Brahma Kamal (*Saussurea obvallata* (DC.) Edgew.), an important Himalayan medicinal plant: Ethnomedicinal, phytochemical and pharmacological overview

Prabhakar Semwal, Sakshi Painuli, Devesh Tewari, Rainer W. Bussmann, Lok Man S. Palni, Ashish Thapliyal

Reviews and Mini-Reviews

Abstract

Background: The genus *Saussurea* of Asteraceae comprises approximately 490 species. Plants belonging to this genus are being used in different countries as traditional medicines. Brahma Kamal (*Saussurea obvallata* (DC.) Edgew.), is one of the most important and endangered species of this genus and is being used in traditional, ornamental and religious purposes. The aim of this work is to review and understand the scientific work conducted so far on *S. obvallata,* which includes its ethnomedicinal uses, pharmacognostic description, phytochemistry, pharmacology, micro-propagation and important conservation aspect.

Methods: Systematic literature searches were performed on *S. obvallata* through various scientific search engines including Agricola, ACS publications, Google patents, Google Scholar, J-STAGE, JSTOR, PubMed, Science Direct, Scopus, SpringerLink, Taylor & Francis, Web of Science, USPTO, Wiley, published books and conference proceedings using keywords *"Saussurea obvallata", "Brahma Kamal" and "Brahma' lotus"* for screening of relevant information.

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Results & Conclusion: The literature analysis revealed diverse traditional uses of *S. obvallata*, against wounds, paralysis, cerebral-ischemia, cardiac and mental disorders. Various extracts (methanol, ethanol, petroleum ether, chloroform, n-butanol, aqueous, etc.) of *S. obvallata* were evaluated for their phytochemicals and pharmacological activities. Additionally, anti-hypoxia, anticancer, radio-protective, antioxidant and antimicrobial activities were also studied using different *in-vitro* and *in-vivo* models. *S. obvallata* is being used widely in traditional medicine and socio-economic applications but scientifically, it is not fully assessed regarding its complete therapeutic effects, toxicity and safety in human body. Further studies are essential and should focus on conservation, cultivation and sustainable utilization of the species.

Keywords: Brahma Kamal, Himalaya, *Saussurea obvallata,* pharmacology, phytochemistry.

Background

High altitude medicinal plants played significant role in the life of local communities of the area, their culture and economy of the Himalayan region by providing food, medicine, fuel, fodder, etc. A variety of wild and cultivated plants (botanicals) have been used as curative agents since ancient times across the globe (Joshi *et al.* 2016). The popularity of botanicals can be judged from the fact that 85% of the traditional medicine involves the use of plant extract and 70% of the modern medicines are derived from medicinal plants (Singhal & Agrawal 2007, Vieira & Skorupa 1993).

Saussurea (Asteraceae) is one of the important genus comprises around 490 species of flowering plants which is indigenous to temperate, cool and arctic parts of Europe, Asia and North America (von Raab-Straube 2017). The species of this genus are being utilized in traditional and folk medicinal system of various countries such as India, Nepal, China, Tibet, Pakistan, Bangladesh, Uyghur, Mongolia and Kazakhstan. Around, 300 species from China (Wang *et al.* 2010), 62 species from Indian Himalayan Region (11 complete + 2 partial states of Indian Republic), and 18 species from Uttarakhand Himalaya (State of Indian Republic) (Butola & Samant 2010) have so far been described. Traditionally, the species of this genus has been used for the cure of various alinments such as *Saussurea bracteata* Decne (Boils, headache, cough, cold, and lungs infection), *Saussurea costus* (*Falc.*) *Lipsch. (*Dysentery, rheumatism, skin disorder, jaundice, and leprosy), *Saussurea graminifolia* Wall. (Blood purifier, potency, and vaginal discharge), *Saussurea simpsoniana* Lipsch. (Nervous debility, snake bite, plague, painful periods, and sexual problems), etc (Butola & Samant 2010). While, *Saussurea costus* (Pandey *et al.* 2007, Zahara *et al.* 2014), *Saussurea medusa* (Fan J-Y *et al.* 2015), and *Saussurea involucrata* (Chik W-I *et al.* 2015, Gong *et al.* 2020) are some of the popular species that have been investigated in respect of ethnobotany, ethnochemistry, ethnopharmacology, and their components have been elucidated. However, little is known about *S. obvallata*. Therefore, in this paper we reviewed and discuss the updated scientific information conducted so far on *S. obvallata,* which includes its ethnomedicinal uses, pharmacognostic description, phytochemistry, pharmacology, reproductive biology, micro-propagation, and updated information on filed patents.

