Folklore use of *Osbeckia* species from Munnar hills, Kerala

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Abstract

The genus *Osbeckia* of Melastomataceae was named by Carl Linnaeus, for the Swedish explorer and naturalist Pehr Osbeck. Osbeckias are native to Eastern Asia, and found in China, Japan, Malaysia, India, Sri Lanka and Australasia. Most species were documented as herbal remedies for curing many human diseases. In India the genus comprises about 22 species out of which 12 species are reported from Kerala. Most of the species are small shrubs and have gained medicinal status in folklore belief from India, China, and Indonesia. Ethnopharmacologically, the leaves, shoots, barks, seeds, and roots of Melastomataceae members have been used to treat diarrhea, dysentery, hemorrhoids, cuts and wounds, toothache, and stomachache. The coloured floral extracts of various genera also revealed wide pharmacological actions such as anti-nociceptive, anti-inflammatory, wound healing, antidiarrhoeal, cytotoxic, and antioxidant activities. Many phytochemical constituents including anthocyanins were isolated and identified from different parts of *Melastoma* and *Memycylon* species. Thus, the aim of this survey is to present comprehensive information on ethnomedicinal uses and pharmacological activities of *Osbeckia* species from Kerala. Floral extracts of most of the *Osbeckia* species contain substantial levels of phenols and anthocyanin. This data validate the folklore use of various species to cure many human disorders. Future studies are planned to evaluate the biological potentialities in terms of anthocyanin profile using *in vitro* cultures of the species such as *O. aspera* and *O. reticulata.*

Key words: Mannan, Anthocyanin, Medicinal potential, *Osbeckia*, Tribal knowledge

Introduction

Out of the 2, 70,000 plant species taxonomically documented so far, about 70,000 plants have medicinal significance (Rajasekharan and Ganeshan, 2004). Globally, about 80% of the traditional drugs used for primary health care are elucidated from herbals (WHO, 2010). In India, nearly 17,500 species of higher plants are recorded, of which 6,000 are considered to have therapeutic properties (Jomy *et al*., 2010). The Munnar region of Kerala harbors about 6,600 plant species, of which about 1,780 were used for medicine either by the pharmaceutical industries or tribals as crude extracts for local health care system (Lawarence and Murukan 2016). At present, the traditional medical practitioners who are dependent only on medicinal plants for their vocation, livelihood dependence are minimum (Barik *et al*. 2008). Therefore, there is ample scope for livelihood enhancement through taking up of medicinal plants cultivation and sustained trade in the region (Jain, 1991). Because of over exploitation and several other associated factors, many of the medicinal plant species were on the verge of extinction. However, no empirical data was available to assess the threat status of these medicinal herbs. The correct threat analysis of the species is crucial for planning conservation prioritization. Rainforests abode not less than 60 % of all flowering plant species known on earth and they provide all aspects needed for human existence. Through several decades of evolution,

plants have synthesized many phytochemical substances to defend themselves against pests and pathogenic diseases. These phytochemicals, at least some of them, can act within the human body against pathogens and other causes of diseases. These molecules represent important sources of natural drugs. They are highly complex and their molecular structures surpass the imagination of the pharmacologists and cannot easily be reproduced in the *in vitro* labs.

The present study was conducted in Munnar hills of Idukki district, Kerala to assess the status of the *Osbeckia* species and to document ethnobotanical importance of the different species and their phytochemical validations.

Materials and methods

Study area

Munnar is a part of the Western Ghats with steep slopes and undulations. The soil is laterite and brown. The altitude range from 750 to 1800 m, latitude 10°N, and longitude 77°E. The area receives rainfall from 250 mm to 425 mm and the temperature range from 15°C to 30°C. Dry period is from December to April.

