

## A comprehensive scientific overview of *Blepharispermum subsessile* DC. (Asteraceae), a conservation concern medicinal plant with promising pharmaceutical potential

Soumendra Kumar Naik<sup>\*,1,#,+</sup>, Shashikanta Behera<sup>1#</sup>, Sakti Kanta Rath<sup>2</sup> & Jayanta Kumar Patra<sup>3</sup>

<sup>1</sup>Department of Botany and Center of Excellence in Environment and Public Health, Ravenshaw University, Cuttack 753 003, Odisha, India

<sup>2</sup>Rama Devi Women's University, Bhubaneswar 751 022, Odisha, India

<sup>3</sup>Research Institute of Biotechnology & Medical Converged Science, Dongguk University-Seoul, Goyang-si, Republic of Korea  
E-mail: <sup>+</sup>sknuu@yahoo.com

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*Blepharispermum subsessile* DC., belonging to family Asteraceae, is known for its uses in the treatment of arthritis and related joint pain in folkloric medicine in different states of India. Additionally, ethnomedicinal uses of this plant include treatment of dysentery, diarrhoea, various ophthalmic problems, common cold, rhinitis, skin diseases, scabies, wound and gynaecological disorders. Most importantly, traditional use of *B. subsessile* to treat arthritis has been recently supported by scientific pharmacological studies including a clinical trial. However, overexploitation, unregulated trade and habitat destruction has put the *B. subsessile* under severe threat in their natural habitats of India in general and Odisha state of India in particular. To conserve the gene pool of this plant species along with other medicinal plants in their wild habitat (*in situ*) a medicinal plant conservation area (MPCA) has already been established in Odisha. Recently, attempt has also been made for development of tissue culture - mediated plant regeneration protocols aiming at its conservation by rehabilitation and *ex situ* means. In the present review, comprehensive and updated information on the distribution, botany, ethnomedicinal uses, phytochemistry and biological activities of *B. subsessile* has been summarized and focus has been given to the attempts made for conservation of this traditional medicinal plant species through conventional means as well as biotechnological intervention. Future research required for *B. subsessile* conservation and pharmaceutical utilization in fullest but in best sustainable manner has also been discussed.

**Keywords:** *Blepharispermum subsessile*, Conservation measures, Ethnomedicinal uses, *In vitro* propagation, Phytochemicals

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*Blepharispermum subsessile* DC. is a valuable folklore medicinal plant of India belonging to family Asteraceae. The word 'Blepharispermum' has been derived from Greek 'blepharis': eyelash and 'sperma': seed, referring to the shape of the seeds<sup>1</sup>. The plant is popular for its use in the treatment of rheumatism in traditional system of medicines<sup>2</sup>. Besides, it is also used in a number of other diseases<sup>3-7</sup>. *B. subsessile* is commonly known as 'Rasanajadi or Raasnaa or Rasna' in different parts of India including Odisha<sup>2,5,8-11</sup>. India is a large country where many different regional languages are spoken. Thus, interestingly, it has been found that besides *B. subsessile* a number of medicinal plants have been referred and used as Raasnaa / Rasna (a ayurvedic drugs) in different parts

of India which includes *Pluchea lanceolata* (Asteraceae) in North and West India; *Alpinia galaga* (Zingiberaceae) and *Alpinia calcarata* (Zingiberaceae) in South India including Kerala; *Vanda roxburghii* (Orchidaceae) in Eastern India including Odisha; *Dodonaea viscosa* (Sapindaceae) in Andhra Pradesh and *Lepidagathis trinevis* (Acanthaceae) in Bihar<sup>2</sup>. However, in North as well as West part of India mostly *P. lanceolata* has been used as 'Raasnaa or Rasna' and known as the accepted source plant<sup>2,12,13</sup> and *Alpinia galaga* has been considered as the main substitute plant<sup>2</sup>. On the other hand, recently it has been reported that *B. subsessile* showed better result compare to *P. lanceolata* for the treatment of *sandhivata* (osteoarthritis)<sup>14,15</sup>.

