Original Research Article

Evaluation of morphological characters and phytochemical properties of *Osbeckia octandra* (L.) with available adulteration materials in Sri Lanka

.

ABSTRACT

.

Aims: *Osbeckia octandra* (L.) is a medicinal plant widely used in traditional therapeutic systems to treat liver disorders due to presence of important phytochemicals. *O. octandra* is usually adulterated due to confusion in vernacular names and lack of accurate morphological identification. Therefore, this study was conducted to investigate morphological characters and phytochemical properties of *O. octandra* with available adulterants namely *Osbeckia aspera* and *Melastoma malabathricum*.

Place and Duration of Study: The study was carried out in the Department of Plant and Molecular Biology, University of Kelaniya from 1st May 2020 to 31st January 2021.

Methodology: Morphological characterization, phytochemical screening, and thin layer chromatography (TLC) were used to distinguish these three species.

Results: *M. malabathricum* can be clearly distinguish from *O*. *octandra* and *O. aspera* using morphological characters. The results of phytochemical screening of leaf extracts have shown the presence of saponin in hexane and distilled water extracts, where phenols, flavonoids and anthocyanin in ethyl acetate, ethanol, distilled water and boiled distilled water extracts for all species. *M. malabathricum* was clearly separated from *O. octandra* and *O. aspera* in TLC profile of ethanol extracts in solvent systems of Hexane (HE): Ethyl acetate (EA); 9:1, HE: EA; 13:7, HE: EA: Ethanol (ET); 6:3:1 and HE: EA: ET; 15:3:2 under visible light. TLC profiles of ethanol leaf extracts in solvent system HE: EA; 13:7 and HE: EA:ET; 15:3:2, and TLC profile of ethyl acetate in solvent system HE: EA; 13:7 have also revealed a clear difference between the phytochemical compositions of *O. octandra*, *O. aspera* and *M. malabathricum* under UV light (365nm).

Conclusion: Adulteration of *O. octandra* from *O. aspera* and *M. malabathricum* can be identified using morphological characters and TLC profiles.

Keywords: Osbeckia octandra, Osbeckia aspera, Melastoma malabathricum, Morphology

TLC

1. INTRODUCTION

Medicinal plants have been extensively used in worldwide ethnomedicine since ancient times due to the presence of wide range of phytochemicals, such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, volatile oils, etc. These phytochemicals obtained from herbal plants are considered as major sources of therapeutically valuable compounds. Therefore, approximately fifty percent of the approved drugs are directly or indirectly produced using herbal plants at present. [1] The therapeutically valuable compound of these herbal plants can be extracted from leaves, roots, flowers, bark, etc. and used either directly or as a decoction, tincture, or mixed with other ingredients to treat diseases.

Osbeckia octandra (L.) DC belongs to family Melastomataceae known as "Vern: Heen bovitiya" is an endemic plant to Sri Lanka widely used in traditional medicine. [2,3] This plant species is distributed in dry, wet, and montane zones in the country. [3] Leaves, bark, and roots of this plant are used to treat diabetes mellitus, hemorrhoids, hepatitis, ascites, liver disorders, jaundice, and hyperlipidemia in Ayurveda. [4]

One of the major drawbacks confronted with the use of *O. octandra* in traditional therapeutic system is adulteration. *O. octandra* can be adulterated with plant materials that resemble morphological, chemical, and therapeutical characteristics of the authentic plant. Confusion in vernacular names, lack of knowledge about authentic plant species, incorrect taxonomical identification, similarity in morphology, lack of authentic plant due to extinction and deforestation, the need for a continuous supply of raw materials for medicinal preparations, and careless collection are identified as major reasons for the adulteration of *O. octandra*. According to reported information, *Osbeckia aspera* referred as "Bovitiya" [2,3] belong to the same family is found in Sri Lanka and used in traditional medicine for the alleviation of jaundice related liver diseases. [5] *Melastoma malabathricum* known as "Maha bovitiya", or "Bovitiya" by the local population [2,3] is also used in traditional medicine. [6] Both *O. aspera* and *M. malabathricum* tend to get misidentified as *O. octandra* in the general public, due to their similar vernacular names and morphological characteristics. Therefore, phenotypic characterization and evaluation of the phytochemical properties of *O. octandra* with adulteration materials is a timely requirement because of the increasing popularity of "Heen bovitiya" as an ingredient in traditional therapeutic systems.

