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ANTHOCYANIN DIVERSITY IN *OSBECKIA* L. SPECIES FROM MUNNAR HILLS

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ABSTRACT

Melastomataceae family is known for colours. The members possess diverse polyphenolic compounds. The major constituents of this family belong to terpenoids, simple phenolics, quinones, lignans, glycosides, tannins or hydrolyzable tannin oligomers of molecular weights up to 4600 Da, and flavonoids and anthocyanins. Anthocyanin show many biological potentialities including food colourant. Therefore, the present study aims to unravel diversity of *Osbeckia*, *viz*. species along Munnar hills and also to analyze their anthocyanin content. Six species and three varieties of *O. aspera* were collected i.e., *O. gracilis*, *O. wynadensis*, *O. leschenaultiana O. aspera* var. aspera, *O. aspera* var. travancorica, *Osbseckia aspera* (L.) var. wightiana, *Osbeckia reticulata, Osbeckia virgata.* A taxonomic key was prepared for the identification of these species and the germ plasm was maintained in the garden as part of conservation. Anthocyanin content showed remarkable variations both in leaves and flowers among the species. Highest level was noticed with *Osbseckia aspera* (L.) var. wightiana and *Osbeckia reticulata.* Habitat of the plant also influences the production of anthocyanins, red or blue coloured pigments. The abiotic stress induction of anthocyanin biosynthesis has evaluated only on a small number of species. In order to meet the demand for this natural product, it's essential to evaluate anthocyanin biosynthesis in terms of their habitat. Thus, further studies are planned in terms of *in vitro* culture, elucidation of anthocyanin and its fractionation from *Osbseckia aspera* (L.) var. wightiana and *Osbeckia reticulata.*

Key words: *Osbeckia*, Munnar hills, anthocyanin, biological properties.

INTRODUCTION

Many ethno botanical herbals were used by the local people for curing various diseases or for cultural/ethnic purposes. Herbal knowledge database was usually transferred from generations to generations through their next kins but it had not been documented. This information remains mostly with the local practitioners of the particular area.

Melastomataceae are dye yielding flowering group, with approximately 174 genera and nearly 5,400 species distributed in tropical and subtropical parts of the earth. Vegetative and floral features were different among the species. Generally morphological characters have been employed for circumscribing the taxa [1].

Anthocyanins are aqueous glycosides or acylglycosides of anthocyanidins, and seen mostly as polyhydroxylated and or methoxylated heterosides which derive from the flavylium ion or 2-phenylbenzopyrilium in nature. Fruits and vegetables contain cyanidin, delphinidin, pelargonidin, peonidin, petunidin and malvidin were the most common anthocyanins. Plants defend themselves from oxidative burst due to UV exposure by synthesizing polyphenolic compounds [2]. Over UV-B radiation triggers the production of flavonoids, anthocyanin pigments in plant tissues [3]. These molecules act as UV screeners and filters absorbing and effectively minimizing the UV flux in plant tissues [4]. Enzymes

associated with synthesis of phenolics were unregulated [5].

In addition, floral colour changes in plants provide visual differentiation to pollinators and influence the visiting pollinators [6]. Pollinators use colour as marker to the availability of floral rewards. By choosing flowers based on colour cue, pollinators increase their foraging caliber. Research attempted on the chemotaxonomy and the properties of flowers during colour change were minimal.

Within the generic limits of *Osbeckia*, the colour variation was interesting. The flower colour ranges from white to pink, purple and violet. The colour diversity of flowers is due to the presence of anthocyanin pigments. Many works proved that anthocyanins have considerable potentialities as taxonomic indicators in plant categorization [7]. However, correlation between anthocyanin distribution patterns and morphological features was reported as an important contribution to grouping and identification of species.

Aims of the study

The present study involves 1. field analysis of *Osbeckia* species

2. anthocyanin content of the species

MATERIALS AND METHODS

Morphological analysis of the samples collected from Munnar hills was carried out using light microscopy and floras. The specimens were authenticated by referring the herbaria of University of Calicut and JNTBGRI, Palode. Herbaria were made and deposited in the herbarium of Department of Botany, University College, Trivandrum.

Quantification of phenols and anthocyanin

Total phenol content in the leaves and flowers of *Osbeckia spp.* was estimated by the method of Mayr *et al.*[8]. 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 [9] was used for the estimation of anthocyanin content in leaves and flowers. The absorbance was read at 510 and 700 nm against distilled water as blank.

Statistical analysis

The entire data were +/_ SD. Significance was noted $p<0.01$ or $p<0.05$ levels.

RESULTS AND DISCUSSION

Initially, morphological analysis was attempted to reveal the following features. Leaves of *Osbeckia* were elliptic to linear or lanceolate, covered with many diverse types of hairs, 3 to 8 ribbed and petiolate. Inflorescence appear as terminal panicles or cymose clusters. Pedicels 3 to 8 mm in length, calyx tube up to 8 mm with long simple to complex hairs used as marker in species discrimination.

Leaves of *O. gracilis* were 3.6 x 2.1 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.

Leaves of *O. wynadensis* 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.

Leaves of *O. leschenaultiana* were 2.5 x 1.5 cm in 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 hairs, petals upto 2.5 cm, obovate; stamens 10, filaments 6 mm, anthers 6 mm, twisted.

Leaves of *O. aspera* var. aspera were simple, opposite, 3.5-8.5 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;, 1.25-1.5 cm long; stigma curved, papillate. 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. *Osbseckia aspera* (L.) var. wightiana shows 9-10 x 3-4 cm, ovate, leaves 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.

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.

In *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 (Table 1).