*B. subsessile* is marketed in Odisha, Madhya Pradesh and Chattisgarh as Rasna or Rasanajadi or Raasnaa<sup>2,9</sup>. Being a shrub with medicinally important root the plant has been uprooted unsustainably in

\*Corresponding author

#Both SKN and SB are equal contributors so considered as joint first author.

wild. The root of the plant has trade value and sold in the local market of different parts of India particularly in Bilaspur and Raipur, Chattisgarh state<sup>9</sup>. Raipur market of *B. subsessile* root is also catered from Nuapada district of Odisha. In recent years the price of the roots which was sold at a cost of Rs. 500/kg in 2007-2008 has gone up. This may be due to both high demand of the plant as well as limited availability of the plant materials. In fact, *B. subsessile* is under serious threat in its natural habitat due to unsustainable harvest for medicinal uses, trading of roots and loss of habitats<sup>9</sup> and merit urgent attention for conservation. Thus to protect the natural populations of this plant species and to achieve sustainability, development of different conservation strategies is of paramount importance.

Here we attempt to compile and present an updated and comprehensive overview on different aspects of *B. subsessile* including occurrence, botany, ethnomedicinal uses, phytochemistry, biological activities and efforts on conservation measures including biotechnological approaches through tissue culture. It was found that very little scientific research documentation is available on this medicinal plant species in the above said research domains. Thus the aim of this review is to bring *B. subsessile*, one of the pharmaceutically underexplored/overlooked but overexploited medicinal plants of India to the notice of the researchers by providing a platform to understand the plant species and design future strategies for its conservation as well as mining down its ethno-pharmacological attribute for potential therapeutic activities in a sustainable manner.

## Botany

### Distribution

The plant was thought to be endemic to India<sup>9,16</sup> and reported to occur in several parts of India including Karnataka<sup>2,8,16,17</sup>, Madhya Pradesh (Undivided), Maharashtra, Tamil Nadu<sup>2,8,16</sup>, Odisha<sup>8,9</sup>, Andhra Pradesh (Undivided), Telengana<sup>16,18</sup> and Chattisgarh<sup>19</sup>. However, the plant is also known to occur in Sri Lanka<sup>20</sup> and therefore its distribution range extends beyond the geographical boundary of India.

### Botanical description

*Blepharispermum subsessile* DC. (Fig. 1a-f) is an erect under-shrub with woody root-stock, growing up to 1.5 m. Stem single or many arising from the root stock; young stem is greenish and solid but mature

ones are hollow due to reduced central part of the pith<sup>11</sup>. The leaves are simple, alternate, glabrous, subsessile or short-petioled, finely reticulate. The leaf blades are ovate, elliptic or obovate, entire and very rarely toothed, obtuse or subacute at the apex. Flowers are borne on capitula or heads. Heads are white in colour, solitary, terminal, aggregated into globose clusters and with broad leafy bracts. Female florets 2 and bisexual florets 3-8 in each head. Achenes of the inner bisexual florets are comparatively smaller than the outer female florets. Both types of achenes have pappus with few bristles or short hairs<sup>8</sup>. The flowering and fruiting in this species have been observed between July - September and August - January respectively.

### Ethnomedicinal uses

*B. subsessile* has been reported to be found in different parts of India; however the ethno-botanical literatures indicated its use mainly in Chhattisgarh (including undivided Madhya Pradesh), Odisha and Karnataka. The ethno-medicinal uses of different plant parts of *B. subsessile* have been presented in Table 1. The literature indicated that the plant is largely valued for its root which is usually used for the treatment of rheumatism and related joint and body pain (Table 1). Besides, the plant is used as tonic and also for treatment of dysentery and delivery related problems. Juice of the leaves is used to treat ophthalmic and ear related problems in Odisha and Karnataka, respectively. However, some examples of *B. subsessile* used in combination with other medicinal plants to treat ailments is also available (Table 1). In undivided Madhya Pradesh (Bastar region, presently in Chhattisgarh) cattle are given plant-juice on the day of 'Pola' festival with a belief of keeping them free from intestinal worms for the entire year<sup>4</sup>.

### Phytochemistry

Comprehensive work is yet to be done on the phytochemistry of *B. subsessile*. Owing to its medicinal properties most of the work till now has been focused on roots/rhizomes. Till date, five chromene compounds and more than twenty other biologically active compounds have been isolated and identified from aerial parts and roots of the plant respectively. The details of the compounds isolated/identified from various plant parts of *B. subsessile* have been enlisted in Table 2.