2. MATERIAL AND METHODS

2.1 Collection of information about *O. octandra* (Vern: Heen bovitiya) and available adulterants

Collection of information about *O. octandra* (Vern: Heen bovitiya) was carried out using online survey and interviews.

2.2 Collection of specimens and morphological data

Fresh plant materials were collected from Polgahawela in Kurunegala district (7.3275° N, 80.2935° E) and Minuwangamuwa in Kegalle district (7.3275° N, 80.2935° E) Sri Lanka. Morphological characters of fresh flower, leaf, fruit, and stem of *O. octandra*, *O. aspera* and *M. malabathricum* were observed and recorded using ten samples of each.

2.3 Preparation of leaf extracts

Air dried leaf samples of each specimen were ground to obtain fine particles. Three grams of each powdered leaf samples were soaked in 30.0 mL of hexane (HE), ethyl acetate (EA), ethanol (ET) and distilled water (DW) separately. Another three grams of each sample were dissolved in 60.0 mL of distilled water and boiled at 100 ^{0}C for 10 minutes (BDW). Then, all samples were kept 24 hours at room temperature with occasional stirring. The extracts were filtered using Whatman no 01-filter paper and stored in 4 ^{0}C for further analysis. [7]

2.4 Phytochemical Screening

Preliminary phytochemical screening of leaf extracts of *O. octandra*, *O. aspera* and *M. malabathricum* was carried out to detect the presence of saponin, phenols, flavonoids and anthocyanin. [8]

2.5 Thin layer chromatography profiles

TLC studies were carried out to screen phytochemicals present in hexane, ethyl acetate and ethanol leaf extracts of *O. octandra*, *O. aspera* and *M. malabathricum*. TLC studies were done using silica TLC-grade; Merck Gel-G plates. Seven solvent systems were used in the study (Table. 01). [7]

Table 01. Composition of different solvent systems

Solvent System	Composition		
	Hexane (mL)	Ethyl acetate (mL)	Ethanol (mL)
HE: EA; 9:1	18.0	2.0	0.0
HE: EA; 17:3	17.0	3.0	0.0
HE: EA; 4:1	12.0	3.0	0.0
HE: EA; 13:7	13.0	7.0	0.0
HE: EA:ET; 18:1:1	18.0	1.0	1.0
HE: EA:ET; 15:3:2	15.0	3.0	2.0
HE: EA:ET; 6:3:1	12.0	6.0	2.0

2.6 Visualization of chromatograms

Developed chromatograms were visualized under visible light and long-length ultraviolet light (365 nm). The retention factor (R_f) values of the developed spots on TLC plates were calculated by using following formula under the two different lightning conditions.

$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by the solutes from the baseline}}{\text{Distance travelled by the solvents from the baseline}}$$

2.7 Analysis of data

Cluster analysis of morphological characters of *O. aspera, O. octandra* and *M. malabathricum* was carried out using single linkage algorithm and Euclidean similarity measure in PAST software version 2.17. Twenty qualitative characters and nine quantitative characters were considered for morphological analysis in the selected three species.

3. RESULTS AND DISCUSSION

3.1 Results of online survey on "Identification of medicinal uses and available adulteration materials of *O. octandra* (Vern: Heen bovitiya) in Sri Lanka".

According to the results of the survey, majority of general public can identify medicinal plants for some extent only (81.9%). Based on the responses for the identification of a photograph of *O. octandra* plant, 30.1% of respondents stated the plant as "Bovitiya", while 25.3% was capable of identifying the herb precisely. However, 44.6% stated that they are unable to identify the plant properly. According to survey results, *O. aspera* and *M. malabathricum* were selected as possible adulterants because of the similar vernacular names and morphological characters compared to *O. octandra*.

