**ASIAN JOURNAL OF PHARMACOGNOSY**
Asian Journal of Pharmacognosy 5(3): 36-43
eISSN-0128-1119

Accepted September 19, 2021

# Phytochemical evaluation, *in-vitro* radical scavenging and antioxidant activities of aqueous leaf extract of Heen Bovitiya (*Osbeckia octandra* L. (DC.) grown in Sri Lanka

Sujeewa Krishanthi Hettihewa* and Pilippenge Dinithi Sanketha Ananda Silva

Department of Pharmacy, Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka.

*For correspondence: krishanthi2001@yahoo.com

***Abstract:*** *Osbeckia octandra* L. (DC.) is an endemic plant which belongs to the family *Melastomataceae* and highly used in Sri Lankan Ayurvedic medicine. The aim of the present study was to evaluate total phenolic (TP), flavonoid (TF) contents, *in-vitro* radical scavenging activity and antioxidant activity of aqueous extract obtained from *Osbeckia octandra* L. (DC.) (*Heen bovitiya*) leaves. The crude aqueous extract was prepared by soaking method in the dark conditions from oven dried Heen bovitiya leaves collected from Galle District in Sri Lanka. The freeze dried powder of the crude extract was obtained and subjected to preliminary phytochemical tests. TP and TF contents of extract were determined by Folin-Ciocalteu assay and Aluminum chloride colorimetric method respectively. *In-vitro* antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Results of the preliminary phytochemical screening exhibited the presence of phenolic compounds, flavonoids, tannins, terpenoids, phytosterols, saponins, alkaloids, carbohydrates, proteins and amino acids. TP and TF contents of aqueous extract were calculated as 5356.501±59.552 mg Gallic acid equivalents (GAE)/100 g dry weight (DW) of leaves and 2004.514±2.206 mg Catechin equivalents (CAE)/100 g DW of leaves. Radical scavenging and antioxidant capacities for the aqueous extract were 11.279±0.516 mmol Trolox equivalents/100 g DW and 33.366±0.674 mmol Fe(II) equivalents/100 g DW of leaves. Results revealed that *O. octandra* leaves have a high TP and TF content which is exhibited through its promising antioxidant activity. Hence, it was concluded that *O. octandra* is a medicinally important herb with remarkable antioxidant activity which could be used to address pathological states related to oxidative stress.

***Keywords:*** Antioxidant; Total flavonoid; Total phenolic; *Osbeckia octandra* L. (DC.)

**ASIAN JOURNAL OF PHARMACOGNOSY**
Asian Journal of Pharmacognosy 5(3): 36-43
eISSN-0128-1119

## INTRODUCTION

Antioxidants are compounds that protect cells from oxidative damage caused by reactive oxygen and nitrogen species which can either delay or inhibit the oxidation of oxidizable substrates, if available in low concentration than the substrates (Hettihewa, 2014). Reactive oxygen and nitrogen species (ROS and RNS) are free radicals formed including; singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxy nitrile during normal aerobic metabolism. Free radicals oxidize cellular components and damage the cellular constituents hence, causing chronic diseases such as; cancer, diabetes mellitus, atherosclerosis, cirrhosis, arthritis, cataracts, brain dysfunction and other aging diseases (Jayasri *et al.*, 2009; Bogahawaththa *et al.*, 2021; Wasana *et al.*, 2021). Though there are many defense mechanisms (endogenous antioxidants) to limit the amount of reactive oxidants in the body, they are insufficient to remove all the free radicals formed in the body which accumulates oxidative stress. It is advisable to consume exogenous antioxidants to remove the excess free radicals from the body to maintain the balance between the pro-oxidants formed and the antioxidants (Hettihewa, 2014). The current trend is to consume exogenous antioxidants through natural sources (fruits and vegetables) or drugs than the intake of synthetic antioxidants to restore the balance antioxidants due to the less or no toxicity and mutagenicity (Jayasri *et al.*, 2009).