In 1987, Kulkarni *et al.*<sup>29</sup> isolated and identified four new compounds [chromenes: 8-methoxy-2, 2-



Fig. 1 — *Blepharispermum subsessile* (a) a seedling plant in forest floor, (b) a mature plant with multiple branches arising from root stock, (c) flower head before opening of florets, (d) flower head with florets, (e) mature heads, (f) seeds, (g) entrance gate of Gurudongar Medicinal Plant Conservation Area (MPCA), Odisha, India.

dimethylchromene; desmethylisoencecalin; 5-hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene; (-)-artemesinol] as well as a known chromene,

desmethoxyencecalin from the acetone extract of the aerial parts of the plant. These compounds were identified using IR,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra.

Table 1 — Ethno-medicinal uses of *B. subsessile*

Plant parts	Country/State/District	Ethno-medicinal use	Method of preparation	Route of administration	References
Plant	Undivided Madhya Pradesh (Bastar <sup>#</sup> )	Tonic and stimulants	Decoction of the plant	-	3
Root	Odisha (Koraput)	Rheumatism, joint pains and body ache	Juice	Internally (Dose:10 mL)	21
Root	Undivided Madhya Pradesh (Bastar <sup>#</sup> )	Scabies and other skin diseases	Powder	5 g	4
Plant	Undivided Madhya Pradesh (Bastar <sup>#</sup> )	Flatulence in cattle (Ethno-veterinary practices) Common cold and rhinitis in children	Plant juice	Orally (50-70 g) -	4
-	India	Diarrhoea	-	-	22
Leaves	Odisha (Sundargarh)	Various ophthalmic diseases	Juice	Eye drop	23
Root	Odisha (Sundargarh)	Backache due to Rheumatism	Water extract of root with honey	Oral	23
-	Madhya Pradesh	Rheumatic affections (due to anti-inflammatory properties)	-	Externally and Internally	2
Root	Chhattisgarh	Irregular menstruation	Decoction of Ashoka ( <i>Saraca asoca</i> ) root, Arjun ( <i>Terminalia arjuna</i> ) bark, Rasna ( <i>Blepharispermum subsessile</i> ) root and Giloy ( <i>Tinospora malabarica</i> ) stem.	Oral; Half glass decoction twice daily for one month	24
-	Chattisgarh (Raipur and Mahasamund)	Arthritis	-	-	25
Root	Chhattisgarh	Well-being of mother after delivery	Decoction made of 'Rasna jadi' (Root of <i>Blepharispermum subsessile</i> ) together with Kulthi beej (seed of Horse gram: <i>Dolichos biflorus</i> ) and soft, apical shoot of <i>Phoenix acaulis</i> (Chhind kanda)	One dose (after two days of delivery)	26
Seed/Fruit	Chhattisgarh (Raigarh)	Wound (antiseptic), Dysuria, Delivery problem	-	-	19
Root	Chhattisgarh (Bastar)	Delivery problem, Dysentery	-	-	27
Root	Chhattisgarh (Bastar)	Delivery problem	-	-	7
Rhizome, whole plant	Chhattisgarh (Bastar)	Dysentery	-	-	7
Leaves	Karnataka (Belgaum, Bellary, Chickmagalore, Dharwad, Mysore, North Kannada)	Otitis and other inflammatory conditions	Juice of leaves	Dropped in ear	28
Root	Karnataka (Belgaum, Bellary, Chickmagalore, Dharwad, Mysore, North Kannada)	Dyspepsia, bronchitis and fevers.  Rheumatism, rheumatic swelling and nervous troubles.	Roots form a constituent of medicated oils.	External	28

<sup>#</sup>Now in Chhattisgarh; - no information

Table 2 — Summary of extracts/ compounds isolated and/ or identified from *B. subsessile* with their biological activities