Materials and Methods

The available information/data about the *S. obvallata* was collected through various scientific search engines including Agricola, ACS publications, Google patents, Google Scholar, J-STAGE, JSTOR, PubMed, Science Direct, Scopus, SpringerLink, Taylor & Francis, USPTO, Web of science, Wiley, published books and conference proceedings using "*Saussurea obvallata", "Brahma Kamal"* and *"Brahma' lotus"* keywords for screening of relevant information.

Results and Discussion

To the best of our knowledge, this is the first review report of *Saussurea obvallata* which provides an updated knowledge on each and every prospect of the species. However, general features of few species of *Saussurea* genus has been reported by Ghosh (2017).

Geographical distribution

Saussurea obvallata is indigenous to India, Pakistan, China, Tibet, Nepal, Myanmar and Bhutan where it grows in the Himalayan region with 3000-4800 m altitude (Kirtikar & Basu 1984, Pant & Semwal 2013, Semwal 2017).

Taxonomy and nomenclature

The name "*Saussurea*" honours the Swiss taxonomist and philosopher, Horace Benedict de Saussure (1740-1799) and "*obvallata*" is derived from a Latin word "*obvallatus",* meaning surrounded by wall signifying to involucral bracts. In a recent revision of the subgenus, *S. obvallata* was split into a series of eight allopatric species, and the populations studied here were the only to retain the original name (von Raab-Straube 2017).

The first samples of *Saussurea obvallata* were collected by the Richard Blinkwoth from the Kumaon Region of Uttarakhand (India), and submitted to

Nathaniel Wallich, a botanist and superintendent of the Calcutta Botanical Garden (1817-1846) in the herbarium of the East India Company in London and now known as the Wallich Herbarium at Kew (von Raab-Straube 2017). The chromosome number in this species has been found to be 32 (n=16) with or without three B chromosomes (Amano & Ohba 2000, Fujikawa *et al.* 2004, Gupta & Gill 1989, Shetty 1967).

An assessment of genetic diversity and structure of *S. obvallata* has been reported by Semwal *et al.* (2020) and according to the study, the species revealed lowest gene flow level amongst all studied populations of *S. obvallata*. In the other words, 60.63% genetic variation has been recorded within population while, 39.37% recorded among populations of *S. obvallata.* von Raab-Straube (2013) has analysed phylogenetic relationships in the genus *Saussurea* by using two DNA sequences (one from the nuclear ribosomal ITS region & other from the chloroplast *trn*L-*trn*F regions) for the investigation of 47 different species of *Saussurea* including *S. obvallata*. The taxonomic hierarchy of *S. obvallata* are presented in Table 1.

Table 1. Taxonomic Hierarchy of *S. obvallata*

Kingdom	Plantae
Subkingdom	Viridaeplantae
Infrakingdom	Streptophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Infradivision	Angiospermae
Class	Magnoliopsida
Superorder	Asteranae
Order	Asterales
Family	Asteraceae
Genus	Saussurea DC.
Subgenus	Amphilaena
Species	*S. obvallata* (DC.) Edgew.

Local names

Hindi: ब्रह्मकमल, Sanskirt: ब्रह्मकमल:, Nepali: ब्रह्मकमल, Khas Chhetri (Farwest Nepal): ब्रह्मकोइला, English: Brahma's lotus, King of Himalayan flowers, Sacred Saussurea.

