A new arsenal of polyphenols to make Parkinson’s disease extinct: HPLC-MS/MS profiling, very interesting MAO-B inhibitory activity and antioxidant activity of *Otostegia fruticosa*

Somaia A. Al-Madhagy\(^{a, b}\), Sameh S. Gad\(^c\), Eman S. Mostafa\(^d\), Simone Angeloni\(^e, f\), Muhammed A. Saad\(^g, h\), Omar M. Sabry\(^a\)\(^\circ\), Giovani Caprioli\(^e\)\(^\circ\) and Seham S. El-Hawarya\(^a\)

\(^a\)Department of Pharmacognosy, College of Pharmacy, Cairo University, Cairo, Egypt; \(^b\)Department of Pharmacognosy, Faculty of Pharmacy, Sana’a University, Sana’a, Yemen; \(^c\)Department of Pharmacology and Toxicology, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt; \(^d\)Department of Pharmacognosy, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt; \(^e\)School of Pharmacy, University of Camerino, Camerino, Italy; \(^f\)RICH – Research and Innovation Coffee Hub, Belforte del Chienti, MC, Italy; \(^g\)Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt; \(^h\)School of Pharmacy, Newgiza University, Giza, Egypt

**ABSTRACT**

Fifteen compounds belong to phenolic acids, derivatives of phenolic acids, iridoids, xanthones and flavonoids were characterized in the methanolic extract of *Otostegia fruticosa* leaves using HPLC-MS/MS. Extract has been also investigated for its MAO-B inhibitory activity, antioxidant activity, total phenolic and total flavonoid content. The extract exhibited interesting MAO-B inhibitory activity (IC\(_{50}\); 2.24 ± 0.08) compared to the reference compound sele-giline (0.55 ± 0.02 \(\mu\)g/mL). It also showed a potent antioxidant activity proven in both DPPH and ORAC assay methods. The extract showed an IC\(_{50}\) of 3.64 ± 1.22 \(\mu\)g/mL in the DPPH test which was significantly lower than that of the standard ascorbic acid which attained an IC\(_{50}\) of 18.3 ± 1.41 \(\mu\)g/mL. Moreover, in the oxygen radical absorbance capacity assay (ORAC) the extract showed a decline in the IC\(_{50}\) to 3.48 ± 1.16 \(\mu\)g/mL as compared to the standard Trolox which exhibited an IC\(_{50}\) of 27.0 ± 13.41.

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1. Introduction

Neurodegenerative disorders are a real challenge nowadays (Noble and Burns 2010). Parkinson’s is the second most prevalent neurodegenerative disease worldwide (Mhyre et al. 2012). During the period between 2000 and 2017, the death rate from Alzheimer’s disease (AD) in the United States, only, climbed by 145 percent (Kozioł et al. 2020). The prevalence of PD is predicted to be 222 percent by 2030 (Kozioł et al. 2020). Parkinson’s disease (PD) affects 10 million individuals globally (Ball et al. 2019). It is a disorder characterized clinically by bradykinesia, stiffness, and asymmetric resting tremor of the distal extremities (Williams and Litvan 2013). As a result, there is a great demand for additional therapeutic targets and medication candidates of a high level of activity against these neurodegenerative disorder (Poewe and Mahlknecht 2020).

Monoamine oxidases (MAOs) are enzymes located in the outer mitochondrial membrane classified as MAO-A and MAO-B (Edmondson and Binda 2018). In mammals, they are in charge of catecholamine and serotonin catabolism. MAOs have a role in the genesis of depression, Parkinson’s disease, and Alzheimer’s disease. As a result, both MAO-A and MAO-B inhibitors have broad neuroprotective benefits (Kozioł et al. 2020). MAO-B enzyme is known to be more linked to neuronal loss and neurotoxic production in Parkinson’s disease. They are well-known therapeutic targets for many central nervous systems (CNS) disorders (Alborghetti and Nicoletti 2019). There are only three MAO-B inhibitors utilized nowadays to treat Parkinson’s disease. They are; selegiline, rasagiline, and safinamide (Nasso et al. 2021). Polyphenols, coenzyme Q10, and vitamins A, C, and E have been proposed as therapeutic agents for preventing and delaying the development of Parkinson’s disease (Lange et al. 2019).