The Ethnobotanical survey of *Osbeckia* species was conducted in six hamlets of Mannan tribe of Munnar viz. Kovilmala, Murikkattukudy, Maniyarankudy, Vallakadavu, Mannakkudy and Thoprankudy. Regular field visits were conducted to the hamlets during 2015 December to 2017 January. The information were collected from elderly experienced men, women, plant collectors and medicine men (Vaidyas), using a detailed questionnaire prepared according to the methodology suggested by Jain (1991). The fresh specimens were collected and identified with the floras and manuals (Nayar T.S. *et al* 2006). The *Osbeckia* species used by Mannan tribes were enumerated with botanical name, family, local name, plant parts used and their medicinal importance in the Table-1. The voucher specimens were deposited in the herbarium of Department of Botany, University College, Trivandrum. Kerala.

Quantification of phenols, flavonoids and anthocyanin

Total phenol content in the leaves and flowers of *Osbeckia spp.* was estimated by the method of Mayr *et al.,* (1995). 1 g tissue was used and the absorbance of the light blue solution was recorded at 650 nm against the reagent blank containing 3 ml 80% methanol, 0.5 ml Folin's reagent and 2 ml 20% Na_2CO_3. The total phenols g^{-1} tissue was calculated from the standard graph.

Protocol by Sutharut and Sudarat (2012) was used for the estimation of anthocyanin content from the leaves and flowers. The absorbance was read at 510 and 700 nm against distilled water as blank.

For the analysis of flavonoid content protocol by Kalitha *et. al* (2013) was used and the OD was read at 340 nm. A standard curve was constructed and the concentration of flavonoids in each sample was calculated. The values of flavonoids were expressed as mg/g sample.

Results

Nine taxa were collected from Munnar hills and identified using morphological and floral characters. They are described here with details (Fig.1).

1. *Osbeckia gracilis* Bedd.

Leaves of *O. gracilis* were 3.5 x 2 cm in size, elliptic-oblong, obtuse at apex and base, cuspidate, yellowish green, densely covered with half-adnated hairs spreading from midrib, 3-ribbed; petiole 3 mm long. Flowers in terminal cymes, pedicelled; calyx tube 7 mm long, densely simple-hairy, lobes 4 x 3 mm, ovate-oblong, obtuse, ciliate; petals 15 x 12 mm, obovate, ciliate, pink; anthers 5 mm long. The aqueous pant extract is effective in skin problems, and also used in throat infections.

2. *Osbeckia wynaadensis* Clarke.

Leaves of *O. wynaadensis* were 1.8 x 3 cm in size, lanceolate, acute, 3-5-nerved, hairy; hairs adnated and spreading from the midrib. Flowers many, in terminal panicled cymes; pedicels 4 mm long; calyx tube 8 mm long, bristles 4 or 5 in a tuft, to 3 mm long; lobes 11 x 5 mm, lanceolate, ciliate; petals 20 x 10 mm, obovate, anthers 7 mm long. Seeds 0.5 x 0.3 mm obovoid, minutely muriculate. The plant has anti-inflammatory properties and used against skin infections.

3. *Osbeckia leschenaultiana* DC.

Leaves of *O. leschenaultiana* shows 2.5 x 1.5 cm size, elliptic-ovate, acute at apex, base rounded, densely pubescent, 3-5 nerved; petiole to 3 mm. Flowers terminal, solitary or capitate, pink; calyx cup with compound rufous hairs, stalked hairs; petals to 2.5 cm, obovate; stamens 10, filaments 6 mm, anthers 6 mm, twisted. The leaf and floral extracts have anti-inflammatory properties.

Fig.1-a. *Osbseckia aspera* (L.) var. *wightiana* (Benth ex Wight & Arn.); b. *Osbeckia wynaadensis* Clarke.; c *Osbeckia leschenaultiana;* d. *Osbeckia gracilis* Bedd.; e. *Osbeckia reticulata* Bedd.; f-g. *Osbeckia parvifolia* Arn. Wight & Arn. g. DC. ; h. *O. aspera* var. *travancorica* (Bedd ex Gamble); i. *O. aspera* var. *aspera*; j. *Osbeckia virgata* D. Don ex Wight & Arn.