Pharmacognosy

Botanical and morphological description

Saussurea obvallata: Perennial. Stem solitary, erect, purplish to reddish brown, 15-60 cm long, 2-8.33 mm diameter, ribbed, hollow; indumentum (0.5-2 mm) long, flagellate, uniseriate, eglandular hairs. Leaves; basal and lower petiolate: leaf blade oblong to blunt lanceolate; 18.7-42.75 mm width (broad) and 8.99-20.83 cm length (long), surface pilose, glandular hairy, glabrescent, base attenuate, margins toothed, distinct mid-rib; purple and upper leaves without petiole called bracts; 10-12, spirally arranged in pseudo whorl, totally enclosing inflorescence, boat shaped, semi-transparent, creamy white or pale yellow. Inflorescence 15-21 cm length and 70-84 mm diameter; capitulae 6-16 per head; corymbiform synflorescence 3.5-9 cm in diam., pedunculate or subsessile, florets hermaphrodite, smell awful; corolla (11-13 mm): actinomorphic, tubular with linear-lanceolate lobes, bluish purple or violet; stamen (6-7 mm), filaments (2-3 mm), anther tube (4-6 mm), appendages (1-2 mm); achenes: dark brown to black, cylindric or obovoid, obtuse, smooth, size 4-4.8×1.6, distinctly ribbed, 5-7 rounded ribs in cross-section, isodiametric (Amano & Ohba 2000, Ghosh 2017, Semwal *et al.* 2019, von Raab-Straube 2013, 2017). Flowering and fruiting: July-September and August-October (Fujikawa 2010, Semwal *et al.* 2019, von Raab-Straube 2017).

Microscopy description

Microscopic examination of leaves reveals the presence of a single layer of epidermis, thick cuticle, stomata, spongy parenchyma, vascular bundles, phloem fibres, ground tissue, well developed palisade tissue, and quite large intercellular spaces among sponge tissue while, the leaf powder showed the presence of cork cells, pitted vessels, fragments of endocarp, leaf fragment, and fragment of xylem. A transverse section of stem had many prominent ridges on the outer side, epidermis, parenchyma, collenchyma cells, cortex, endodermis, pericycle, and vascular bundles while, the powdered stem showed presence of cork cells, pitted vessels, annular vessels, calcium oxalate crystals, iodine, and group of lignified fibres. A transverse section of roots showed the presence of epidermis, cortex, endodermis, single layered pericycle, vascular bundles, and a small pith region while, the powdered rhizome showed cork cells, tracheids, starch, tracheids reticulate vessels, and fibres. The powered flower shows the presence of tracheid, pitted vessels, parenchyma cells, prismatic crystals, fibres, and oil globules in *S. obvallata* (Yadav *et al.* 2018). These types of anatomical structures established a relationship between leaf anatomy and their adaptation in alpine environment (Gui-fang 2012).

Conservation status

The majority of the species are categorized as threatened due to their extensive use in different applications. A total of forty-four species of this genus are categorized as rare, and two as endangered (*S. obvallata* (DC.) Edgew; *S. simpsoniana* (Field&Gard.) Lipsch.) and two as critically endangered (*S. gossypiphora* D. Don; *S. costus* (Falc.) Lipsch.) due to heavy pressure on these species for their extensive traditional uses (Butola & Samant 2010). Three species of this genus are mentioned under IUCN Red List including, *Saussurea costus* (critically endangered), *Saussurea seoulensis* (least concern), and *Saussurea pinnatiphylla* (data deficient) (The IUCN Red List of Threatened Species 2019-1; https://www.iucnredlist.org/search?query=Saussurea&searchType=species; accessed on dated 07/07/2019). Every part of *S. obvallata* is being used for different traditional, religious, medicinal, ornamental and socio-economic applications (Pant & Semwal 2013). Due to the multiple uses, the species has been categorized as an endangered species by the Conservation Assessment Management Plan (CAMP) and identified as one of the top-ranking priority species for conservation programs (Bisht *et al.* 1988, Negi *et al.* 1999, Semwal 2017).

Ethnomedicinal importance

The species is used widely in traditional medicine, and still playing a major role in curing various old and new diseases. Many species of the genus *Saussurea* are being used in different traditional/folk medicine systems of India, China, Tibet, Nepal, Pakistan, Bangladesh, Uyghur, Mongolia, Kazakhstan, and other Asian countries (Butola & Samant 2010, von Raab-Straube 2017). Traditionally, *S. obvallata* is being used by the Himalayan communities of Tibet, China, Nepal and Indian Himalaya for the cure/treatment of various diseases and disorders including paralysis, cerebral ischemia, wounds, cardiac and mental disorders; some people also use it as antiseptic and in healing cuts, etc. (Kirtikar & Basu 1984, Pant & Semwal 2013, Negi *et al.* 1999, Phondani *et al.* 2010, Tsarong 1986). Table 2 includes the details information of traditional uses of *S. obvallata*.

