Biocatalysis and Agricultural Biotechnology 18 (2019) 101039

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

# Clinical enzymes inhibitory activities, antioxidant potential and phytochemical profile of *Vernonia oligocephala* (DC.) Sch.Bip. ex Walp roots

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ARTICLE INFO

*Keywords:*
Tyrosinase
Lipoxygenase
Urease
Cholinesterases
Phytochemical
Antioxidant

ABSTRACT

We investigated into the different solvent extracts (methanol, dichloromethane, ethyl acetate, *n*-hexane and *n*-butanol) of *Vernonia oligocephala* (DC.) Sch.Bip. ex Walp roots for their phytochemical composition, antioxidant and enzyme inhibition potential. Phytochemical analysis showed the presence of flavonoids, saponins, terpenoids, and phenolics as secondary metabolites. Methanol extract exhibited the highest phenolic (113.11 mg GAE/g) and flavonoid (97.35 mg QE/g) contents as well as ferric reducing antioxidant power (71.92 GAE/g). The ethyl acetate extract exhibited maximum DPPH ($IC_{50}$; 39.11 μg/mL), total antioxidant capacity (73.07 mg GAE/g) and urease inhibition ($IC_{50}$; 55.89 μg/mL). The *n*-hexane fraction was most active against cholinesterases. All extracts showed least activity against tyrosinase and lipoxygenase (except butanol fraction, $IC_{50}$: 132.2 μg/mL). This study showed that *V. oligocephala* extracts can be regarded as perspective material for isolating bioactive molecules.

## 1. Introduction

Bioactive molecules are the phytochemicals mostly containing functional compounds present in plants or food, possessing the capabilities to regulate several metabolic activities or pathways in the living organisms which results in benefits for health and well-being promotion (Mocan et al., 2017). Currently, the antioxidant potential of natural products along with their enzyme inhibition properties are highly emphasized against diabetes mellitus, cardiovascular disorders and Alzheimer's disease (Xiao and Hogger, 2015). As a consequence, the potential role of natural products to act as antioxidants and to protect against various free radical induced diseases has been explored (Shahidi and Ambigaipalan, 2015). *V. oligocephala* is one of the important medicinal plant traditionally used in treating cough asthma, colics, inflammation, ophthalmology, pruritus, and to prevent gonorrhea, tetanus and urinary tract infections (Duke, 2008). Previously, this plant has been reported for antiplasmodial activity (Clarkson et al., 2004) and the presence of a triterpene compound olicephlate with antidiabetic potential (Riaz et al., 2013). Moreover, different solvent extracts from aerial parts of this plant were also reported for mild cholinesterase inhibition potential (Mahmood et al., 2018). However, information about phytochemical composition or further bioactivities of *V. oligocephala* root part is not comprehended and quite inadequate. Considering the underestimated biological and phytochemical potential, this study was targeted at exploring the bioactive contents, antioxidant and enzyme inhibition potential of this plant. Antioxidant potential was assessed utilizing radical scavenging (DPPH), reducing power (FRAP) and phosphomolybdenum assays. Moreover, inhibition potential against key enzymes involved in common human pathologies like neurodegenerative disorders (cholinesterases), inflammation (lipoxygenase), ulcers (urease) and skin problems (tyrosinase) was also evaluated.

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https://doi.org/10.1016/j.bcab.2019.101039
Received 6 November 2018; Received in revised form 3 February 2019; Accepted 10 February 2019
Available online 12 February 2019
1878-8181/ 

**Table 1**
Total bioactive contents *V. oligocephala* root extracts.

| Solvent | Yield (%) | Total phenolic content (mg GAE/g) | Total flavonoid content (mg QE/g) |
|---|---|---|---|
| Methanol | 9.8 | 113.11 ± 1.2 [d] | 97.35 ± 0.19 [d] |
| DCM | 6.4 | 59.08 ± 0.82 [c] | 37.67 ± 1.59 [c] |
| EA | 4.9 | 27.01 ± 0.54 [b] | 19.43 ± 0.54 [b] |
| *n*-hexane | 3.1 | 4.76 ± 1.6 [a] | 1.07 ± 0.25 [a] |
| *n*-butanol | 2.7 | 3.12 ± 0.97 [a] | 0.97 ± 1.02 [a] |

Data from three repetitions, with mean ± S.D, means with different superscript letters in the same column are significantly ($p < 0.05$) different.

## 2. Material and methods

### 2.1. Plant materials

*V. oligocephala* roots were collected from Bahawalpur, Pakistan. The plant was identified by Dr. M. Arshad Chaudhry (late), The Islamia University of Bahawalpur and deposited in the university herbarium for future reference (VO-WP-01-12-135). The shade dried roots of *V. oligocephala* were first extracted in methanol (80% w/v) for 7 days with periodic shaking, filtered and concentrated using Rotavapor-R20 at 37 ºC. Resultant methanol extract was further subjected to fractionation with dichloromethane (DCM), ethyl acetate, *n*-hexane and *n*-butanol, respectively. The respective fractions were then concentrated by Rotavapor-R20 at 37 °C.