4. Osbeckia aspera L.

Osbeckia aspera L. is the most extensively explored species of *Osbeckia* and there are many reports regarding their anti-oxidant, anti-diabetic anti-inflammatory and hepatoprotective effects (Thabrew *et al*., 1998). Three different varieties were collected *viz*, *Osbeckia aspera, O. aspera* var. aspera, *O. aspera* var. *travancorica* (Bedd ex Gamble) and *O. aspera* (L.) var. *wightiana* (Benth ex Wight & Arn) All the species were used by Mannan tribes of Munnar against many disorders.

4a. O. aspera (L) var. aspera

Leaves of *O. aspera* var. aspera are simple, opposite, 3.5-9 x 1.5-3.2 cm, elliptic-lanceolate, acute to shortly acuminate at apex, base attenuate, basally 5-ribbed, more or less pubescent with appressed short hairs on both sides, yellowish-green; petiole to 1 cm long. Flowers ca 2 cm across, in terminal cymes, sometimes elongated clusters, pentamerous. Calyx tube 6-8 mm long, ca. 5 mm wide, cupular, with dense short bristle-like hairs; lobes 5, oblong, obtuse. Petals 5, pink, 1-1.5 x 0.8-1 cm, ovate, apex rounded. Stamens 10; anthers 5-6 mm long. Ovary hairy at apex; style exserted, 1.25-1.5 cm long; stigma curved, papillate. Plant extract is used to treat Diarrhea and Liver diseases. It has anti-inflammatory and anti oxidant properties also.

4b. **O. aspera (L.) var. travancorica (Bedd ex Gamble)**

Leaves of *O. aspera* var. *travancorica* were 15 x 5 cm, ovate-oblong, acuminate, 7-ribbed, covered with adpressed hairs above and below; petiole 1 cm long. Flowers 6 cm across, 5-15 together in terminal cymes, bracts 1 cm across, orbicular; calyx cup 13 mm long, densely covered with stalked bristles, lobes 7 x 4 mm, obovate, emarginate, bristled; intersepalar emergences with stellate bristles; petals 3.5 x 2.5 cm, obovate, pink; filaments 13 mm long, anthers 9 mm long, acuminate, constricted at base. The plant is used in skin infections and inflammations.

4c. O. aspera (L.) var. wightiana (Benth ex Wight & Arn.)

Leaves of *Osbseckia aspera* (L.) var. *wightiana* (Benth ex Wight & Arn.) shows 9-10 x 3-4 cm, ovate, rounded at base, acute at apex, 5-ribbed, densely hairy; hairs half adnated above and below; petiole 1 cm long. Flowers 3-5-together in terminal sessile clusters; bracts densely rufous hairy; calyx tube 8 mm long, densely tufted hairy, hairs rufous brown; lobes 3 x 2 mm, bristled at tip; anthers 9 mm long, acuminate. Mature fruit pulp is used for soothing effect in tooth ache, to cure ulcers in mouth, and a decoction is used to cure jaundice.

5. Osbeckia reticulata Bedd.

Osbeckia reticulata leaves were 5-8 x 2.5-4.5 cm in size, coriaceous, strigose, dark green above, paler below, 5-7-nerved, base obtuse, margin serrulate, apex acute; petiole 1 cm. Cymes terminal, to 6(8) cm, 4-6-flowered; peduncle 0. Flowers 5-merous with 6 cm wide. Hypanthium urceolate, 1.5 x 1.4 cm; intersepalar emergences stalked, cupular, 7 x 3 mm; lobes 5, oblong, 9 x 4 mm; bristle hairs both simple and stellate. Petals 5, purple-rose, orbicular, 4 x 3 cm. Stamens 10, to 2 cm; filaments 1 cm; anthers equal to, or slightly longer than, filaments, oblong, slightly beaked. Ovary 5-celled, 10-lobed; style 2.5 cm. The flower extract in water has anti-inflammatory and anti-microbial properties. Leaf is used to treat skin infections. The ripened fruit is effective in treating ulcers in mouth.