Extract	Plant parts used	Area of plant sample collection	Compounds isolated and/or identified	Biological activities	References
Acetone	Aerial parts	Pune (Katraj Ghat), Maharashtra, India	8-Methoxy-2,2-dimethylchromene* Desmethyloencecalin*	- Oviposition deterrent activity against the potato tuber moth ( <i>Phthorimaea operculella</i> )	29
			Desmethoxyencecalin	-	
			5-Hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene*	-	
			(-)-Artemesinol*	-	
Methanol	Rhizome	Raipur <sup>#</sup> , Undivided Madhya Pradesh, India	Desmethyloencecalin	Anti-implantation activity	30
			Two minor chromenes	-	
Methanol	Rhizome	Cuttack, Odisha, India	Desmethyloencecalin	Antifeedant activity against larvae <i>Spilarctia oblique</i> and antifungal activity against <i>Candida albicans</i> & <i>Cryptococcus neoformans</i> .	31
			5-Hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene	Antifeedant activity against larvae <i>Spilarctia oblique</i> and antifungal activity against <i>Candida albicans</i> & <i>Cryptococcus neoformans</i> .	
Ethyl acetate	Root	Gurudongar Reserve Forest, Odisha, India	Betulin	Tumor disorders, skin irritations, insect bites and tuberculosis**	32
			Dronabinol	Analgesic, anti-inflammatory**	
			Benzoic acid, 4-heptyl-, 4-cyanophenyl ester	---	
			Benzamide, 4-chloro, o-(2,6-dimethoxybenzoyl) oxime	---	
			9,12-Octadecadienoic acid (Z,Z)-	Anti-inflammatory, acne reductive, antioxidant**	
			n-Propyl 9, 12-octadecadienoate	---	
			n-Hexadecanoic acid	Anti-inflammatory**	
			2-Amino-4-(2-cyclohexyl-ethyl)-7-methyl-	---	
			5-oxo-4H,5H-pyrano (4, 3-b) pyran-3-carbonitrile		
			Carbamult	Pesticide**	
			Carvacrol.	Nematocide**	
Methanol	Root	Gurudongar Reserve Forest, Odisha, India	Clindamycin	-	33
			m-Guaiacol	-	
			Carvacrol	-	
			Syringol	-	
			Methyl 4-methoxysalicylate	-	
			Carbamult	-	
			Benzoic acid, 2-hydroxy-6-methoxy	-	
			2-(3,4-Dimethoxyphenyl)-6-methyl-3,4-chromanediol	-	
			Phenol, 4-allyl-2,6-dimethoxy	-	
			2-Amino-4-(2-cyclohexyl-ethyl)-7-methyl-	-	
			5-oxo-4H,5H- pyrano (4, 3-b) pyran-3-carbonitrile	-	
			4-(1E)-3-Hydroxy-1-propapenyl-2-methoxyphenol	-	
			Piperine	-	
			n-Hexadecanoic acid (Palmitic acid)	-	
			9,12- Octadecadienoic acid (Z,Z)- (Linoleic acid)	-	
			n-Propyl 9,12-Octadecadienoate	-	
			Totarol	-	
			Anthiaergostan-5,7,9,22-tetraen-14-ol-15-one	-	

Contd.



Table 2 — Summary of extracts/ compounds isolated and/ or identified from <i>B. subsessile</i> with their biological activities (Contd.)					
Methanol	Root	Gurudongar Reserve Forest, Odisha, India	Crude extract	Anti-arthritis activities	34

<sup>#</sup>Now in Chhattisgarh; <sup>\*</sup>New compound isolated; <sup>\*\*</sup>The biological activities are based on Dr Duke's Phytochemical and Ethnobotanical Databases (online database) by Dr Jim Duke of the Agricultural Research Service/USDA; --- : No data; - : Not tested

The chemical structures of four new chromenes isolated from *B. subsessile* have been provided in Fig. 2. As the root/rhizome of *B. subsessile* has been used mainly in folkloric medicine most of the subsequent works related to compound isolation were based on root/rhizome. Besides two minor chromenes, Agarwal *et al.*<sup>30</sup> isolated and reported desmethylisoencecalin from the methanolic extract of rhizome. In addition to desmethylisoencecalin, Agarwal *et al.*<sup>31</sup> reported 5-hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene from methanolic rhizome extracts. These compounds were isolated using chromatographic purification of the chloroform fraction on silica gel and identified by comparing the mp, IR, <sup>1</sup>H NMR and MS spectra with that of the known compounds. However, both these chromenes have already been reported from the aerial part of the plant by Kulkarni *et al.*<sup>29</sup>. Das and Suresh Kumar<sup>32</sup> identified ten different compounds from ethyl acetate root extract of *B. subsessile*. Identity of these compounds were established by comparing their retention time and peak area with that of literature and GC-MS spectra. Recently, Das and Sureshkumar<sup>33</sup> have successfully identified 19 compounds from the methanolic root extracts using GC-MS analysis. However, about six of these nineteen compounds have also been identified in ethyl acetate root extract by Das and Suresh Kumar<sup>32</sup> (Table 2).