3.2 Qualitative and quantitative morphological characterization

Morphological characterization of twenty qualitative characters and nine quantitative characters of selected three species are summarized in Table 02 and Table 03, respectively. According to the results, *O. octandra* and *O. aspera* share similar qualitative characters compared to *M. malabathricum* (Fig.1). Therefore, *M. malabathricum* can be clearly distinguished from *O. octandra* and *O. aspera* using both qualitative and quantitative morphological characters. A close relationship was observed between *O. octandra* and *O. aspera* according to the cluster analysis of qualitative and quantitative morphological characters (Fig. 2).

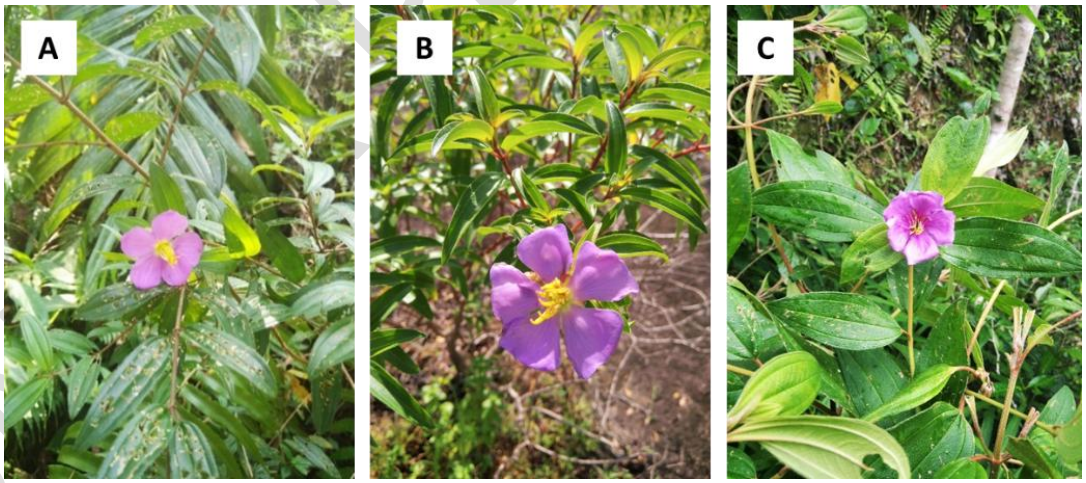

Fig. 1. Habit of A). *O. octandra,* B) *O. aspera* and C). *M. malabathricum*

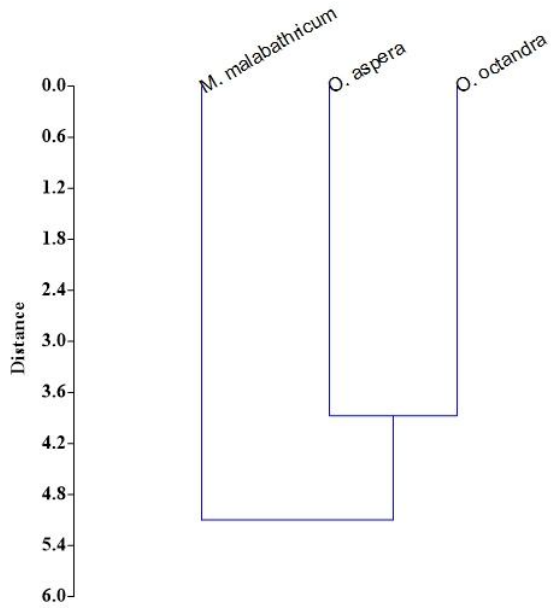

Fig. 2. Cluster analysis of qualitative and quantitative morphological characters *O. octandra, O. aspera* and *M. malabathricum*

Table 02. Qualitative morphological character comparison of *O. octandra, O. aspera*, and *M. malabathricum*