Table 2 Traditional uses of *Saussurea obvallata*

Plant Parts	Ethno-pharmacological uses	Dosage form	Country	References
Whole Plant	It is used in the treatment of paralysis of limbs and cerebral ischemia	-	Tibet	Tsarong 1986
	It is used for the treatment of headache and other body pain	The paste prepared from whole plant is applied	India	Bisht *et al.* 2013
	It is used to protect woolen clothes from the damages caused by insects	Whole inflorescence	India	Chauhan 1999
	It is used for the treatment of bruises and cuts	The paste prepared from whole plant is applied	India	Sharma & Samant 2014
Roots	It is used as antiseptic and also used for healing cuts and bruises	The paste prepared from root is applied	India	Kirtikar & Basu 1984, Negi *et al.* 2015
	It is used to cure boils, cuts and bruises	The paste prepared from root is applied	Pakistan	Bano *et al.* 2014
	It is used to cure leucoderma	The paste prepared from root is applied	India	Sharma 2016
	It is used to cure fever and cough	-	India	Bhat 2013, Singh & Rawat 2011
	It is used to cure cardiac disorders	Decoction of roots (200 ml) mixed with 2-3 spoons full of the oil of *Cedrus deodar* and applied externally to treat the heart; (100 ml)	India	Phondani *et al.* 2010
	It is used to cure bruises and fractures	Decoction of roots (200 ml) mixed with 2-3 spoons full of the oil of *Cedrus deodar;* (100 ml)	India	Maikhuri *et al.* 2000
Leaves	It is used to cure boils, cuts and wounds	Decoction of dried leaves (100ml) mixed with half spoonful of salt and few drops of this, applied in the infected area; (20 ml × 3 days)	India	Maikhuri *et al.* 2000, Phondani *et al.* 2010
	It is used to cure bruises and fractures	Decoction of roots (200 ml) mixed with 2-3 spoons full of the oil of *Cedrus deodar;* (100 ml)	India	Phondani *et al.* 2010
	It is used to cure wounds and cuts	Dried leaves (100 gm) mixed with salt (10 gm) and used in infected area	India	Kala *et al.* 2006
Flower buds	Used to treat boils, hydrocele and reproductive disorders	-	India	Kala *et al.* 2006

	It is used to cure boils, cuts and bruises	The paste prepared from flower is applied	Pakistan	Bano *et al.* 2014
	It is used to treat bone-ache, intestinal ailments, urinary track problems and coughs	-	India	Negi *et al.*1999
	It is used to treat urinary infections in cattle.	Raw form	India	Saklani & Rao 1996
	Flower heads are used to cure hydrocele	Flower heads are roasted with ghee and one to two tea spoons (full) are given to patients in the morning for three to six days	India	Gupta *et al.* 2013
Bracts	It is used to treat cough and respiratory problems	-	India	Gupta *et al.* 2013
Seeds	It is used to treat mental disorders	The powder of seeds steeped in water overnight then filtered (1 cupful)	India	Phondani *et al.* 2010

Religious and ceremonial use

In Indian traditional system, *S. obvallata* holds immense sacred value in the Himalayan region and it is offered in different temples to local deities of holy shrines for instance Kedarnath, Badrinath, Tungnath, Rudranath, and Triyuginarayan where it is distributed as *Prasaad* (the vegetarian material substance distributed in pilgrimage) (Semwal *et al.* 2019a). Locally, inflorescences are collected and soled as single or in garland forms (Semwal *et al.* 2019a). Collection of *S. obvallata* is presented in figure 1. Most people of the Himalayan valley hang these flowers in their houses for protection from evil spirits.

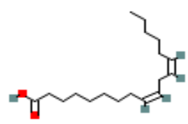
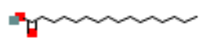
Inorganic components

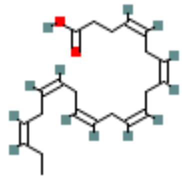
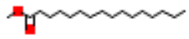
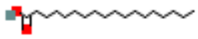
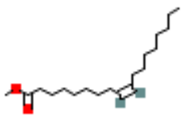
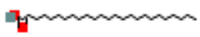
The first report on physicochemical investigations of *S. obvallata* including, protein, crude fibre, ash value, phosphorus, calcium, magnesium, potassium, iron, silica, reducing sugars and amino acid content was published by Tiwari *et al.* (1986). They observed the highest percentage of protein (26.25%), iron (0.042%) and crude fibres (20.00%) in inflorescence, leaves and stem respectively. The stem part contained higher quantities of amino acids than the values reported for leaves and inflorescence. The amino acids were identified as aspartic acid, alpha-alanine, beta-alanine, glycine, histidine, leucine, iso-leusine, lysine, methionine, phenylalanine, serine, threonine and tryptophan.