**Biological activities**

*B. subsessile* has been used to treat a number of diseases as reported in different ethnomedicinal studies. However, till date, only a few studies have been carried out to test the bioactivities of various extracts as well as compounds isolated from *B. subsessile* to substantiate its folkloric uses (Table 2). This plant possesses antifeedant, antifungal as well as antifertility activities due to the presence of bio-active constituents called chromene. Kulkarni *et al.*<sup>29</sup> demonstrated that desmethylisoencecalin has oviposition deterrent activity against the *Phthorimaea operculella* (potato tuber moth). Agarwal *et al.*<sup>30</sup> studied the antifertility activity of *B. subsessile* (rhizome) using crude methanol extract, its chloroform/ methanol/ water soluble fractions and chromene (desmethylisoencecalin) isolated from the

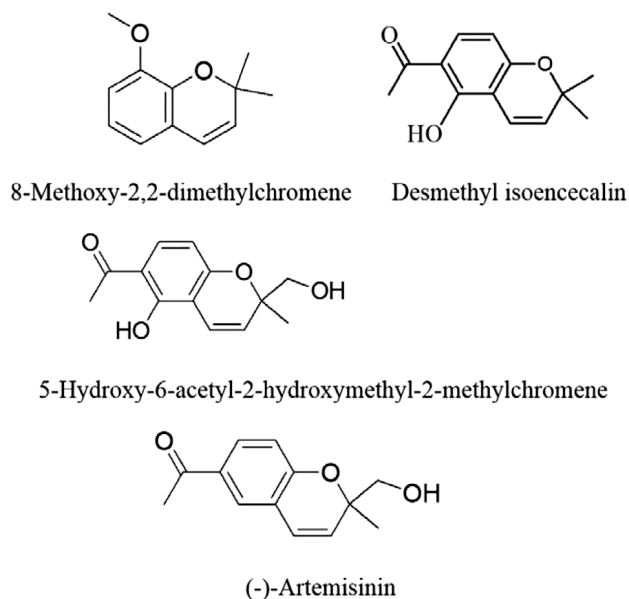


Fig. 2 — New chromenes isolated from *B. subsessile* (Original source: Kulkarni *et al.*, 1987)

chloroform soluble fraction on virgin female rats and adult Sprague-Dawley rats. Oral doses (250 mg/kg) of methanol extract (crude) and its chloroform fraction exhibited 75% and 50% antifertility activity respectively. However, the methanol and water soluble fractions showed no infertility activity. At the same time chromene (desmethylisoencecalin) at a significantly lower dose (50 mg/kg) showed 50% antifertility activity, which reflected its potential for use as active constituent in antifertility related issues. One year later, Agarwal *et al.*<sup>31</sup> reported the antifungal and antifeedant activities of the rhizome of *B. subsessile*. Crude methanol extract of rhizome and two chromenes isolated from there i.e. desmethylisoencecalin and 5-hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene, showed anti-*Candida albicans* and *Cryptococcus neoformans* activity, with MIC values ranging between 25-250 µg/mL. Ketoconazole (0.1-0.3 µg/mL) was taken as positive control in the study. Further it was found that both the chromenes have antifeedant activity against larvae *Spilarctia obliqua*. However, of the two chromenes at equal concentration, desmethylisoencecalin was found to be 22% more

efficient than 5-hydroxy-6-acetyl-2-hydroxymethyl-2-methylchromene.

A recent study by Jadhav<sup>14</sup> on humans supported the traditional use of *B. subsessile* for remedy of arthritis. They found that *B. subsessile* was more effective than *P. lanceolata* for treatment of osteoarthritis (sandhivata). During the clinical trial study, Jadhav<sup>14</sup> divided thirty patients suffering from the said diseases into two groups of fifteen patients each. The decoction (at a dose of 50 mL for 3 weeks) of *P. lanceolata* root was compared with that of the *B. subsessile* for relief of osteoarthritis (sandhivata). It was observed that the fifteen patients treated with the decoction of *B. subsessile* exhibited higher relief (85.72%) compared to the other group administered with *P. lanceolata* (71.43%).