Character	***O. octandra***	***O. aspera***	***M. malabathricum***
Habit	Shrub	Shrub	Shrub
Hairs on branchlets	Less pubescent with appressed to spreading hair	Less pubescent with appressed to spreading hair	Absent
Scales on branchlets	Absent	Absent	Present
Shape of leaf	Elliptic	Elliptic to elliptic ovate	Elliptic
Shape of leaf base	Rounded to acute	Rounded to acute	Acute to obtuse
Shape of leaf apex	Acute	Acute to acuminate	Acute or shortly acuminate
Leaf margin	Entire	Entire	Entire
Arrangement of leaves	Opposite	Whorled	Alternate
Hairs on leaf	Present	Present	Present
Scales on leaf	Absent	Absent	Present
Vain pattern	3-nerved	3-5 nerved	5 nerved
Flower arrangement	Few in loose clusters	Few in dense clusters	Few in dense clusters
Color of petals	Pink to mauve and violet	Pink to mauve and violet	Pink to mauve and violet
Shape of sepals	Triangular	Triangular to ovate	Ovate-triangular

Shape of anthers	Narrowly ovate	Narrowly ovate	Narrowly ovate
Arrangement of anthers	Twisted	Twisted	Basally projected connectivity
Color of stamens	Yellow	Yellow	Pink
Fruit type	Dry capsule	Dry capsule	Ovoid fleshy capsule
Scales on fruit	Absent	Absent	Present
Number of seeds present in the fruit	Numerous	Numerous	Numerous

Table 03. Quantitative morphological character comparison of *O. octandra, O. aspera*, and *M. malabathricum.*

Character	*O. octandra*	*O. aspera*	*M. malabathricum*
Height (cm)	120.33±0.67	150.0±0.58	213.10±1.36
Width of leaf (mm)	3.41±0.10	9.33±0.26	52.47±0.38
Length of leaf (mm)	8.17±0.18	32.60±0.38	118.57±0.43
Length of petiole (mm)	2.54±0.07	4.10±0.12	11.63±0.09
Width of hypanthium (mm)	2.03±0.03	4.30±0.06	6.27±0.03
Number of petals	5.00±0.00	5.00±0.00	5.00±0.00
Length of Petal (mm)	18.07±0.09	20.07±0.09	23.23±0.29
Length of anthers (mm)	8.33±0.09	9.13±0.09	8.90±0.12
Number of anthers	11.00±0.00	10.00±0.00	10.00±0.00

3.3 Phytochemical screening of *O. octandra, O. aspera* and *M. malabathricum*

Presence of phytochemicals in the different leaf extracts of *O. octandra, O. aspera* and *M. malabathricum* were recorded in the Table 04.

Table 04. Phytochemical Screening of different leaf extracts of *O. octandra*, *O. aspera* and *M. Malabathricum*

Test	*O. octandra*					*O. aspera*					*M. malabathricum*				
	HE	EA	ET	DW	BDW	HE	EA	ET	DW	BDW	HE	EA	ET	DW	BDW
Saponins	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-
Phenols	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Flavonoids	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Anthocyanin	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+

** "+" indicates presence and "-" indicates absence. (HE= Hexane, EA= Ethyl acetate, ET= Ethanol, DW= Distilled Water and BDW= Boiled Distilled Water*.

3.4 TLC profiles of *O. octandra*, *O. aspera* and *M. malabathricum*

Considerable difference among R_f values under visible light were observed in ethanol leaf extracts of *O. octandra, O. aspera* and *M. malabathricum* developed in HE: EA; 9:1, HE: EA; 13:7, HE: EA:ET; 6:3:1 and HE: EA:ET; 15:3:2 solvent systems (Table. 05). And also, significant differences among the R_f values under UV light (365 nm) were seen for ethanol and ethyl acetate leaf extracts of *O. octandra, O. aspera* and *M. malabathricum* developed in HE: EA; 13:7 and HE: EA:ET; 15:3:2 (Table. 06).