Flavonoids and polyphenol compounds in herbal medicine showed potent reducing effects in oxidative stress and risk of developing related neurodegenerative diseases such as Parkinson’s disease, Alzheimer’s disease, multiple sclerosis, stroke, and...
Huntington’s disease via modulating various neurotransmitter systems in the brain (Nasso et al. 2021; Tahir et al. 2021).

Lamiaceae plants are known for their notable anti-inflammatory, antioxidant, and neuroprotective effects (Khankandi et al. 2019). It is well-recognized that the powerful therapeutic properties of Lamiaceae species is due to their polyphenolic compounds (Hossain et al. 2010). *Otostegia fruticosa* (Forssk.) is one of Lamiaceae plants that grows wildly in certain Yemeni governates.

This study aimed to investigate the chemical constituents of *O. fruticosa* using HPLC-MS/MS, antioxidant activity, total phenolic and total flavonoid content. Also, we tried to correlate the phytochemical composition with neurological activity against Parkinson’s disease.

### 2. Results and discussion

#### 2.1. HPLC-MS/MS analysis of the dried extract

HPLC-MS/MS analysis of the methanolic extract of *O. fruticosa* leaves showed characteristic bioactive compounds belong to phenolic acids, derivatives of phenolic acids, iridoids, xanthones and flavonoids. Table S1 reports the content of the compounds detected in the extract, expressed in μg/g of dried weight extract. Fifteen out of thirty reference standard compounds were detected in *O. fruticosa*. The major identified compounds are 3-caffeoylquinic acid, 5-caffeoylquinic acid, loganic acid (iridoid glucoside), and caffeic acid (381.05, 231.57, 35.75, and 17.09 μg/g, respectively).

#### 2.2. Total phenolic and flavonoid contents

The crude methanolic extracts of *O. fruticosa* was found to be good source of wide range of potent phenolics and flavonoids Table S2. The total phenolic content (TPC) in *O. fruticosa* was (413 ± 2.01 mg GAE/g). The equation for standard curve was $y = 0.0242x + 0.0211$, where $R^2 = 0.9985$. Meanwhile, the total flavonoid content (TFC) in the same extract was (168 ± 3.01 mg RTE/g extract). The equation for standard curve was $y = 0.0048x + 0.0091$, where $R^2 = 0.9989$.

#### 2.3. Antioxidant activity

Tables S4 and S5 as well as Figures S1 and S2 illustrates some of the main characteristics of the methanolic extract of *O. fruticosa* as it disclosed a promising antioxidant activity proven by a decline in the IC50 of the DPPH assay after the adminstration of the extract to 3.64 ± 1.22 μg/mL, as compared to the standard ascorbic acid which reached an IC50 of 18.3 ± 1.41 μg/mL. Besides, ORAC assay test confirmed the antioxidant potential of the extract by attaining an IC50 of 3.48 ± 1.16 μg/mL, inferior to the reference standard Trolox which gotten an IC50 of 27.0 ± 13.41.
2.4. In-Vitro MAO-B inhibitor activity

The inhibition potency of the methanolic extract of *O. fruticosa* leaves were screened against MAO-B enzyme using an in-vitro fluorometric assay. Percentages of MAO-B inhibition were calculated at three different concentrations compared to standard MAO-B inhibitor, Selegiline. It can be seen from the data in Table S6 and Figure S3 that 100 µg/mL of methanolic extract of *O. fruticosa* leaves reported a significantly higher IC50 by about four folds the selegiline treated group.