Plants contain a variety of secondary metabolites which are biologically active, naturally occurring and providing health benefits for humans. Phytochemicals can act as antioxidants by scavenging the free radicals, thus have a therapeutic potential for free radicals associated disorders (Hausladen and Stamer, 2009; Wasana *et al.*, 2021; Bogahawaththa *et al.*, 2021). Naturally occurring phenolic compounds, flavonoids, vitamins and carotenoids are the major contributors to antioxidant potential in plants (Firuzi *et al.*, 2005; Thaipong *et al.*, 2006; Hettihewa, 2014). There is a long history of plant extracts being used as a treatment for many ailments; since they have many medicinal properties such as antioxidant, anti-tumour, anti-mutagenic, anti-inflammatory, anticancer, and antimicrobial properties (Das and Coku, 2013).

*Osbeckia octandra* L. (DC.) or *Melastoma octandra* belongs to the family *Melastomataceae,* is a rare, endemic plant which is widely used in Traditional and Ayurveda medicine systems. It is commonly called as Ayurveda bush-tree, *Heen Bovitiya* in Sanskrit/ Sinhala, *Kathtoo mukhtohulai* in Tamil and Eight Stamen *Osbeckia* in English. It is an erect more branched shrublet which is about 2 m in height with 4-angled stems covered with strigose or velutinous hairs. Leaves are 1.5-6 cm in size. They are opposite, entire, strigose or subcoriaceous in shape and have 3-5 nerves (Ediriweera and Ratnasooriya, 2009) (**Figure 1**). It is used to treat type -2 diabetes mellitus, viral hepatitis, jaundice and other hepatic disorders (Balasooriya*et al.*, 2020). This plant is well-known to have antidiabetic, anticancer, antimicrobial, anti-inflammatory, anti-mutagenic, anti-clastogenic, anti-aggregant, antispasmodic, anti-anaemic, analgesic, hepatoprotective, anesthetic and immune-stimulant properties (Thabrew and Jayatilaka, 1999; Perera *et al*, 2013).

Many medicinal plants which are used in Ayurveda and Traditional medicine systems are not reported to have proper and adequate information about their composition, active principles available or mechanisms of action (Jayasri *et al*, 2009). Only few of the plants have been validated (Bnouham *et al.*, 2006). Furthermore, most of the natural sources are rich in antioxidants in which their contents are not fully analyzed due to the vast varieties of compounds showing antioxidant properties (Pisoschi and Negulescu, 2011). Therefore, the present study was aimed at the determination of

**ASIAN JOURNAL OF PHARMACOGNOSY**
Asian Journal of Pharmacognosy 5(3): 36-43
eISSN-0128-1119

phytochemical profiles, evaluation of total phenolic, flavonoid contents and *in-vitro* antioxidant activity of aqueous extract obtained from *O. octandra* L. (DC.) (*Heen Bovitya*) leaves as there are a few numbers of studies have been carried out in Sri Lanka.

**Figure 1**: *Octandra* plant and the flower

## MATERIALS AND METHODS

The fresh leaves of matured *O. octandra* without any insect or microbial attacks were collected in three batches from Galle District (geographical coordinates; latitude:6.053519; Longitude:80.220978), Southern Province, Sri Lanka during the period from November 2019 to January 2020 and authenticated from National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. This study was conducted using the chemicals of Folin-Ciocalteu phenol reagent, hydrochloric acid, catechin, sodium carbonate, sodium hydroxide, sodium nitrite, aluminium chloride, gallic acid, 2-2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), trolox, sulphuric acid, nitric acid, sodium hydroxide, ferric chloride, lead acetate, ammonia, benzene, copper acetate, chloroform, acetic anhydride, sodium carbonate, ethanol, sodium nitrite, ferrus sulphate, sodium phosphate, starch, n-hexane of analytical grade were purchased from Sigma Aldrich local agencies in Sri Lanka. Mayer's reagent, Wagner's reagent,

Fehling's A, Fehling's B, Dragendroff's reagent, Ninhydrin reagent, Benedict's reagent were prepared by using the chemicals of routine laboratory work.