Table 05. R_f values for ethanol extracts of *O. octandra*, *O. aspera* and *M. malabathricum* visualized under visible light

Extract	Solvent System	*O. octandra*	*O. aspera*	*M. malabathricum*
Ethanol	HE: EA; 9:1	0.19, 0.34, 0.98	0.19, 0.34, 0.98	0.11, 0.19, 0.34, 0.98
	HE: EA; 13:7	0.38, 0.75, 0.87, 0.96	0.38, 0.75, 0.87, 0.96	0.38, 0.44, 0.75, 0.87, 0.96
	HE: EA:ET; 15:3:2	0.27, 0.50, 0.64, 0.94	0.27, 0.5, 0.64, 0.94	0.27, 0.43, 0.31, 0.5, 0.64, 0.94
	HE: EA:ET; 6:3:1	0.55, 0.75, 0.85, 0.93	0.55, 0.75, 0.85, 0.93	0.55, 0.69, 0.75, 0.85, 0.93

Table 06. R_f values for ethanol and ethyl acetate extracts of *O. octandra*, *O. aspera* and *M. malabathricum* visualized under ultraviolet light (365 nm)

Extract	Solvent System	*O. octandra*	*O. aspera*	*M. malabathricum*
Ethanol	HE: EA; 13:7	0.64, 0.70, 0.75, 0.83	0.24,0.55, 0.64, 0.70,0.75, 0.83	0.43, 0.49, 0.55, 0.64, 0.70, 0.75, 0.83
	HE: EA:ET; 15:3:2	0.32, 0.42, 0.56, 0.60	0.17, 0.42, 0.45, 0.56, 0.60	0.26, 0.32, 0.36, 0.42, 0.45, 0.56, 0.60
Ethyl acetate	HE: EA; 13:7	0.56, 0.58, 0.63, 0.73	0.16, 0.56, 0.58, 0.63, 0.73	0.37, 0.56, 0.58, 0.63, 0.73

4. DISCUSSION

Most of the people (44.58 %) failed to identify *O. octandra,* while nearly 30.0% of the people have identified *O. octandra* as “Bovitiya’’. Only 25. 3% stated this plant as “Heen bovitiya”. Therefore, majority of the general public is familiar with the vernacular name “Bovitiya” for this plant. Vernacular name “Bovitiya” is used for *O. octandra*, *O. aspera* and *M. malabathricum* by the local community. [3] Having same vernacular name for different species and different vernacular names for same species create confusion and invite adulteration. [9]

According to the qualitative and quantitative morphological characterization of this study, *M. malabathricum* can be clearly identified and distinguished from the other two species using leaf and flower characters. Similar morphological characters for this plant were also recorded in previous studies. [10] *O. octandra* and *O. aspera* can be clearly distinguished from each other using shape of leaf and leaf apex, shape of sepals, arrangement of leaves, vain patterns, height of plant, width of leaf, length of leaf, length of petiole, width of hypanthium, length of petals and length of anthers. In addition to that, more prominent hairs on both sides of the leaves were observed in *O. octandra* compared to *O. aspera*. This study is in accordance with the study of morphological and anatomical characterization of *Osbeckia* species. [11] According to the cluster analysis of qualitative and quantitative traits *O. octandra*, *O. aspera* and *M. malabathricum* grouped into two clusters. It reveals, *O. octandra* and *O. aspera* are more closely related compared to *M. malabathricum* as both species

belong to the same genus. [3] Morphological characterization studies were carried out to identify varieties of Oats and Brazilian *Theobroma* L. species. [12, 13]

These three species have similar phytochemical profiles according to the preliminary screening used in the study. Water extract of all three species have shown better extraction potential as it shows the presence of all phytochemicals considered in the study. Ethyl acetate, ethanol and boiled distilled water extracts shows the presence of more phytochemicals compared to hexane. [7, 14, 15,16, 17, 18]. In the present study, saponins were also extracted in the hexane extracts apart from water extracts. Similar observations were reported for petroleum ether leaf extract of *Acrostichum aureum* and hexane extracts of *Guiera senegalensis* (Egyptian mimosa). [19, 20]