6. Osbeckia virgata D. Don ex Wight & Arn.

Osbeckia virgata leaves were 1.5-3.3 x 0.5 - 1.8 cm, elliptic to linear-lanceolate, base attenuate, apex acute, hairy below on nerves, prominently 3-ribbed; petiole up to 5 mm long. Flowers in terminal few-flowered clusters, pentamerous, c. 2 cm across. Calyx tube 3-4 mm long, subglobose with stalked stellate and simple bristles; lobes 5, lanceolate, acute. Petals pink, 6-7 x 3-4 mm, obovate. Stamens 10; anthers 4-5 mm long. Ovary 5-locular, with tufted stiff hairs. The leaf extract is anti-inflammatory and helps in wound healing.

7. Osbeckia parvifolia Arn. Wight & Arn.

Osbeckia parvifolia Arn. Wight & Arn. Syn. *O. cupularis* D. Don ex Mal : Cherukadali is distributed in Southern montane wet scrub and Southern montane wet grasslands. Commonly grow in marshy areas; favours sandy loam soils, medium acidic. low in potash and phosphate and high in organic carbon. It is a small herb. Leaves simple, opposite ovate or elliptic-ovate, acute, 3-ribbed. up to 4 x 2 cm. Flowers

white or pink in capitate heads. Fruit ovate-oblong, obscurely ribbed. Whole plant is pounded and applied to swellings.

The polyphenols among the six species screened shows significantly varied values in flowers and leaves. Flowers of *O. aspera* (L.) var. *wightiana* (Benth ex Wight & Arn) showed outstanding phenolic content i.e., TPC of 24.7 mg GAE / g (Table 2). Values were significant at 1% level i.e., $P < 0.01$. *O. virgata* showed 44.3 mg/ g anthocyanin content with 53.4 mg GAE / g of phenols. *O. aspera* var. *aspera* retain the third position in terms of anthocyanin and phenol content i.e., 36.6 mg/ g and 46.6 mg GAE / g of phenols respectively. *O. leschenaultiana* showed least level of the anthocyanin among the species analyzed (19.2 mg/g.). The remarkable level of polyphenols indirectly suggests the pharmaceutical potentialities of the species. For example anthocyanin of eggplant was proved for potent metal-chelating activity (Sadao and Yataro 2014). Gulcin *et al*. (2005) revealed that the leaves of *Perilla frutescens* var. *nankinensis displayed ideal correlation between polyphenols and antioxidant property*. Figueiredo, and Lima (2015), assessed antioxidant activity of anthocyanins from *Sideroxylon obtusifolium* fruits. Vaidya, (2014) compared antioxidant capacity of fresh and dry leaf extracts of sixteen *Scutellaria* species. Gandhiappan and Rengasamy (2012) compared antioxidant activity of different species of Solanaceae. Medina-Medrano *et al*., (2015) fractionated phenolic constituents and antioxidant properties of five wild species of *Physalis*. Sulniute *et al* (2016) comprehensively evaluated antioxidant potential of selected *Salvia* species using high pressure methods for the isolation of lipophilic and hydrophilic plant fractions. *In the present study of* the *Osbeckia* species screened, flowers showed higher anthocyanin level compared to their leaves.

Table 1. *Osbeckia* species used by tribal communities in Munnar

Sl. No.	Botanical name	Local name	Plant parts used	Medicinal importance	Method of use	Dose and time
1	*Osbeckia aspera* (L.) var. *travancorica* (Bedd ex Gamble)	Cheru kalathi	Leaf,	Skin infections	Boil in water and use for extended wash	Several times a day
			Flower ,	inflammations	Chewed or a paste is applied	Several times a day
			Fruit	Wound healing in mouth	Chewed or a paste is applied	Several times a day
2	*Osbeckia aspera* (L.) var. *wightiana* (Benth ex Wight & Arn)	Kalathi	Leaf,	Skin infections	Boil in water and use for extended wash	Several times a day
			Flower ,	Tooth ache,	Chewed or a paste is applied	Several times a day
			Fruit	Ulcers in mouth	Chewed or a paste is applied	Several times a day

