentle heating.

Test for sterols and triterpenoids
Libermann- Buchard test: Plant extract was treated with few drops of acetic anhydride, boiled and cooled, then conc. sulfuric acid was added from the sides of the test tube by which a brown ring appeared at the junction of two layers and the upper layer turned green which showed the presence of steroids and formation of deep red color indicated the presence of triterpenoids.

Salkowski's test: 1ml of chloroform was added to 2ml of each extract followed by a few drops of conc. sulphuric acid. A reddish brown precipitate produce immediately indicates the presence of terpenoids.

Test for flavonoid (Alkaline reagent test):
2ml of extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute hydrochloric acid indicate the presence of flavonoid.

Test for carbohydrate:
Molish's test: Drops of Molish's reagent were added to 2ml portion of the extract. This was followed by addition of 2ml of conc.H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for 2-3 minutes. Formation of a red or dull violet color at the interphase of the two layers gave a positive test.

RESULTS AND DISCUSSION

Preliminary phytochemical screening
It involved testing of different extracts of *Arnebia benthamii* for their contents of different classes of phytochemical compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical tests to give general idea regarding the nature of phytochemical constituents present in crude drug and the efficiency of different solvents used for the extraction of phytochemicals from various plant parts (inflorescence, root and leaf) was compared. The qualitative chemical tests for various phytoconstituents were carried out for all the extracts of *A. benthamii* as mentioned below in Table-1, 2 and 3:

Table 1: Qualitative analysis of the phytochemicals in inflorescence

Phytochemicals	Tests	Methanol	Ethanol	Acetone	Chloroform
Alkaloids	Dragendorff's test	++	+++	+	++
	Mayer's test	+	++	+	+
	Wagner's test	+	++	++	+
Saponins	Froth test	+	++	+	+
Tannins and Phenolics	Alkaline reagent test	+	+++	+++	++
Glycosides	Legal's test	+	++	–	+++
Proteins and amino acids	Millon's test	++	+++	+++	++
	Xanthoproteic test	+	++	+	+++
Sterols and Terpenoids	Libermann-Buchard test	–	+++	–	–
Flavonoids	Alkaline reagent test	++	++	+++	++
Carbohydrates	Molischs Test	+++	+++	+++	+++

Table 2: Qualitative analysis of the phytochemicals in root

Phytochemicals	Tests	Methanol	Ethanol	Acetone	Chloroform
Alkaloids	Dragendorff's test	++	++	++	+
	Mayer's test	++	++	++	+
	Wagner's test	++	+++	+	+
Saponins	Froth test	–	+	–	–
Tanins and phenolics	Alkaline reagent test	–	+	–	–
	Ferric chloride test	–	+	_	+
Glycosides	Legal's test	+	++	–	+
Proteins and amino acids	Xanthoproteic test	++	+++	+	+
Sterols and terpenoids	Salkowski test	+	+	–	–
	Libermann- Buchard test	–	–	–	–
Flavonoids	Alkaline reagent test	++	+++	++	–
Carbohydrates	Molisch's test	+++	++	+++	++

Table- 3: Qualitative analysis of the phytochemicals in leaf

Phytochemicals	Tests	Methanol	Ethanol	Acetone	Chloroform
Alkaloids	Dragendorff's test	++	++	+++	+++
	Mayer's test	+	++	++	++
	Wagner's test	++	++	++	++
Saponins	Froth Test	++	++	+	_
Tanins and Phenolics	Alkaline reagent test	+++	+++	++	+
Glycosides	Raymond's Test	+	+	+	_
	Legal's Test	+	+	+	_
Proteins and amino acids	Xanthoproteic test	++	++	++	+
	Millons test	+	+++	++	+
Sterols and terpenoids	Libermann- Buchard test	+	+++	_	_
Flavonoids	Alkaline reagent test	+++	+++	+	++
Carbohydrates	Molisch's test	++	+++	+	++