TLC profiles of present study have shown that ethanol and ethyl acetate leaf extracts have revealed a large array of phytochemicals present in the leaves of *O. octandra*, *O. aspera* and *M. malabathricum*. Ethanol leaf extracts of *O. octandra*, *O. aspera* and *M. malabathricum* developed in the solvent systems of HE: EA; 9:1, HE: EA; 13:7, HE: EA:ET; 6:3:1 and HE: EA:ET; 15:3:2 have resulted separation of different phytochemicals with different R_f values observable under visible light. And also, TLC profiles of ethanol leaf extracts in solvent system HE: EA; 13:7 and HE: EA:ET; 15:3:2, and TLC profile of ethyl acetate in solvent system HE: EA; 13:7 observed under UV light also shows a wide range of phytochemicals. According to the results highest number of spots and clear separation were observed in ethanol leaf extracts of solvent system HE: EA; 13:7. TLC profiles also provide an idea about the polarity of various chemical constituents. [21] Therefore, this combination of extract and solvent system can be used for better phytochemical extraction and separation in the selected species.

The ethanol TLC profiles in this study can be used to distinguish *M. malabathricum* from both *O. octandra* and *O. aspera* as it shows unique R_f values for *M. malabathricum* in visible light. [7] TLC profiles of ethanol leaf extracts in solvent system HE: EA; 13:7 and HE: EA:ET; 15:3:2, and TLC profile of ethyl acetate in solvent system HE: EA; 13:7 observed under UV light also show unique spots for *O. aspera* and *M. malabathricum*. Therefore, these profiles can be used as a basic tool to distinguish *O. aspera* and *M. malabathricum* from *O. octandra*. TLC profiles have served as a characteristic fingerprint for leaf and root extracts of *Hypochaeris radicata*. [22]

5. CONCLUSIONS

O. aspera and *M. malabathricum* are identified as the available adulteration materials due to confusion in vernacular name used by the general public and similar morphological characteristics. Practitioners in traditional medicine can use qualitative and quantitative morphological characterization in this study to identify *O. octandra*, *O. aspera* and *M. malabathricum* when preparing herbal medications. Leaf extracts of *O. octandra*, *O. aspera* and *M. malabathricum* contain saponin, phenols, flavonoids, and anthocyanins. TLC profile of ethanol leaf extracts in solvent system HE: EA; 13:7 and HE: EA:ET; 15:3:2, and TLC profile of ethyl acetate in solvent system HE: EA; 13:7 observed under UV light (365 nm) can be used to distinguish phytochemical compositions of *O. octandra*, *O. aspera* and *M. malabathricum.* The study highlights the efficacy of " herbal plants " which is an ancient tradition, used in South Asia. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

REFERENCES

1. Chen GT, Lu Y, Yang M, Li JL, Fan BY. Medicinal uses, pharmacology, and phytochemistry of Convolvulaceae plants with central nervous system efficacies: A systematic review. Phytotherapy Research, 2018; 32(05): 823–864.
2. Senarathne L. A Check List of the Flowering Plants of Sri Lanka.1st ed. Colombo; National Science Foundation: 2001, 213-218.
3. Dassanayake MD, Fosberg FR. Revised Handbook to the Flora of Ceylon (VI). N. Delhi:1996,162-168.
4. Wijerathne C, Siriweera E, Jayasena HMCK, Wijayagunawardane MPB. Hepatoprotective Effect of *Osbeckia octandra* (Heen Bowitiya) Against CCl_4 Induced Chronic Liver Damage in ICR Mice. Proceedings of the 1st Faculty of Agriculture Undergraduate Research Symposium, Faculty of Agriculture, University of Peradeniya, vol. 01, 2014;110.
5. Thabrew, M, Jayatilaka K. A comparative study of the beneficial effects of *Osbeckia octandra* and *Osbeckia aspera* in liver dysfunction in rats. Ceylon Journal of Medical Science, 1999; 42(1):1.
6. Mamat S, Kamarolzaman M, Yahya F, Mahmood N, Shahril M, Jakius K, Mohtarrudin, N, Ching S, Susanti D, Taher M, Zakaria Z. Methanol extract *of Melastoma malabathricum* leaves exerted antioxidant and liver protective activity in rats. BMC Complementary and Alternative Medicine, 2013; 13(1): 326.
7. Nath D, Choudhury M, Mazumder P, Mitra A. Phytochemical profiling of *Melastoma malabathricum* Linnaeus (Melastomataceae): an ethnomedicinally important plant of Eastern Himalaya. Pleione, 2014; 8(2): 478 - 485.