*Preparation of plant materials:* Leaves were rinsed with distilled water to remove dirt and dust, and the weight was recorded. The leaves were oven dried until a constant weight was obtained at a temperature below 45 ºC and powdered using the grinder (SISIL, Mixer, Grinder 550W) (Zakaria *et al.*, 2011).

*Preparation of crude extract:* Extraction was performed by following the methods published (Zakaria *et al.*, 2011; Hettihewa, 2014) with slight modifications. Briefly, the powdered dried leaves (20.00 g) were soaked in distilled water (150.0 mL) in a Schott Duran bottle, keeping in the shaker (GEMMYCO shaker, Lab shaking incubator, model:IN-666) for 24 hours at room temperature (30±2 $^0$C). The water extract was obtained after filtration through three layers of muslin cloth and filtrate was subjected to liquid-liquid partition with hexane to remove chlorophylls. The defatted crude extract was concentrated by rotary evaporator (HAHNVAPOR, HS-20055) at 40 ºC and was freeze-dried (BK-FD 10PT) to store in the freezer (Haier Deep, Germany) (Hettihewa, 2014; Johari and Khong, 2019).

*Preliminary phytochemical screening:* The freeze-dried powder of the crude extract was obtained and subjected to preliminary phytochemical screening tests to screen for the phytoconstituents available in the aqueous extract including; alkaloids, phenolic compounds, tannins, flavonoids, steroids, phytosterols, terpenoids, diterpenes, glycosides, saponins, carbohydrates, reducing sugars, proteins and amino acids (Visweswari *et al.*, 2013; Keo *et al.*, 2017).

*Determination of TP, TF contents and in-vitro antioxidant activity:* Total phenolic (TP) content of the aqueous defatted crude extract was evaluated by Folin-Ciocalteu assay and expressed as mg Gallic acid equivalent (GAE)/100 g dry weight (DW) of the leaves. Total flavonoid content (TF) was evaluated by Aluminium chloride method and the results were expressed as mg Catechin equivalent (CAE)/100 g DW of the leaves. *In-vitro* radical scavenging activity and antioxidant activity were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays respectively (Hettihewa, 2014).

*Determination of TP content:* Distilled water (7.90 mL), sample (0.01 mL) and 0.20 N Folin-Cioclateu reagent (0.50 mL; 1:1 with water) were mixed and allowed to stand. Sodium carbonate (1.50 mL; 20.00 g DW/ 100.0 mL) was added after one minute and vortexed. The mixture was allowed to stand in dark at room temperature for 2 hours. The absorbance was measured at wavelength, 765 nm using SHIMADZU UV-1800 double beam spectrophotometer. The mixture of reagent and distilled water were used as the blank sample. The standard Gallic acid calibration curve was prepared and TP content was determined as mg Gallic acid equivalents (GAE) to 100 g dry weight (DW) of leaves.

*Determination of TF content:* The defatted crude extract (0.03 mL), 30% ethanol (3.40 mL), 0.50 M $NaNO_2$ (0.15 mL) and 0.30 M $AlCl_3.6H_2O$ (0.15 mL) were mixed and NaOH (1 M; 1.00 mL) was added after 5 minutes. Absorbance was measured at wavelength 506 nm using SHIMADZU UV-1800 double beam spectrophotometer. The standard Catechin calibration curve was prepared and TF content was determined as mg Catechin equivalents (CAE) to 100 g DW of leaves.

**ASIAN JOURNAL OF PHARMACOGNOSY**
Asian Journal of Pharmacognosy 5(3): 36-43
eISSN-0128-1119

*In-vitro radical scavenging activity by DPPH Assay:* DPPH stock solution ($6.25 \times 10^{-5}$ M) was prepared by dissolving DPPH (2.40 mg) in methanol (100.0 mL) and stored at -20 ºC. The sample (0.025 mL) was added to DPPH solution (2.00 mL) and incubated in dark for 30 minutes. The blank was prepared containing the same volume of solvent in the place of test sample. Absorbance was measured at wavelength 517 nm using SHIMADZU UV-1800 double beam spectrophotometer with reference to methanol to determine the concentration of remaining DPPH. Results were expressed as mmol Trolox equivalents (TE) to 100 g DW of leaves.