### 2.2. Phytochemical screening and total bioactive components

Qualitative phytochemical evaluation were assessed using standard protocols (Evans, 2009). Folin-Ciocalteu method was employed to determine total phenolics (Kahkonen et al., 1999) and the results were expressed as mg GAE/g extract. While, total flavonoid content assay was done by Aluminum chloride colorimetric method (Chew et al., 2009). The data was expressed as quercetin equivalents (QE/g extract).

### 2.3. Antioxidant assays

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) assay was performed as described by Koleva et al. (2002). The FRAP (ferric reducing antioxidant power) assay was performed according to standard method as described previously (Saeed et al., 2012). Total antioxidant capacity was evaluated by phosphomolybdenum method (Prieto et al., 1999). The reducing power and total antioxidant capacity was expressed as equivalent of gallic acid (mg GAE/g extract).

### 2.4. Enzyme inhibitory assays

Cholinesterases (acetylcholinesterase (from eel fish) and butyrylcholinesterase (from equine serum), jack-bean urease, soybean lipoxygenase and mushroom tyrosinase inhibition activities were determined spectrophotometrically according to standard methods as described by Ellman et al. (1961) (Ellman et al., 1961), Weatherburn (1967) (Weatherburn, 1967), Baylac and Racine (2003) (Baylac and Racine, 2003) and Orhan et al. (2012) (Orhan et al., 2012), respectively. Eserine was used as control for acetylcholinesterase (AChE) and BChE (butyrylcholinesterase), baicialin for lipoxygenase and kojic acid for urease and tyrosinase.

### 2.5. Statistical analysis

The results obtained were expressed as mean ± S.D. All the tests were performed for three times and the data analysis was carried out in triplicates. One-way analysis of variance (ANOVA) and Tukey's significant difference post hoc test ($p < 0.05$) were used to calculated differences. EZ–Fit Enzyme kinetics software was used to calculate $IC_{50}$ values (Perrella Scientific Inc. Amherst, USA).

## 3. Results and discussion

### 3.1. Phytochemical composition

Preliminary phytochemical evaluation of *V. oligocephala* roots reveals the presence of secondary metabolites like glycosides, saponins, flavonoids, terpenoids, triterpenoids, phenolic compounds and steroids, whereas, alkaloids and tannins were absent. Maximum phenolic and flavonoid contents were observed for methanol extract i.e., 113.11 mg GAE/g extract and 97.35 mg QE/g extract (Table 1). The solvent methanol has already been reported to be more efficient in extraction of plant polyphenols (Anokwuru et al., 2011).

### 3.2. Antioxidant activities

Free radicals provoke a wider range of chronic and degenerative disorders, such as Alzheimer's disease, diabetes and cancer. Table 2 reveals antioxidant activities of *V. oligocephala* extracts.

In DPPH assay, ethyl acetate extract showed superior scavenging activity ($IC_{50;}$ 39.11 µg/mL), followed by *n*-butanol ($IC_{50}$; 56.31 µg/mL), methanol ($IC_{50}$; 51.21 µg/mL) and DCM extract ($IC_{50}$; 88.69 µg/mL). This can be attributed to the higher yields of phenolic compounds presented by these extract.

Similarly, the methanol extract which contains the highest values in terms of total bioactive contents also exhibited the highest ferric reducing power (71.92 ± 0.43 mg GAE/g). Similar results concerning solvent polarity-related antioxidant capacity were observed by previous researchers (Llorent-Martínez et al., 2016). Similarly, for TAC assay, the ethyl acetate (73.07 ± 1.06 mg GAE/g) and methanol (71.11 ± 0.45 mg GAE/g) extracts exhibited highest total antioxidant capacity, whereas DCM extract presented the lowest one.

### 3.3. Enzyme inhibition activities

All the extracts were tested for enzyme inhibition activities

**Table 2**
Antioxidant activities of *V. oligocephala* root extracts.

| Solvent | %RSC (0.5 mg/mL) | DPPH $IC_{50}$ (µg/mL) | AAEAC (mg AAE/g) | FRAP (mg GAE/g) | TAC (mg GAE/g) |
|---|---|---|---|---|---|
| Methanol | 89.61 ± 0.27 [c] | 51.21 ± 0.15 [c] | 75.88 ± 0.45 [c] | 71.92 ± 0.43 [e] | 71.11 ± 0.45 [d] |
| DCM | 79.02 ± 0.31 [b] | 88.69 ± 0.11 [e] | 43.63 ± 0.92 [a] | 54.17 ± 0.99 [d] | 19.94 ± 0.91 [a] |
| EA | 90.93 ± 0.66 [d] | 39.11 ± 0.19 [b] | 98.95 ± 0.18 [d] | 42.76 ± 0.63 [c] | 73.07 ± 1.06 [d] |
| *n*-hexane | 25.74 ± 0.14 [a] | > 500** | nt | 21.84 ± 0.53 [b] | 26.59 ± 1.3 [b] |
| *n*-butanol | 88.95 ± 0.21 [c] | 56.31 ± 0.22 [d] | 68.7 ± 0.66 [b] | 13.77 ± 1.16 [a] | 49.63 ± 0.87 [c] |
| Ascorbic acid | 93.21 ± 0.97 [e] | 16.96 ± 0.14 [a] | nt | nt | nt |

Values are expressed as means ± S.D. of three replicates, means with different superscript letters in the same column are significantly ($p < 0.05$) different, nt: not tested, **The $IC_{50}$ value was higher than 500 µg/mL. RSC: radical scavenging capacity, AAEAC: Ascorbic acid equivalent anti-oxidant capacity, FRAP: ferric reducing anti-oxidant power, TAC: total antioxidant capacity.