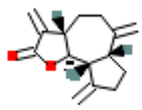
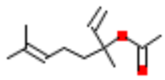
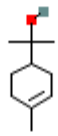
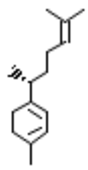
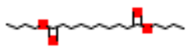
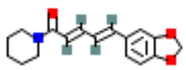
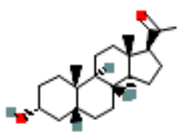
Phytochemistry

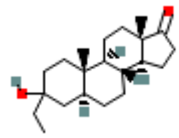
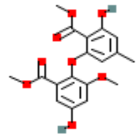
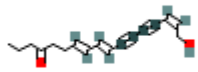
Generally, medicinal plant produces a large number of secondary metabolites, but many Himalayan species of medicinal plants produces more due to cold stress caused by critical weather conditions (Alonso-Amelot *et al.* 2004, Zlatić & Stanković 2017). These plants are gaining importance due to their unique therapeutic applications (Mangal *et al.* 2018, Tewari 2014). Previous reports on phytochemical investigations identified, saponins, phenol, tannins, terpenoids, flavonoids, glycosides, proteins, and alkaloids in the leaves and flowers extracts of *S. obvallata* (Semwal *et al.* 2014). The GC-MS methods reported the presence of squalene and α-linolenic acid methyl ester in petroleum ether extract of *S. obvallata* (Mishra *et al.* 2018). Another study on phytochemical investigations reported the qualitative and quantitative analysis of chemical constituents present in leaves and flower extracts of *S. obvallata* (Semwal *et al.* 2014, Semwal & Painuli 2019). The results of these studies showed the presence of 78 phytoconstituents in methanolic leaves and flowers extracts of *S. obvallata.* Few major compounds are presented in the Table 3.

Table 3. Some of the major constituents in *S. obvallata* extracts (Semwal & Painuli 2019)

Compounds	**Molecular Formula**	**PubChem CID**	**Chemical Structure (2D)**
Linoleic acid	$C_{18}H_{32}O_2$	5280450	
Palmitic acid	$C_{16}H_{32}O_2$	985	

Doconexent	$C_{22}H_{32}O_2$	445580	
Methyl palmitate	$C_{17}H_{34}O_2$	8181	
Stearic acid	$C_{18}H_{36}O_2$	5281	
1-Docosanol	$C_{22}H_{46}O$	12620	
Gondoic acid	$C_{20}H_{38}O_2$	5282768	
Henicosanoic acid	$C_{21}H_{42}O_2$	16898	
Methyl oleate	$C_{19}H_{36}O_2$	5364509	
Pentacosanoic Acid	$C_{25}H_{50}O_2$	10468	

Dehydrocostus lactone	$C_{15}H_{18}O_2$	73174	
Linalyl acetate	$C_{12}H_{20}O_2$	8294	
Litsomentol	$C_{30}H_{52}O_2$	12311315	
α –Terpineol	$C_{10}H_{18}O$	17100	
γ-Curcumene	$C_{15}H_{24}$	12304273	
Dibutylsebacate	$C_{18}H_{34}O_4$	7986	
Piperine	$C_{17}H_{19}NO_3$	638024	
Eltanolone	$C_{21}H_{34}O_2$	31402	

Androstan-17-one, 3-ethyl-3-hydroxy-, (5alpha)-	$C_{21}H_{34}O_2$	14681481	
Methyl asterrate	$C_{18}H_{18}O_8$	5249326	
Bupleuronol	$C_{17}H_{20}O_2$	46881232	