8. Harborne J. Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis, 1st ed., New York; Chapman and Hall in association with Methuen Inc: 1973,4-119.
9. Kumar JS, Krishna V, Seethapathy GS, Ganesan R., Ravikanth G, Shaanker R.U. Assessment of adulteration in raw herbal trade of important medicinal plants of India using DNA barcoding. 3 Biotech, 2018; 8 (135): 1–8.
10. Wu R, Zou P, Tan G, Hu Z, Wang Y, Ning Z, Wu W, Liu Y, He S, Zhou R. Molecular identification of natural hybridization between *Melastoma malabathricum* and *Melastoama beccarianum i*n Sarawak, Malaysia. Ecology and Evolution, 2019; 9(10): 5766-5776.
11. Lawarence B, Murugan K. Comparison of Analgesic and Anti- Inflammatory Activities of Purified Anthocyanin from *Osbeckia aspera* (L.) Blume and *Osbeckia reticulata* Bedd. Using Animal models. Asian Journal of Pharmaceutical and Clinical Research, 2019; 12(1):106.
12. Sumathi S, Balamurugan P. Usefulness of morphological characters for varietal identification in oats (*Avena sativa* L.). International Journal of Plant Sciences, 2014; 9: 7-12.
13. Santos RC, Pires JL, Correa RX. Morphological characterization of leaf, flower, fruit and seed traits among Brazilian *Theobroma* L. species. Genetic Resources and Crop Evolution, 2012; 59: 327-345.
14. Akter K., Barnes E, Brophy J, Harrington D, Community Elders Y, Vemulpad S, Jamie J. Phytochemical Profile and Antibacterial and Antioxidant Activities of Medicinal Plants Used by Aboriginal People of New South Wales, Australia. Evidence-Based Complementary and Alternative Medicine, 2016; 1-14.
15. Prasadani M, Bogahawaththa S, Illeperuma R, Kodithuwakku S. Leaf Extract of *Osbeckia octandra* L. (Heen Bovitiya) Suppresses Human Oral Squamous Cell Carcinoma Cells Migration and Induces Cellular DNA Damage. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology, 2021; 33(2): 215-220.
16. Lawarence B, Murugan K. Comprehensive Evaluation of Antioxidant Potential of Selected *Osbeckia* species and their *in vitro* Culture, Purification and Fractionation. Pharmacognosy Journal, 2017; 9(5): 674-682.
17. Grayer R, Thabrew M, Hughes R, Bretherton S, Lever A, Veitch N, Kite G, Lelli R, Simmonds M. Phenolic and Terpenoid Constituents from the Sri Lankan Medicinal Plant *Osbeckia aspera*. Pharmaceutical Biology, 2008; 46(3): 154-161.
18. Giri D, Rajbhandari M. Phytochemical Analysis and Constituents of Hexane Extract of *Melastoma malabathricum* L. Journal of Institute of Science and Technology, 2018; 23(1): 18-25.
19. Raja S, Ravindranadh K. Preliminary phytochemical screening of different solvent extracts of whole plant of *Acrostichum aureum*, World Journal of Pharmaceutical Sciences, 2014; 2 (12): 1753-1759.
20. Abubakar N, Shehu K, Yahaya M, Tafinta Y, Imonikhe M. Phytochemical Screening and Thin Layer Chromatographic Studies of *Guiera senegalensis* G.F Gmel (Egyptian mimosa). Annals of Biological Sciences, 2016; 4(1): 26-30.
21. Biradar SR, Rachetti BD. Extraction of some secondary metabolites & thin layer chromatography from different parts of *Centella asiatica* L. (URB). American Journal of Life Sciences, 2013; 1: 243-247.
22. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for in vitro antioxidant activities. Asian Pacific Journal of Tropical Biomedicine, 2014; 4(1): 359–3