In addition, the calculated IC50 of MAO-B inhibition showed that the methanolic extract of *O. fruticosa* leaves has potent inhibition of MAO-B enzyme with IC50 2.24 ± 0.08 µg/mL (compared to the standard inhibitor Selegiline IC50 0.55 ± 0.02 µg/mL).

Biological activities of plant extract containing several compounds are usually due to the synergistic effect between their metabolites rather than the abundance and nature of particular metabolite (Cafaro et al. 2020; Sabry et al. 2021). The methanol extract of the plant under study showed high polyphenol content. Meanwhile, many clinical studies have revealed that consumption of polyphenols rich diets have valuable effects on numerous diseases such as obesity, cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases (Fukutomi et al. 2021).

Loganic acid, 3-caffeoylquinic acid, 5-caffeoylquinic acid, caffeic acid, and syringic acid, were found to be present in the methanolic extract of *O. fruticosa* leaves in high concentration. Interestingly, there are number of in-vitro studies that confirmed neuroprotective mechanisms of these compounds which may empower the evidence of the therapeutic potential of the plant against Parkinson’s disease. A study showed a significant neuroprotective activity of 3-caffeoylquinic acid and 5-caffeoylquinic acid against oxidative stress-induced cell death (Nakajima et al. 2007). Another study revealed that 2.5 µg/mL of caffeic acid exhibit dose-dependently protection activity on neuronal PC12 cells against H2O2-induced neurotoxicity with best protection (48%) at 40 µg/mL (Jeong et al. 2011). As *O. fruticosa* leaves extract, in this study, contains 17.09 µg/mL of caffeic acid, a potential neuroprotective activity is predicted via similar mechanisms.

The noticeable potential anti-Parkinson’s effect of *O. fruticosa* is also supported by a recent in-vitro study of syringic acid. 0.1, 1, 10, and 20 µM of syringic acid reduced OGD/R-induced neuronal damage, increased cell viability, and decreased LDH leakage in a dose-dependent manner (Cao et al. 2016).

In-vivo and in-vitro studies showed that the use of particular dietary foods containing quercetin, rutin, kaempferol, and isoquercitrin has proved superior improvement in cognitive capabilities by limiting neuropathological circumstances like protein aggregation, free radical scavenging, lipid peroxidation, mitochondrial molecular impairment, and another cellular dysfunction (Tahir et al. 2021).

Quercetin that was identified in the sample can pass the blood-brain barrier which has a consistent direct effect on neurons and glia; this includes not only the cerebral vasculature but also the parenchyma of brain cells (Singh et al. 2020). In addition, hyperoside showed neuroprotective effects through enhancing the antioxidant defense (Kääriäinen et al. 2008; Kwon et al. 2019). These compounds are present in
the extract under investigation. They might potentiate the activity of other components through synergistic action (Ramadan et al. 2021).

3. Conclusions

Using HPLC-MS/MS technique, methanolic extract of *Otostegia fruticosa* leaves was found to contain fifteen compounds out of thirty polyphenolic and flavonoidal reference standards. This extract showed fantastic MAO-B inhibitory activity, *in-vitro* antioxidant activity, high total phenolics and high total flavonoid contents. Fantastic MAO-B inhibitory activity can be explained by the synergistic effect of its phenolics and flavonoidal compounds as well as the high amounts of 3-caffeoylquinic acid, 5-caffeoylquinic acid, caffeic acid, and syringic acid. It is predicted that *O. fruticosa* leaves extract have promising neuroprotective activity against Parkinson’s disease, as it contains compounds have proved potent neuroprotective activity in numerous previous *in-vitro* studies. These promising activities give the opportunity for this plant to be used as a drug source making Parkinson’s disease an extinct ailment. It is recommended to conduct a well-designed *in-vivo* studies to confirm these biological activities as well as ensure plant’s safety.

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ORCID

Omar M. Sabry  http://orcid.org/0000-0002-5796-2708
Giovani Caprioli  http://orcid.org/0000-0002-5530-877X

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