*In-vitro antioxidant activity by FRAP Assay:* FRAP reagent was prepared by mixing 100.0 mL of 0.30 M sodium acetate buffer (pH=3.6), 10.00 mL 0.02 M ferric chloride, 10.00 mL of 0.01 M 2,4,6-tripyridyl-s-triazine in 0.04 M HCl and 12.00 mL of distilled water. Reagent was warmed at 30 ºC for 2 hours before use. FRAP reagent (2000.0 µL) was mixed with sample (60 µL), vortexed and incubated at 30 ºC for 5-10 minutes. Absorbance was measured at wavelength 593 nm using SHIMADZU UV-1800 double beam spectrophotometer. Blank was prepared with FRAP reagent (2000.00 µL) and distilled water (60.00 µL). Standard $FeSO_4$ calibration curve was prepared and antioxidant content was determined as mg Fe(II) equivalents (FE) to 100 g DW of leaves.

*Statistical analysis:* All experimental measurements were conducted in triplicate and the results were expressed as mean ± standard deviation. Statistical Package for Social Sciences (SPSS) 25 was used to analyze the results at 95% confidence interval (Sathiyawelu *et al.*, 2013).

## RESULTS

The average freeze dried yield of the aqueous extract obtained from the *Heen bovitiya* leaves was 12.25±1.061%. The qualitative phytochemical analysis revealed that the phytoconstituents including; phenolic compounds, flavonoids, tannins, diterpenes, terpenoids, phytosterols, saponins, alkaloids, carbohydrates, proteins and amino acids were present in the aqueous extract of *O. octandra* leaves. TP content of the aqueous extract was 5356.500±59.552 mg Gallic acid equivalents (GAE)/100 g DW of leaves while TF content of the aqueous extract was 2004.514±2.2058 mg Catechin equivalents (CAE)/100 g DW of leaves. *In-vitro* radical scavenging activity of the aqueous extract evaluated by DPPH assay was 11.279±0.516 mmol Trolox equivalents/100 g DW of leaves. Ferric reducing power of the aqueous extract was 33.366±0.674 mmol Fe(II) equivalents/100 g DW of leaves (**Table 1**).

**Table 1:** TP, TF contents and *in-vitro* antioxidant activity of *O. octandra* leaf extract

| Content | Average±Standard deviation |
|---|---|
| TPC | 5356.500±59.552 mg GAE/100 g DW |
| TFC | 2004.514±2.206 mg CAE/100 g DW |
| Radical scavenging activity | 11.279±0.516 mmol Trolox equivalents/100 g DW |
| Ferric reducing power | 33.366±0.674 mmol Fe(II) equivalents/100 g DW |