3	*Osbeckia aspera* (L.) var. *aspera*	-	Leaf, ,	Skin problems, Diarrhea, Liver diseases Jaundice	Boil in water and use for extended wash for skin problems. Used internally in liver diseases	Several times a day. Once daily swallowed with water
			Flower ,	Tooth ache,	Chewed or a paste is applied	
			Fruit	Ulcers in mouth	Chewed or a paste is applied	Several times a day
			Root	Liver diseases Jaundice	Used internally in early morning	Once daily in early morning
4	*Osbeckia gracilis* Bedd.	-	Leaf,	Skin problems,	Boil in water and use for extended wash	Several times a day
			Flower ,	Throat infections	Boil in water and gargled	Several times a day
			Fruit	Inflammation in mouth	Chewed or a paste is applied	Several times a day
5	*Osbeckia leschenaultiana* DC.	-	Leaf,	Skin problems, Anti inflammatory	Boil in water and use for extended wash	Several times a day
			Flower ,	Anti inflammatory	Chewed or a paste is applied	Several times a day
			Fruit	Anti inflammatory	Chewed or a paste is applied	Several times a day
6	*Osbeckia reticulata* Bedd.	perum kadali	Leaf,	Skin problems, Anti inflammatory, Skin infections,	Boil in water and use for extended wash	Several times a day
			Flower ,	Ulcers in mouth	Chewed or a paste is applied	Several times a day
			Fruit,	Anti inflammatory, wound healing	Chewed or a paste is applied	Several times a day
			Bark	Skin infections,	Chewed or a paste is applied	Several times a day
7	*Osbeckia virgata* D. Don ex Wight & Arn.	-	Leaf	Skin problems, Anti inflammatory, wound healing	Boil in water and use for extended wash	Several times a day

8	*Osbeckia wynaadensis* Clarke	Vaatta kalathi	Leaf,	Anti inflammatory	Leaf extract applied in affected areas	Several times a day
			Flower ,	Skin infections	Chewed or a paste is applied	Several times a day
			Fruit	Anti-inflammatory	Chewed or a paste is applied	Several times a day
9	*Osbeckia parvifolia* Arn. Wight & Arn.	Cherukadhali	Leaf	Anti inflammatory	Leaf extract applied in affected areas	Several times a day

Table -2 Flavonoid, Phenol and Anthocyanin content of *Osbeckia* species

Sl. No.	NAME	PART USED	FLAVANOID (mg/gm)	PHENOL (mg/gm)	ANTHOCYANIN (mg/gm)
1	*Osbeckia aspera* (L.) var. *aspera*	leaf	3.82	5.07	1.085
		flower	5.58	46.66	36.57
2	*Osbeckia aspera* (L.) var. *wightiana* (Benth ex Wight & Arn.)	leaf	10.32	15.55	NIL
		flower	8.17	24.76	57.61
3	*Osbeckia aspera* (L.) var. *travancorica* (Bedd ex Gamble)	leaf	6.82	8.07	3.085
		flower	8.58	49.63	42.57
4	*Osbeckia reticulata* Bedd.	leaf	9.03	39.68	NIL
		flower	8.87	57.77	32.56
5	*Osbeckia gracilis* Bedd.	leaf	8.25	31.58	NIL
		flower	4.875	11.42	31.22
6	*Osbeckia virgata* D. Don ex Wight & Arn.	leaf	6.9	41.11	NIL
		flower	4.68	53.49	44.33
7	*Osbeckia leschenaultiana* DC.	leaf	9.47	42.06	4.5
		flower	4.17	34.92	19.2
8	*Osbeckia wynaadensis* Clarke	leaf	10.27	44.07	6.3
		flower	6.18	36.87	24.76
9	*Osbeckia parvifolia* Arn. Wight & Arn.	leaf	7.1	38.7	5.4
		flower	5.2	32.1	23.2