Pharmacology

Few studies have been carried out to establish the pharmacological description of *S. obvallata* (Table 4). The *in-vivo* radio-protective effects of *S. obvallata* extract (aqueous) were evaluated in mice models by Liang-wen *et al.* (2009a). The experimental models were prepared by radiation exposure of 60Coγ-rays at a dose of 6Gy and after irradiation, the mice models were treated with the extract (6Gy). After the treatment, it has been observed that, the plant extract of *S. obvallata* significantly (P <0.05) encouraged the recovery of haematological functions and the number of karyota of femur compared to control group. The radio-protective effects of aqueous extract of *S. obvallata* (bracts) were also reported by the same group (Liang-wen *et al.* 2009b). In this study, the aqueous plant extract of bracts promotes the recovery of damage hematopoietic system in radiation-damaged mice compared to control group (P <0.05). Both the studies showed dose dependant radio-protective effects. Ying *et al.* (2015) reported the radio-protective effects of the aqueous extract of *S. obvallata* and *Crataegus* on mice models. The experimental models were prepared by the radiation exposure of X-rays at a dose of 4Gy. After irradiation, the mice models were treated with extract (4Gy) up to 14 days. The results of the study demonstrated that, *S. obvallata* have moderate radio-protective effects in mice while *Crataegus* showed better results (P<0.01).

The anti-hypoxic activity of *S. obvallata* was examined in hypoxia mice models. In addition, adenosine triphosphate (ATP) and adenosine triphosphatase (ATPase) activity in brain and cardiac muscle; lactic acid (LAC) and lactate dehydrogenase (LDH) in blood and cardiac muscles; blood sugar and glycogen content in liver and skeletal muscles were also examined in these models (Ma H-P *et al.* 2011). In this study, *Saussurea obvallata* demonstrated the significant results in terms of survival time (36.34 min) and prolongation rate (20.52%), while, *Saussurea involcucrata* (Kar. et Kir.) Sch.-Bip demonstrated highest anti-hypoxic activity (survival time=40.78 min, prolongation rate=33.13%) at the dose of 1000 mg/kg. In addition, LAC content of mice plasma was recorded as 2.84 mmol/L for *S. obvallata* while, 1.93 mmol/L for *S. involucrata* at the dose of 1000 mg/kg and the dose may be effective in the treatment of acute mountain sickness. Hai-li LI *et al.* (2016) also evaluated the anti-hypoxic activity of *S. obvallata* (crude extract) on EA.hy926 cells. The experimental model of hypoxic injury was prepared with sodium dithionite. The results indicated that, the plant extract increased the EA.hy926 cell survival rate in the model of hypoxia injury (from the concentration of 1.25 mg/mL) when compared with control.

The anticancer activity of leaves and flower extracts of *S. obvallata* were evaluated against MCF-7 breast cancer cell lines and a considerable activity of the extracts was recorded when compared with a positive control (unpublished data). The antibacterial activity of petroleum ether extract of *S. obvallata* (1-3 mg/disk) was determined against the bacterial strains namely, *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus,* and *Bacillus subtilis*, respectively (Mishra *et al.* 2018). The antibacterial assay was performed by using disk diffusion method. In this study, the leaves extract exhibited the highest zone of inhibition against the *S. aureus* (15.2 mm) at 3.0 mg/disk, whereas *P. aeruginosa* was the less sensitive bacterial strain. The MIC value of *S. obvallata* extract was measured against different bacterial strains from a range of 87.2 to > 100 µg/mL.

Semwal & Painuli (2019) also reported the antimicrobial activity of *S. obvallata* extracts (20 µl of 5mg/mL) against four bacterial (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* & *Klebsiella pneumoniae*) and three fungal (*Candida albicans, Candida glabrata, & Candida tropicalis*) strains, respectively. The antimicrobial assay was performed by using well diffusion method. In this study, methanolic and aqueous extracts exhibited judicious antibacterial and antifungal activity against all the strains in terms of zone of inhibition from a range of 8.87 to 20.50 mm and 8.30 to 15.90 mm, respectively (Table 5). The antioxidant activity of *S. obvallata* extracts (20 µl of 1mg/mL) was measured by two *in-vitro* assays namely, DPPH and H_2O_2 methods and a considerable antioxidant activity was recorded in both the extracts from a range of 29.25 to 82.88% and 39.75 to 41.05%, respectively (Semwal & Painuli 2019).

