IJP (2014), Vol. 1, Issue 4 **(Research Article)**

Received on 10 January 2014; received in revised form, 26 February 2014; accepted, 30 March 2014; published 01 April 2014

PRELIMINARY INVESTIGATION OF PHYTOCHEMICALS OF *SAUSSUREA OBVALLATA* (BRAHM KAMAL) AND *PITTOSPORUM ERIOCARPUM* (AGNI): TWO ENDANGERED MEDICINAL PLANT SPECIES OF UTTARAKHAND

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Keywords:

Saussurea obvallata, *Pittosporum eriocarpum*, Alzheimer, Stroke and Epilepsy

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ABSTRACT: Aim of this study was to determine the presence of phytochemical components in *Saussurea obvallata* and *Pittosporum eriocarpum*, two endangered medicinal plant species of Uttarakhand. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. These components are produced by plants to protect themselves, but recent researches demonstrate that they can also protect humans against diseases. Our research shows the presence of active components in Brahma kamal and Agni extract with different solvents like methanol, ethanol, chloroform, and distilled water. Traditionally these herbs had been used for the treatment of various disorders like paralysis of limbs, cerebral ischemia, narcotic, expectorant, bronchitis, urinary tract problems, cough, *etc*. Our results indicated the presence of active components like alkaloids, flavonoids, terpenoids, *etc*. These components are responsible for the modulation in different disorders like Alzheimer, stroke, and epilepsy, *etc*. Our research team is carrying out a detailed investigation of components and its role in different disorders.

INTRODUCTION: This is the first report of phytochemical analysis ("to the best of our knowledge") for *Saussurea obvallata* (DC.) Edgew. (Asteraceae), a rare, threatened and endemic medicinal herb and *Pittosporum eriocarpum* Royal endangered medicinal plant species of the Indian Himalayan region found at high altitudes (*S. obvallata*) and middle altitudes (*P. eriocarpum*). However, the essential oil in *S. obvallata* has been reported in a dissertation only [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.1(4).266-69

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.1(4).266-69

Brahma Kamal (*Saussurea obvallata*), the state flower of Uttarakhand (India), is an endemic herb of the Indian Himalayan region and is also known as the king of Himalayan flowers. The plant is distributed between elevations of (3,800 - 4,800 M). It is a hermaphrodite herb which achieves an average height of about 5-10 cm. Flowers start blooming from mid monsoon (mid-July) to (mid-October), after flowering the plant perishes and becomes visible again on April [2].

In Uttarakhand, Brahma Kamal is generally found in the region of Kedarnath, Tungnath, Valley of Flower, Hemkund Sahib and Gangotri [3]. The plant holds immense sacred value in the region. It is offered to Lord Shiva at Kedarnath, Lord Vishnu at Badrinath and distributed as 'prasada.' As per the popular mythological story (Vedas & Purana's)

Lord Shiva (Father of Lord Ganesha) the Hindu God of destruction, after getting angry cut the head of Ganesha then Brahm Kamal was created by Lord Bhrama (Hindu god of creation) after geodes Parvati (Mother of Lord Ganesha) pleased to him. Brahma Kamal was used to transplanting the head of an elephant onto the body of Lord Ganesha. There is Brahma Kamal mentioned in Mahabharata and Ramayana (holy books of India).

Brahma Kamal is used for the preparation of traditional medicines by the local peoples in Tibet and other places including Garhwal Himalayas. The flowers, rhizomes, and leaves are used for the treatment of bone ache, an intestinal ailment, urinary tract problems and cold/a cough. Rhizomes particularly used as antiseptic, healing cuts and bruises [4, 5, 6]. It is also used for the treatment of wounds, cut and boils (dried leaves), cardiac disorder (roots and leaves), and mental disorder (seeds) [7]. In the Tibetan system of medicine, it is used for the treatment of paralysis of limbs and cerebral ischemia [8].

Pittosporum is a genus of about 200 species in the *Pittosporaceae* family & *Pittosporum erriocarpum* commonly known as Agni. The species has been categorized as an endangered species by the "International Union for Conservation of Nature" (IUCN, 1998 - Walter and Gillett) which is the world's main authority on the conservation status of species. This plant species is recorded from Shastradhara (Dehradun) and Mussoorie in Uttarakhand (India). It is found in the form of a shrub or small tree and grown on hot rocky slopes up to 2,400 M. This species is widely used for the preparation of traditional medicines, which are used widely in the treatment of narcotic, expectorant, bronchitis, *etc*. [9] The species can be artificially regenerated by sowing or layering. It is classified as a multipurpose species and is lopped for fodder, fuel wood, suitable for soil conservation and reclamation of degraded sites [10]. In 2010 H. Padalia *et al.,* reported a case study about its habitat and distributions (Geospatial multiple logistic regression approach for habitat characterization of scarce plant population) [11].

MATERIAL AND METHODS:

Preparation of Plant Extract: The plant samples collected from the Kedarnath valley (30.73 N latitude and 79.06 E longitudes) and Doon valley. The sample was identified by Dr. Anup Chandra (Scientist - D) Systemic Botany, Forest Research Institute Dehradun, India (Ref. no. GEU/DBT/AT-1PS/2013). The sample was store in ice until being transported to the laboratory.