Discussion

Traditionally different species of *Osbeckia* are used for different medicinal purposes. Aqueous extracts of *Osbeckia octandra* and *O. aspera*, have been traditionally used for treatment of viral hepatitis by Ayurvedic practitioners in Sri Lanka (Jayaweera, 1982). These plant extracts also exhibited hepato-protective effects (Thabrew *et al*., 1995; 1998). Another species *Osbeckia chinensis* has long been used as an anti-inflammatory agent and antipyretic in China (Su *et al*., 1987, 1988). Phenolics were major antioxidants in *O. aspera* (Grayer *et al*., 2008). There were sparse reports of *O. nepalensis* for its anti-hyperglycemic activity of therapeutic compounds from aqueous and ethanolic extracts in alloxan induced diabetic rats (Devi *et al*., 2012).

O. stellata leaves was reported for its antimicrobial and antioxidant efficacy. The petroleum ether extract has better antimicrobial efficacy against most of microbes examined. Petroleum ether extract also showed strongest antioxidant activity. Differential antimicrobial or antioxidant activity of extracts against different bacteria might be due to presence of different active phytochemicals. Among those, phenolic compounds, terpenoids, and steroids are important compounds in antimicrobial or antioxidant effects. Further study is required to isolate the different active compounds from this plant to confirm their full spectrum of efficacy. This wild plant has the prospect of being a new clinically efficient source of antimicrobial or antioxidant compounds (Das and Anjeza Coku, 2013).

The leaves of most *Osbeckia* species were chewed up and applied as paste on cuts or wounds or finely chopped up and squeezed to apply the juice onto the wound to stop bleeding. Similarly, the leaves can also be used to prevent scarring from smallpox, to treat dysentery, diarrhea, and piles, and as a tonic. The young leaves were eaten to treat diarrhea while the young premature leaves were consumed raw to cure dysentery. The shoots can be ingested to treat puerperal infections, high blood pressure and diabetes while the shoots juice can also be used as a mouthwash to relieve a tooth ache or to treat leucorrhea. Other than those mentioned above, the leaves were also medicinally useful to treat ulcers, gastric ulcers, scar, pimple, and black spots on the skin. The roots can also be used as mouthwash to relieve a toothache and to treat epilepsy, given to postpartum women aid healing and womb strengthening or to alleviate rheumatism, arthritis, and tenderness in the legs. The decoction of the roots was used to treat diarrhea. In addition, the roots' decoction can be applied to lessen the soreness due to thrush in children. The barks were medicinally useful for the treatment of various skin diseases. The flowers were also used in India to treat cancer.

Other than that, the powdered leaves and roots can be applied to wounds and pox scars to aid the healing process or used to relieve the discomfort of hemorrhoids with the former also used as astringent for dysentery. The juice of leaves and roots was used as a digestive aid. Furthermore, the leaves and flowers were useful for the treatment of cholera, diarrhea, prolonged fever, dysentery, leucorrhoea, wounds, and skin diseases and for the preparation of gargles. The

decoction of roots and leaves or roots alone were also traditionally used to tone up the uterus after childbirth in order to strengthen the womb and accelerate wound healing. Women also use this herb for excessive menstrual bleeding and cramps, to relieve postmenstrual syndrome, stomach ache and white discharge, and to enhance fertility. Its flowers, seeds and leaves were used to reduce white vaginal discharge and indigestion. The flowers of most species were also used as a sedative and for hemorrhoidal bleeding. The combination of leaves and flowers were used as astringent in leucorrhea and chronic diarrhea. Despite being a traditional medicinal herb that was widely used, particularly, in Malay culture, there was not much scientific study carried out on most of the species.

Conclusion

Traditional healthcare practices of indigenous people pertaining to human health are termed as ethnomedicine. Ethnomedicine is the mother of all other systems of medicine. Recently the importance of these traditional medicines has been realized worldwide as some of them proved to be very effective. Mannan tribes use *Osbeckia* species for their health care and as colouring agent. This work also gives scope for appropriate scientific studies on the phytochemical and pharmacological activities of the recorded species for drug design.

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