+ = trace amount, ++ =moderate amount, +++ =good amount, – = completely absent

Ethanol extract of all the three plant parts gave positive tests for ten phytochemical compounds viz. alkaloids, saponins, tannins, phenolics, glycosides, proteins and amino acids, sterols, terpenoids, flavonoids and carbohydrates. In the same way methanolic extract gave positive tests for eight compounds viz. alkaloids, saponins, tannins, phenolics, glycosides, proteins and amino acids, flavonoids and carbohydrates. Seven compounds were shown by acetone extract viz. alkaloids, saponins, tannins, phenolics, proteins and amino acids, flavonoids and carbohydrates. Chloroform extract gave positive tests for eight compounds viz. alkaloids, saponins, tannins, phenolics, glycosides, proteins and amino acids, flavonoids and carbohydrates. Alkaloids were tested in all the four solvents (methanol,ethanol, acetone and chloroform) and in all the three selected plant parts (inflorescence, root and leaf). All the three plant parts in all solvents showed positive tests for alkaloids. Presence of saponins was indicated by the formation of froth. Inflorescence part showed positive tests for saponins. Only ethanol extract of root part was tested positive for presence of saponins (in traces). All extracts except chloroform solvent extract tested positive in the leaf part. The inflorescence and leaf showed positive results in all the extracts for the presence of tannins and phenolics which were indicated by the formation of yellow to red precipitate within short time. In case of root except ethanolic extract all other three solvent extracts showed the absence of tannins and phenolics. Glycosides were

tested in all the selected solvents in inflorescence and root parts. Leaf showed positive tests for glycosides in all solvent extracts except chloroform extract where the test was negative. Each plant part in each extract showed positive tests for proteins and amino acids.

In case of inflorescence only ethanolic extract showed positive result for sterols and terpenoids. Negative test was shown by methanol, acetone and chloroform extract of inflorescence. In roots sterols and terpenoids were shown in ethanolic and methanolic extracts whereas leaves also showed presence of sterols and terpenoids in methanolic and ethanolic extracts only but with intense red coloration. All the selected solvent extracts of inflorescence and leaf showed the presence of flavonoids which was indicated by the appearance of yellow to red color. Except chloroform all other extracts were tested positive. Each plant part extract in all the solvents showed the presence of carbohydrates which was recorded by the formation of a purple to violet color ring at the junction.

CONCLUSION

The outcome of the present work indicates that methanol is a good solvent for the extraction of alkaloid, glycosides, proteins and amino acids, flavonoids and carbohydrates asit was able to extract them from the three plant parts. Ethanol is a good solvent for the extraction of alkaloid, saponis, tannins and phenolics, glycosides, proteins and amino acids, steroids and terpenoids, flavonoids and carbohydrates, and acetone is a good solvent for the extraction of alkaloid proteins and amino acids, flavonoids and carbohydrates and chloroform also proved to be a good solvent for alkaloids, tannins, phenolics and carbohydrates. Furthermore, the presence of different phytochemicals in the plant parts (inflorescence, root and leaf) of *Arnebia benthamii* validates the ability of the plant to provide a source of natural medicines. Moreover, isolation, purification and quantification of the phytochemicals found present will allow researchers value from the plant.

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REFERENCES

[1] P Tiwari; B Kumar; M Kaur; G Kaur; H Kaur. *Internationale pharmaceutica sciencia*, **2011**, 1(1), 98-106.
[2] American Cancer Society. *Phytochemicals*, **2000**.
[3] E Meagher; C Thomson. Medical Nutrition and Disease, 2nd edition, G Morrison and L Hark, Malden, Massachusetts: Blackwell Science Inc.; **1999**. 33-58.
[4] B Schneeman. *J Nutr*, **2007**, 137(2), 493-494.
[5] NS Ncube; AJ Afolayan; AI Okoh. *Afr J Biotechnol*, **2008**, 7 (12): 1797-1806.
[6] A Srivastava; YH Shukla; S Kumar. *J Med Arom Plant Sci*, **1999,** 21, 1131-1138.
[7] S Manjkhola; U Dhar. *Curr Sci India*, **2002**, 83 (4), 484-488.
[8] JB Singh; P Kachroo. Forest Flora of Srinagar and Plants of Neighbourhood. 1st edition, Bishen Singh Mahendra Pal Singh, Dehradun, India, ISBN: **1976**, 81-7622-131-7.
[9] PA Wani; AR Dar; GG Mohi-ud-din; KA Ganaie; IA Nawchoo; BA Wafai. *Int J Bot*, **2006**, 2, 402-408.
[10] U Dhar; P Kachroo. Alpine flora of Kashmir. Himalaya. Scientific Publishers, Jodhpur, India. **1983,** pp**:** 280.
[11] A Ganai. Assessment and reproductive strategies of *Arnebia benthamii*. Ph.D. thesis. University of Kashmir, Srinagar. **2005**, 62-63.
[12] S Khatoon; S Mehrotra; U Shome; BN Mehrotra. *Int J Pharmacog*, **1993**. 31, 269-277.