**ASIAN JOURNAL OF PHARMACOGNOSY**
Asian Journal of Pharmacognosy 5(3): 36-43
eISSN-0128-1119

## DISCUSSION

Preliminary phytochemical screening of the present study revealed that the aqueous extract of *O. octandra* leaves is rich in many phytochemicals; phenols, flavonoids, tannins, diterpenes, terpenoids, phytosterols, saponins, alkaloids, carbohydrates, proteins and amino acids. There were no literature reports on phytochemical analysis of water extract of *O. octandra* leaves to the best of our knowledge. However, our research findings are supported by the literature reported by Wijesinghe and Priyardarshan in 2020, showing that phytochemicals including, saponins, phenols, flavonoids and anthocyanins are present in leaves of *O. octandra* extracted into ethanol (Wijesinghe and Priyardarshan, 2020). These secondary metabolites including; plant derived alkaloids, flavonoids, tannins, phenolics, terpenoids and steroids are therapeutically important phytochemicals (Samaradivakara *et al*., 2016; Wijesooriya *et al*., 2019). The findings of the present study revealed that *O. octandra* leaves contain a high content of phenolics, flavonoids and antioxidants and they are in the agreement with a previous study in which *O. octandra* leaves had shown a remarkably high concentration of phenolics, flavonoids and antioxidant capacity. Out of twelve medicinal plants, *O. octandra* had shown the highest TP, TF contents and the best radical scavenging activity (Perera *et al*., 2013). It was revealed from a previous study that TP, TF contents, percent inhibition of DPPH radical and Ferric reducing power of *O. octandra* leaves obtained from Uva Province in Sri Lanka were 94.53±8.30 mg GAE/DW, 111.49±4.70 mg Rutin equivalents/g DW, 87.57±0.29 % and 8.49±0.56 mg Ascorbic acid equivalents/g DW respectively (Safeena and Samarakoon, 2020). Another study had evaluated the TP content and DPPH radical scavenging activity as 658 mg GAE/g and 55.7-98.4 µg/mL for *O. octandra* leaves purchased from the traditional herbal market (Perera *et al*., 2015). A comparative study had determined that TP content and DPPH values for *O. octandra* leaves obtained from Galle (Southern Province), Rukmale (Western Province) and Bandarawela (Uva Province) are significantly different in which TP contents were 608±4.8, 666±5.1 and 483±3.2 mg/GAE/g while DPPH antioxidant activity was 55.5±2.4, 97.7±3.1 and 98.4±4.0 µg/mL for the three samples respectively (Perera *et al*., 2013). The values of TP, TF, and antioxidant activities could be different from the findings of the present study due to the various reasons, such as the use of different extraction techniques, geography of the sampling location, the way of expression of the results. Furthermore, *O. octandra* leaf extracts can be used for therapeutic as well as nutraceutical purposes due to high content of phenolics, flavonoids and antioxidants (Balasooriya *et al*., 2020).

## CONCLUSION

The results indicated that *O. octandra* leaves have high amount of total phenolic and flavonoid content which is exhibited through its promising antioxidant activity. Hence, it is concluded that *O. octandra* is a medicinally important herb with promising antioxidant activity and that the leaves can be used to address pathological states related to oxidative stress by developing nutraceuticals such as herbal teas.

## ACKNOWLEDGMENTS

We, authors would like to thank all the nonacademic staff members of Department of Pharmacy, Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka for the support rendered to make this research successful.

## DECLARATION OF CONFLICT OF INTEREST

We hereby declare that the study does not encompass any conflict of interest.

## REFERENCES

Hettihewa S (2014) Extraction, characterization and *in vitro* testing of flavonoids rich fractions obtained from *Actinidia macrosperma* fruit. PhD Thesis – University of Auckland.

Bogahawaththa S, Kodithuwakku S, Wijesundera K, Siriweera E, Jayasinghe L, Dissanayaka W, Rajapakse J, Herath C, Tsujita T, Wijayagunawardane M (2021) Anti-Fibrotic and Anti-Angiogenic Activities of *Osbeckia octandra* Leaf Extracts in Thioacetamide-Induced Experimental Liver Cirrhosis. Molecules 26(16):1-14.

Jayasri M, Mathew L, Radha A (2009) A report on the antioxidant activity of leaves and rhizomes of *Costus pictus* D.Don. International Journal of Integrative Biology 5(1):20-26.

Wasana K, Attanayake A, Jayatilaka K, Priyantha T (2021) Antidiabetic Activity of Widely Used Medicinal Plants in the Sri Lankan Traditional Healthcare System: New Insight to Medicinal Flora in Sri Lanka. Evidence-Based Complementary and Alternative Medicine 2021.

Hausladen A, Stamer J (1999) Nirosative stress. Method in Enzymology 300:389-395.

Firuzi O, Lacanna A, Petrucci R, Marrosu G, Saso L (2005) Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. *Biochimica et Biophysica Acta* 1721:174-184.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Bryne D (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19:669-675.

Das S, Coku A (2013) Antimicrobial and Antioxidant Activities of *Osbeckia stellate* Buch.-Ham. Ex D. (Melastomataceae) Prevalent of Darjeeling Hills. International Journal of Pharmacy and Pharmaceutical Sciences 5(2):551-554.