Besides, Das and Suresh Kumar<sup>32</sup> identified ten compounds and reported the biological activities of six of them based on Dr Duke's Phytochemical and Ethnobotanical Databases (online database) by Dr Jim Duke of Agricultural Research Service/USDA, (Table 2). Among the isolated compounds 'dronabinol', "9, 12-octadecadienoic acid" and "n-hexadecanoic acid" have been known for their anti-inflammatory properties. May be these are among the active principles of *B. subsessile* roots responsible for its effectiveness against arthritis, which is an inflammatory disorder mainly affecting bone and cartilages around joints. A recent work by Das and Sureshkumar<sup>34</sup> also supported the use of *B. subsessile* root to treat arthritis in folklore medicine of India. They studied the anti-arthritic potential of methanolic root extract using both *in vitro* models (Human Red Blood Cell [HRBC] membrane stabilization, inhibition of protein denaturation and proteinase inhibitory activities) as well as *in vivo* models (Freund's Complete Adjuvant (FCA)-, Formaldehyde- and carrageenan-induced arthritis models). According to their report the methanolic root extract showed membrane stabilization properties by inhibiting the lysis of HRBC membrane, which indicated the anti-inflammatory properties of the root extract. The methanolic root extract also showed inhibition of protein denaturation and proteinase inhibitory activity. Anti-inflammatory activity of *B. subsessile* root extract was further ascertained by Das and Sureshkumar<sup>34</sup> using FCA, Formaldehyde- and carrageenan-induced models of arthritis. In all the three models, the root extract was found to inhibit the paw edema in Male albino wistar rats. According to Das and Sureshkumar<sup>34</sup> FCA-induced paw

inflammation was significantly reduced by methanolic root extract (100 mg/kg) of *B. subsessile* after 28 days of administration. Radiographic examinations also confirmed the usefulness of root extract in preventing the FCA- induced soft tissue swelling and destruction ankle joints<sup>34</sup>. Highest inhibitory effect (43.9%) of methanolic root extract of *B. subsessile* was observed with an oral dose of 100 mg/kg at 4 h post-carrageenan injection<sup>34</sup>.

Till date only a few compounds have been isolated from the plant and yet a number of compounds identified from this plant have not been evaluated for their biological activity. In light of ethno-medicinal data, further bioactivity studies of these compounds, can start a new course of action for development of drugs to cure ailments like arthritis, cancer, neurodegenerative disorders etc.

### Toxicity

Some of the important and well known drugs have natural origin. One of the prominent natural sources is medicinal plants. But for a compound (either natural or synthetic in origin) to become an efficient and safe drug, toxicological studies are essential. Till date, only a single report on the toxicity study of *B. subsessile* is available. The acute toxicity study of *B. subsessile* methanolic root extract (2000 mg/kg) by oral administration showed no symptoms of toxicity and mortality after 14 days in male albino wistar rats<sup>34</sup>. However, toxicity studies have already been carried out in other plants for some of the compounds present in *B. subsessile*. For example, acute toxicity studies of Betulin (a pentacyclic triterpene alcohol) on rats and mice revealed no significant effect on body weight of animals and no lethal effect were observed during 14 days administration in rats and mice in all doses tested<sup>35</sup>. The median lethal dose of Carvacrol (a monoterpenic phenol) in rats was 810 mg/kg of body weight when administered by oral gavage while this dose administered intravenously or intraperitoneally to mice has been estimated at 80.0 and 73.3 mg/kg of body weight respectively<sup>36</sup>. At the same time, toxicity studies on Piperine (alkaloid) have also been carried out on mice, rats and hamsters<sup>37</sup>. Further, toxicity studies on other bioactive compounds from *B. subsessile* need to be evaluated in order to prove this plant as a potential source of drugs.

### Conservation measures

The plant has been identified as an endangered species in the state of Odisha, India in the Conservation

Assessment and Management Prioritisation Workshop (CAMP), 2007 due to overexploitation and habitat destruction<sup>9</sup>. Thus, conservation efforts and sustainable utilization of *B. subsessile* are necessary. It was also found that the wild gene pool of *B. subsessile* is present in Gurudongar area (latitude 20°.25' and longitude 82°.55') of Nuapada district, Odisha, India along with numerous other medicinal plants. Thus, subsequently, Gurudongar has been declared as Medicinal Plant Conservation Area (MPCA) in 2008-2009 with United Nations Development Programme (UNDP) assistance through The Foundation for Revitalisation of Local Health Traditions (FRLHT), Bangalore to conserve the gene pool of this valuable medicinal plant species along with other medicinal plants in its natural habitat as *in situ* conservation measures<sup>38</sup> (Fig. 1g).

Like any other species, the conservation of *B. subsessile* required the involvement of both the Government and local community. Thus awareness among local people near to the natural gene pool of *B. subsessile* should be created for its conservation and sustainable uses. Natural propagation of this plant is through seed, which is hampered by low seed setting, viability and germination rates<sup>6</sup>. Unfortunately no cultivation practises at commercial scale have been reported for the plant species<sup>9</sup>. Thus, cultivation methods for this plant species must be standardized. Once developed it should be promoted among local populations so that they can be benefited without depending on wild populations for harvest.