Anthocyanin among the six species screened shows significantly higher values in flowers than leaves. Flowers of *O. aspera* (L.) var. wightiana showed outstanding anthocyanin level with 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 with 46.6 mg GAE / g of phenols respectively. *O. leschenaultiana* showed least levels of the polyphenols among the species analyzed (19.2 mg/g.). The level of polyphenols indirectly suggests the pharmaceutical potentialities of the species. For example anthocyanin of egg plant was proved for potent metal-chelating activity [10]. Gulcin *et al.* [11] revealed that the leaves of *Perilla frutescens* var. nankinensis displayed ideal correlation between polyphenols and antioxidant property. Of the species screened, flowers showed higher anthocyanin level compared to their leaves.

The plausible explanation for diversity of anthocyanin content in different species is that habitat of the species influence anthocyanin biosynthesis. *O. aspera* (L.) var. wightiana being a temperate species that produce high range of chemical defenses against herbivores and infections [12]. Further, *O. leschenaultiana* showed highest level of total phenols in leaves followed by *O. reticulata*. However, these two species showed relatively low profile of anthocyanin content in flowers. Gene-expression and genetic mapping among the species via cis-regulatory change at the LAR1 gene played crucial role in the evolution of pigmentation patterns among the species [13].

Generally, the functional value of leaf and flowers were different and therefore in determining their relationships related with anthocyanin will be different. Phytochemical characters usually correlate in genetic surveys satisfactorily with one or more biological characters. Harborne [14] revealed flavonoid pigments survey in 45% of the genera in *Gesneriaceae* against the classification proposed based on morphological grounds. The diversity at the subfamily category indicated a positive correlation between anthocyanin and their phytogeography. Usually, anthocyanin molecules were stable and the chromatograms resulted were highly reproducible under the same situations. Therefore, the amount of anthyocyanins in *Osbeckia* species may vary yet the qualitative aspect of it may remain relatively consistent, thus making them ideal taxonomic markers.

Phylogenetic significance of anthocyanins reveals that there is a trend for delphinidin gets replaced by cyanidin, which is considered to be the primitive anthocyanin. O-methylation seems an advanced feature when compared with simple hydroxylation reaction. The means of total anthocyanin concentration in the present study ranged from 19.2 to 57.7 mg/ g. This is in accordance with anthocyanin level in species of *Musa* [15]. However, in the present study there is no significant differences in anthocyanin content between the locations were detected.

Anthocyanins form the major fraction of the total phenolics in most flowering species for performing multiple of functions. Anthocyanin synthetic cycle is responsible for the synthesis of these pigments in plant cells and shares many enzymes. The six core structural genes of this pathway were characterized in taxonomically in maize, snapdragon glory, *Ipomoea purpurea*. They reported that upstream genes have evolved substantially more slowly than downstream genes and suggest the difference (Yuan et al., 2016) [16]. So, the present variation may be due o the up and down regulation of genes in their biosynthetic pathways.

CONCLUSIONS

Among the *Osbeckia* species screened, anthocyanin content was significantly highest in flowers of *O. aspera* (L.) var. wightiana followed by *O. virgata* and *O. aspera* var. aspera. Leaves displayed low profile.

The phylogeny of novel flower colours and patterns has played vital role in the adaptive radiation of flowering plants via their specialized interactions with different pollinators. These data could provide valuable information for the development of antioxidant pharmaceutical products. Further studies are warranted to extract, purify and fractionate the anthocyanin content and its biological characterization.

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Table 1. Identifying features of *Osbeckia spp*.

Osbeckia gracilis	Leaf 3.5 x 2 cm in size, elliptic-oblong, obtuse at apex and base, cuspidate, 3-ribbed; petiole 3 mm long. Flowers in terminal cymes, anthers 5 mm long.
Osbeckia wynadensis	Leaves are 1.8 x 3 cm in size , lanceolate, acute, 3-5-nerved, hairy; calyx tube 8 mm long, bristles 4 or 5 in a tuft, to 3 mm long; anthers 7 mm long. Seeds 0.5 x 0.3 mm obovoid, minutely muriculate.
O. leschenaultiana	Leaves of 2.5 x 1.5 cm size, elliptic-ovate, acute at apex, base rounded, densely pubescent, 3-5 nerved; stamens 10, filaments 6 mm, anthers 6 mm, twisted.
O. aspera var. aspera	Leaves were 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, anthers 5-6 mm long. Ovary hairy at apex; style exserted, 1.25-1.5 cm long; stigma curved, papillate.
O. aspera var. travancorica	Leaves were 15 x 5 cm, ovate-oblong, acuminate, 7-ribbed, bracts 1 cm across, orbicular; calyx cup 13 mm long, densely covered with stalked bristles, anthers 9 mm long, acuminate, constricted at base.
Osbseckia aspera var. wightiana	leaves 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; anthers 9 mm long, acuminate.
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, Petals 5, purple-rose, orbicular, 4 x 3 cm.
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. Stamens 10; anthers 4-5 mm long. Ovary 5-locular, with tufted stiff hairs

Table 2. Anthocyanin and Total Phenol content in leaves and flowers of *Osbeckia* spp.(mg/g.)

Osbeckia species/variety	Anthocyanin in leaf	Anthocyanin in flower	Total phenolics in leaf	Total phenolics in flower
Osbeckia aspera var. aspera	2.3	36.5	5.07	46.6
Osbeckia aspera var.whitiana	2.6	57.6	15.5	24.7
Osbeckia leschenaultiana	4.5	19.2	42.0	34.9
Osbeckia reticulata	2.1	32.5	39.6	57.7
Osbeckia gracilis	3.6	31.2	31.5	11.4
Osbeckia virgata	2.9	44.3	41.1	53.4
O. wynadensis	2.7	28.0	35.0	42.0
O. aspera var. travancorica	1.5	30.0	36.0	46.0

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