Ediriweera E, Ratnasooriya W (2009) Herbs used in treatment of Diabetes Mellitus by Sri Lankan Ayurvedic and Traditional Physicians: A review. Ayurveda 30(4):373-391.

Balasooriya M, Karunarathna W, Wickramarachchi W (2020) Evaluation of the clinical efficacy of *Heen Bowitiya* leaves (*Osbeckia octandra*) powder in the management of Diabetes Mellitus. World Journal of Pharmaceutical Research 9(7):2541-2547.

Perera P, Ekanayake S, Ranaweera K (2013) *In vitro* study on antiglycation activity, antioxidant activity and phenolic content of *Osbeckia octandra* L. leaf decoction. Journal of Pharmacognosy and Phytochemistry 2(4):158-161.

Thabrew M, Jayatilaka K (1999) A comparative study of the beneficial effects of *Osbeckia octandra* and *Osbeckia aspera* in liver dysfunction in rats. The Ceylon Journal of Medical Science 42:1-6.

Bnouham M, Ziyyat A, Mekhfi H, Legssyer A (2006) Medicinal plants with potential antidiabetic activity – A review of ten years of herbal medicine research (1990-2000). International Journal of Diabetes & Metabolism 14:1-25.

Pisoschi A, Negulescu G (2011) Methods for Total Antioxidant Activity Determination: A Review. Biochemistry & Analytical Biochemistry 1(1):1-10.

Zakaria Z, Rofiee M, Mohamed A, The L, Salleh M (2011) *In-vitro* anti proliferative and antioxidant activities and total phenolic contents of *Melastoma malabathricum* leaves. Journal of Acupuncture and Meridian Studies 4(4):248-256.

Johari M, Khong H (2019) Total Phenolic Content and Antioxidant and Antibacterial Activities of *Pereskia bleo*. Advances in Pharmacological Sciences 2019:1-4.

Keo S, Meng C, Oeung S, Nov V, Lon S, Vichet T, Va T, Sourn M, Chea S (2017) Preliminary phytochemical screening of selected Medicinal Plants of Cambodia. Asian Journal of Pharmacognosy 1(4):16-23.

Visweswari G, Rita C,Rajendra W (2013) Phytochemical screening of active secondary metabolites present in *Withania somnifera* root: Role in Traditional medicine. International Journal of Pharmaceutical Sciences and Research 4(7):2770-2776.

Sathiavelu A, Sangeetha S, Archit R, Mythili S (2013) *In Vitro* anti-diabetic activity of aqueous extract of the medicinal plants *Nigella sativa*, *Eugenia jambolana*, *Andrographis paniculata* and *Gymnema sylvestre*. International Journal of Drug Development & Research 5(2):323-328.

Wijesinghe S, Priyadarshan A (2020) Phytochemical screening and TLC profiles to identify adulteration of *Osbeckia octandra* (L.) (Heen bovitiya) with *Osbeckia aspera* and *Melastoma malabathricum*. International Conference on Applied and Pure Sciences Proceedings 8.

Samaradivakara S, Samarasekara R, Handunnetti S, Weerasena J (2016) Cholinesterase, protease inhibitory and antioxidant capacities of Sri Lankan medicinal plants. Industrial Crops and Products 83:227-234.

Wijesooriya A, Deraniyagala S, Hettiarachchi C (2019) Antioxidant, Anti-inflammatory and Antibacterial Activities of the seeds of a Sri Lankan Variety of *Carica papaya*. Biomedical & Pharmacology Journal 12(2):539-547.

Safeena M, Samarakoon J (2020) Antioxidant potential of 12 medicinal plants of Sri Lanka. Journal of Medicinal Plants Studies 8(5):170-177.

Perera P, Ekanayake S, Ranaweera K (2015) Comparison of Antiglycation and Antioxidant Potentials and Total Phenolic Contents of Decoctions from Antidiabetic Plants. Procedia Chemistry 16:519-524.