Extraction Methods: The extract was prepared by the methods to describe in [12, 13] with slight modification. The flower sample and leaf sample were washed in tap water, dried, and placed into a blender to be ground into powder. Four solvents (chloroform, methanol, ethanol, and water) were used for the Soxhlet extraction procedure in different ratio. After 6 to 8 h of extract collected, filter with the muslin cloth and transferred to 50 ml tubes and centrifuged for 15 min at 4,000 rpm at 25ºC. The supernatant was collected and kept for drying. After drying, it was mixed with 10% DMSO and used for the experiments.

Phytochemical Analysis: Chemical tests for the screening and identification of active components in the flower extract using standard protocols as described [12, 13]. For each test, 100 µl of each solvent extract was used for analysis.

Test for Saponins: The extract was taken in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Phenols: Extract mixed with 2 ml of 2% solution of $FeCl_3$. Blue/green color indicated the presence of phenols.

Test for Tannins: Extract mixed with 2 ml of 2% solution of $FeCl_3$. Black color indicated the presence of tannins.

Test for Terpenoids: The extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated Sulfuric acid was added carefully and shaken gently. Reddish brown colors observed in the inter-phase indicate the presence of terpenoids.

Test for Flavonoids: Extract was treated with few drops of sodium hydroxide solution, the formation of intense yellow color. Which becomes colorless on the addition of dilute acid indicate the presence of flavonoids.

Test for Glycosides: The extract was mixed with 2 ml of glacial acetic acid containing few drops of 2% $FeCl_3$; mixture poured into another tube containing 2 ml of concentrated sulfuric acids. A brown ring at the inter-phase indicates the presence of glycosides.

Test for Protein: The extract treated with few drops of concentrated nitric acid, the formation of yellow color indicates the presence of proteins.

Test for Alkaloids: The extract was dissolved individually in diluted HCl and filter was treated with saturated picric acids and formation of brown precipitate indicates the presence of alkaloids.

Test for Steroids: Extract mixed with 2 ml of chloroform then carefully added H_2SO_4, the formation of reddish-brown color indicates the presence of steroids.

RESULTS AND DISCUSSION: The study of chemical constituents of the medicinal plants has acquired a lot of importance all over the world. In the present study, a plant sample collected from Kedarnath valley and Dun valley and was authenticated.

Then they were dried, powdered and subjected to phytochemical screening. Powders were subjected to extraction with ethanol, methanol, chloroform, and distilled water. The qualitative tests for four different solvents were performed. The investigation showed that positive (+) and negative (-) indicates the presence or absence of active components in leaves extract and flower extract with different solvents like methanol, ethanol, chloroform, and distilled water respectively.

Both these medicinal plant species contain many active components, these secondary metabolites/ components used in various disorders for treatment/modulation with minimum side effects. The results were given in **Table 1** and **2** respectively.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF *SAUSSUREA OBVALLATA* EXTRACT

Extracts	Saponins	Phenol	Tannins	Terpenoids	Flavonoids	Glycosides	Proteins	Alkaloids	Steroids
Chloroform	+	_	+	+	+	_	+	+	+
Methanol	+	+	_	+	+	+	+	+	+
Ethanol	+	_	_	+	+	+	_	+	_
Distilled Water	+	+	_	+	+	+	+	+	+

Positive (+) show the presence of constituents; whenever negative (-) show the absence of constituents in the flower extract

TABLE 2: PHYTOCHEMICAL CONSTITUENTS OF *PITTOSPORUM ERIOCARPUM* EXTRACT

Extracts	Saponins	Phenol	Tannins	Terpenoids	Flavonoids	Glycosides	Proteins	Alkaloids	Steroids
Chloroform	-	+	+	-	+	_	+	+	-
Methanol	+	+	_	+	+	+	+	+	+
Ethanol	-	_	_	+	+	-	_	-	_
Distilled Water	+	+	+	+	-	+	+	+	+

Positive (+) show the presence of constituents; whenever negative (-) show the absence of constituents in the leaves extract.

CONCLUSION: The primary screening process involved investigating bioactive compounds present in flower extract of *Saussurea obvallata* and leaf extract of *Pittosporum eriocarpum* in different solvents. These bioactive components might play an important role in modulation in a different disease like Alzheimer, stoke, Parkinson and diabetes *etc*. Our research team is carrying out detailed investigations of the chemical composition and mechanism of actions of these herbal plants including *in-vitro* and *in-vivo* studies, and its role in neurodegenerative disorders.

ACKNOWLEDGEMENT: We would like to thank the Forest Research Institute, Dehradun and Graphic Era University, Dehradun for supporting this research.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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How to cite this article:
Semwal P, Anthwal P, Kapoor T and Thapliyal A: Preliminary investigation of phytochemicals of *Saussurea obvallata* (Brahm Kamal) and *Pittosporum eriocarpum* (Agni): two endangered medicinal plant species of Uttarakhand. Int J Pharmacognosy 2014; 1(4): 266-69. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.1(4).266-69.