**Table 3**
Enzyme inhibition activities of *V. oligocephala* roots extracts.

| Solvent | AChE | | BChE | | LOX | | Urease | | Tyrosinase | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Inhibition (%) (500 µg/mL) | $IC_{50}$ (500µg/mL) | Inhibition (%) (500 µg/mL) | $IC_{50}$ (µg/mL) | Inhibition (%) (500 µg/mL) | $IC_{50}$ (µg/mL) | Inhibition (%) (500 µg/mL) | $IC_{50}$ (µg/mL) | Inhibition (%) (500 µg/mL) | $IC_{50}$ (µg/mL) |
| Methanol | 49.77 ± 0.64 | > 500** | 38.32 ± 0.34 | > 500** | 42.88 ± 0.29 | > 500** | 74.82 ± 0.31 | 145.81 ± 0.05 | 29.23 ± 0.397 | > 500** |
| DCM | 41.96 ± 0.31 | > 500** | 26.56 ± 0.44 | > 500** | 57.56 ± 0.82 | > 500** | 35.28 ± 0.39 | > 500** | 3.57 ± 0.541 | > 500** |
| EA | 60.93 ± 0.15 | 302 ± 0.30 | 38.22 ± 0.21 | > 500** | 57.27 ± 0.62 | > 500** | 88.89 ± 0.43 | 55.89 ± 0.04 | 8.73 ± 0.512 | > 500** |
| *n*-hexane | 68.49 ± 0.28 | 215 ± 0.06 | 69.68 ± 0.21 | 159.11 ± 0.18 | 42.15 ± 0.33 | > 500** | 79.82 ± 0.83 | 75.42 ± 0.07 | 31.91 ± 0.382 | > 500** |
| *n*-butanol | 50.81 ± 0.82 | > 500** | 69.38 ± 0.11 | 163.91 ± 0.25 | 78.51 ± 0.25 | 132.21 ± 0.22 | 34.41 ± 0.53 | > 500** | 37.61 ± 0.35 | > 500** |
| Eserine | 91.29 ± 1.17 | 0.04 ± 0.01 | 82.82 ± 1.09 | 0.85 ± 0.0001 | nt | nt | nt | nt | nt | nt |
| Baicalein | nt | nt | nt | nt | 93.79 ± 1.27 | 22.4 ± 1.3 | nt | nt | nt | nt |
| Kojic Acid | nt | nt | nt | nt | nt | nt | 98.12 ± 0.31 | 21.11 ± 0.36 | 93.50 ± 0.91 | 6.04 ± 0.11 |

Values are expressed as means ± S.D. of three replicates; ** $IC_{50}$ values more than 500 µg/mL.
AChE: acetylcholinesterase; BChE: butyrylcholinesterase; nt: not tested.

(cholinesterase, lipoxygenase, urease and tyrosinase) using spectrophotometric methods. The enzyme inhibition (%) and $IC_{50}$ values of the extracts were calculated (Table 3). Hexane and ethyl acetate extracts showed maximum AChE inhibition ($IC_{50}$; 215 and 302, respectively). Whereas, *n*-hexane and *n*-butanol fractions showed highest BChE inhibition with $IC_{50}$ values of 159.11 and, 163.91 µg/mL, respectively. All the other extracts were less active for both cholinesterases. The ethyl acetate fraction showed maximum activity for urease ($IC_{50}$; 55.89 µg/mL) followed by *n*-hexane ($IC_{50}$; 75.42 µg/mL) and methanol ($IC_{50}$; 145.81 µg/mL) extracts. The *n*-butanol and DCM extracts were least active against urease. Only *n*-butanol extract was considerably active for lipoxygenase inhibition ($IC_{50}$; 132.21 ± 0.22). All the extracts were least active against tyrosinase.

## 4. Conclusion

In this study, we investigated the antioxidant capacity, enzyme inhibition potential and phytochemical composition of *Vernonia olala* roots. Saponins, flavonoids, terpenoids, triterpenoids and phenolic compounds were detected as second metabolites. Methanol extract showed highest total bioactive contents and reducing power antioxidant activity. Ethyl acetate extract exhibited prominent DPPH, phosphomolybdenum as well urease inhibition potential. Findings of current research revealed the potential of *V. oligocephala* to discover and develop novel pharmaceutical bioactive compounds with enzyme inhibition and antioxidant capabilities. However, further studies are needed to isolate as well as characterize the potential bioactive constituents.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101039.

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