Reproductive biology and micro-propagation

Reproduction is a vital process which ensures the perpetuation of life. Under the reproductive biology, pollination is an important parameter in the life cycle and history of flowering plants that indicates the success of sexual reproduction (Gopalakrishnan & Thomas, 2014). The impact of harvesting and pollen limitation in two species of *Saussurea* genus (*Saussurea medusa* Maxim. and *Saussurea laniceps* Hand.-Mazz.) has been reported by Law *et al.* (2010). The results of this investigation suggested that, pollinators are required for seed production and *S. medusa* showed pollen limitations.

However, *S. obvallata* did not showed any type of limitations (Semwal 2017, Semwal *et al.* 2019b). Pollination experiments indicated that *S. obvallata* is naturally self-compatible species and preferred for mixed mating (Semwal *et al.* 2019b). Bumble bee was the only insect pollinator observed visiting the flower of this species and other species of this genus (Law *et al.* 2010, Saini *et al.* 2012, Spackman *et al.* 2001, Tsukaya *et al.* 2002). Phenology, pollination biology, and pollinators of *S. obvallata* have been studied in the Uttarakhand Himalaya (India) by Semwal (2017).

Plant tissue culture has become an important technique in the field of plant biotechnology. A number of researchers reported on large-scale production of plant material and production of biologically active constituents using plant tissue culture techniques. In the case of *S. obvallata*, only two reports on micro-propagation have been published from the Indian Himalayan Region. Joshi & Dhar (2003) described the first protocol for micro-propagation of *S. obvallata*. The epicotyle segments of juvenile aseptic seedlings were used for the multiplication purposes due to the non-availability of mature explants. The multiple shoots were formed (five shoots per explant) from epicotyle explants on Murashige and Skoog (MS) medium. The results indicated that 15 days of *in-vitro* rooting and 12 days of *ex-vitro* acclimatization of the plantlets is optimum for field transplantation.

Dhar & Joshi (2005) again described the first successful plant regeneration protocol for *S. obvallata* through indirect organogenesis using a range of explants. The seeds, roots, hypocotyl, cotyledon, and leaf tissue were used as explants and a combination of 6-Benzyladenine (BA) and α-Naphthalene acetic acid (NAA) in MS medium was found to be suitable for induction of callus and shoot differentiation. The maximum callus induction was recorded from 10 to 15-day-old seedlings (explants) and the leaf explants showed 100% callusing (12 shoots per explant) in MS medium (with BA and NAA).

Patent studies

A systematic patent search revealed that only few innovations related to *S. obvallata* have been filed in China. The first invention is related to increasing the survival rate of *S. obvallata* through artificial breeding (CN103141293B). It includes the steps of seed selection, insulation seed germination seedling, tissue culture, land transplant survival and plantation management. The second patent focuses on a method to improve the yield of the extract of *S. obvallata* (CN103191166B). Isolation of effective components from traditional Chinese medicine and its application to treat disorder also described in this method. In third invention, extraction of compounds from the effective part of *S. obvallata* and its role as antitumor drug has been described (CN106236809A).

A fourth invention is related with the cleaning method of *S. obvallata* fruits by using a seed separator method (CN106385867A). It includes a few steps like drying and rubbing the inflorescences, and pappus mixture, etc. Few other inventions related with extraction of pharmaceutical composition and its applications in drug development have been reported in *S. obvallata* and its combination with other herbs (CN102106889B, CN104288286B and CN107252479A).

Table 4. Pharmacological/biological activities of the *S. obvallata*

Plant Part	Activity studied	Mode of experiment	Tested extract	Administered Dose	Possible Mechanism	References
Leaves	Antimicrobial	*In-vitro*	Aqueous and Methanol	5 mg/mL	Not studied	Semwal & Painuli 2019
Flowers	Antioxidant	*In-vitro*	Aqueous and Methanol	1 mg/mL	Not studied	Semwal & Painuli 2019
Leaves	Antibacterial	*In-vitro*	Petroleum ether	1-3 mg/disk	Not studied	Mishra *et al.* 2018
Whole plant	Anti-Hypoxic	*In-vitro*	Aqueous	1.25 mg/mL	Increase the cell survival rate in hypoxia injury model (EA.hy926)	Hai-li Li *et al.* 2016
Whole plant	Radio-protective	*In-vivo*	Aqueous	4 Gy	Promote the repair of DNA damage	Ying Liu *et al.* 2015
Whole plant	Anti-Hypoxic	*In-vivo*	Aqueous	1000 mg/kg	Increase the survival time and prolongation rate	Hui-Ping Ma *et al.* 2011
Whole plant	Radio-protective	*In-vivo*	Aqueous	6 Gy	Promote the repair of DNA damage	Liang-wen *et al.* 2009a
Bracts	Radio-protective	*In-vivo*	Aqueous	-	Promote the repair of DNA damage	Liang-wen *et al.* 2009b