At the same time plant tissue culture has been successful in a number of medicinal plant species to serve as an alternative source of different bioactive compounds for pharmaceutical use<sup>39-41</sup>. Development of large number of plants through tissue culture with same biochemical and genetic profile has potential to be used in place of wild populations. Thus, with the development of such plant regeneration protocol (s) the demand of this plant species from its wild habitat will be reduced and sustainability could be achieved. Besides they have the ability to overcome the limitations of the conventional propagation and regenerate large number of plants which can be used for reintroduction of threatened medicinal plants in their natural habitat. Recently two *in vitro* plant regeneration protocols have been reported on *B. subsessile*. Nayak and Kalidass<sup>6</sup> developed a plant regeneration system in *B. subsessile* by adventitious shoot organogenesis. They recorded best shoot organogenesis from cotyledon explants derived from axenic seedlings on Murashige and Skoog's (MS)

medium supplemented with 2.5 mg/L 6-benzylaminopurine (BAP) and the shoots were rooted on ½ MS medium augmented with 1.0 mg/L indole-3-acetic acid (IAA). Subsequently another micropropagation protocol was also reported by the same group using axenic shoot tip explants<sup>42</sup>. Best shoot proliferation was observed from shoot tip explants on 1.5 mg/L BAP and 0.2 mg/L IAA supplemented MS medium. They reported best rooting of these shoots on ½ MS medium supplemented with 1.0 mg/L IAA. The success of tissue culture depends upon the ability of regenerated plantlets to acclimatize themselves in outdoor free-living autotrophic conditions with negligible mortality following transfer from culture medium<sup>43</sup>. But only about 30-40% of the *in vitro* regenerated plantlets of *B. subsessile* were successfully acclimatized<sup>6,42</sup>. Thus, these protocols have their limitations and further work is necessary to improve the acclimatization rate of the plants prior to their use in conservation and validation of ethno-pharmacological aspects.

#### Future prospects

*B. subsessile* may be underexplored pharmaceutically yet overexploited in its wild habitat due to its popularity in ethno-medicinal uses. Ethnobotanical studies indicated that *B. subsessile* has been prescribed to treat different ailments in India and the most prevalent medicinal use of the plant has been for the treatment of arthritis and related problems. But the ethno-medicinal uses of this plant has not yet been pharmacologically exploited fully. Thus, sincere efforts should be given to isolate more unexplored compounds and evaluate their pharmacological activities. In spite of its limited distribution, the plant could be of potential interest for development of drugs especially for arthritis and joint pain. But as *B. subsessile* has no known cultivation practice and is suffering from destructive harvesting, formulation of strategies for sustainable use is imperative to avoid resource exhaustion and even species extinction. To achieve sustainability emphasis should be given to develop good cultivation methods including organic farming. Medicinal plants grown by organic farming have the potential to produce materials with high productivity and better quality<sup>41</sup>. For example, Kaur *et al.*<sup>44</sup> reported that organic cultivation of *Withania somnifera*, a valuable medicinal plant in Ayurvedic system of medicine, resulted in plants with high content of secondary metabolites (withanolides, withanone, withanolide A and withaferin A) and improved



biomass. Efforts should also be made to develop efficient tissue culture mediated - plant regeneration protocols with improved acclimatization rate, which will open new avenues for large scale plant production for reintroduction or pharmaceutical usages and research in a sustainable manner. More importantly cell suspension and hairy root culture systems, which can be the source of pharmaceutically important metabolites, have not yet been established in *B. subsessile*. It is well known by now that a number of secondary metabolites are produced in a tissue specific manner<sup>45</sup>. Keeping this and importance of root of *B. subsessile* in mind, the development of hairy root system for this plant species seems to be imperative.

In conclusion, further extensive research including isolation and identification of bioactive compounds, pharmacological activities of these compounds with their mechanism of action, and strategies for conservation is necessary to explore the pharmaceutical potential of *B. subsessile* in a sustainable way.

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#### Authors contribution

SKN conceptualized the topic. SKN and SB collected the literature and wrote the manuscript. SKN, SKR and JKP checked and edited the manuscript. All the authors read and approved the manuscript.

#### Conflict of interest

Authors declare no conflict of interest.

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