Table 5. Previous antimicrobial studies in *S. obvallata*

Plant part	Solvent	Concentration of the extract	Microbial strains	Results (MIC/ ZOI)	References
Leaves [WDM]	Aqueous and Methanol	[20 µl of 5 mg/ml]	*Pseudomonas aeruginosa* [MTCC 4306]	13.90 -16.60 ZOI (mm)	Semwal & Painuli 2019
			Escherichia coli [MTCC 1698]	10.83 -11.57 ZOI (mm)	
			Staphylococcus aureus [MTCC 6908]	13.60 -20.43 ZOI (mm)	
			Klebsiella pneumoniae [MTCC 9544]	14.90-19.90 ZOI (mm)	
			Candida albicans [MTCC 3017]	13.90-14.57 ZOI (mm)	
			Candida glabrata [MTCC 3019]	15.43-15.90 ZOI (mm)	
			Candida tropicalis [MTCC 3416]	8.30-13.17 ZOI (mm)	
Flowers [WDM]	Aqueous and Methanol	[20 µl of 5 mg/ml]	*Pseudomonas aeruginosa* [MTCC 4306]	16.33 -20.50 ZOI (mm)	Semwal & Painuli 2019
			Escherichia coli [MTCC 1698]	8.87-13.90 ZOI (mm)	
			Staphylococcus aureus [MTCC 6908]	12.83 -16.53 ZOI (mm)	
			Klebsiella pneumoniae [MTCC 9544]	11.53 -18.53 ZOI (mm)	
			Candida albicans [MTCC 3017]	14.30 ZOI (mm)	
			Candida glabrata [MTCC 3019]	14.27-15.27 ZOI (mm)	
			Candida tropicalis [MTCC 3416]	10.90-12.33 ZOI (mm)	
Leaves [DDM]	Petroleum ether	[500 µl of 1.0-3.0 mg/disk]	*Pseudomonas aeruginosa* [MTCC 1934]	>100 MIC (µg/ml)	Mishra *et al.* 2018
			*Escherichia coli [*MTCC 1610*]*	98.4 MIC (µg/ml)	
			Staphylococcus aureus [MTCC 0902]	87.2 MIC (µg/ml)	
			Salmonella typhi [MTCC 3231]	>100 MIC (µg/ml)	
			Bacillus cereus [MTCC 0430*]*	90.2 MIC (µg/ml)	
			Bacillus subtilis [MTCC 0121]	>100 MIC (µg/ml)	

[DDM: Disk diffusion method; WDM: Well diffusion method; ZOI: Zone of inhibition; MIC: Minimum inhibitory concentration]

Figure 1. Collection of *S. obvallata* for religious purposes

Conclusions

The present paper reports traditional uses, pharmacognosy, phytochemistry, pharmacology, reproductive biology, micro-propagation and patent studies in *Saussurea obvallata*. The literature survey revealed that, *S. obvallata* is one of the most important species of Himalayan regions and used in different traditional systems but their active compounds needs to be further elucidated and authenticated by bioassay-guided isolation. Chemical characterization of the compounds and their mechanism of action remain unclear and should be further investigated by using genomics, proteomics, meta-bolomics and molecular techniques. Moreover, very limited studies are available for this species, not only in terms of chemical characterization but also in terms of pharmacological evaluation as well. However, only a few reports are available for their evidence based pharmacognosy, and phytochemical standardization. Most of the studies were limited to the *in-vitro* screening and a few for *in-vivo.* Detailed information on toxicological study is important to understand the safety profile of any herbal drugs and such toxicological studies on *S. obvallata* are limited. Due to the multiple applications, and very limited availability the species has been categorized as an endangered species by the Conservation Assessment Management Plan (CAMP) and identified as one of the top-ranking priority species for both in situ and ex situ conservation programs. Educating the local people regarding the cultivation, conservation and sustainable utilization of this plant will definitely be helpful in improving the population size of endangered species.

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