SHORT COMMUNICATION

Dismantling Parkinson’s disease with herbs: MAO-B inhibitory activity and quantification of chemical constituents using HPLC-MS/MS of Egyptian local market plants

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ABSTRACT

Withania somnifera, Angelica sinensis, Glycyrrhiza glabra, and Simmondsia chinensis were acquired from the Egyptian market, profiled for their chemical constituents, screened for the in-vitro MAO-B inhibitory activity and evaluated for the total phenolic content. Thirty compounds were characterized in the selected herbs using HPLC-MS/MS. In-vitro MAO-B inhibitory activity and total phenolic content of the acquired herbs were compared with those of a prepared herbal formula consisting of a mixture of equal amounts of the four mentioned herbs. The most potent MAO-B inhibitory activity was exerted by the methanol extract of the prepared formula (IC50 of 712.19 ± 13.90 ng/mL) compared to selegiline (IC50 of 581.69 ± 11.35 ng/mL). The highest value of the total phenolic content was shown by Angelica sinensis methanolic extract (76.15 ± 0.1 mg/g) followed by Glycyrrhiza glabra methanolic extract (65.74 ± 0.1 mg/g), then the mixture’s methanolic extract of the four herbs (37.04 ± 0.1 mg/g).

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1. Introduction

Parkinson’s disease (PD) represents a common debilitating age-related disease (middle or old age) resulting from massive degenerative depletion of dopamine (DA) in neurons, particularly in the substantia nigra (Alexander 2004). PD causes an imbalance between the inhibitory (DA) and the excitatory neurotransmitter acetylcholine (Liu et al. 2021).

Monoamine oxidases (MAOs) types A and B catalyze the oxidative deamination of different neurotransmitters like noradrenaline, dopamine, serotonin, tyramine and benzylamine to the corresponding aldehydes, while MAO-B has a greater affinity for phenylethylamine and benzylamine (Lin et al. 2003). MAO-B activity is elevated in aged humans particularly in those suffering of certain neurodegenerative diseases (Saura et al. 1997; Lin et al. 2003; Hathout et al. 2018). Consequently, inhibition of MAO-B activity could enhance the quality of life of the geriatrics.

The main treatment mechanisms of PD rely on pharmacological management involving administration of levodopa, dopamine agonists, monoamine oxidase B (MAO-B) inhibitors, anti-cholinergics, catechol-O-methyltransferase (COMT) inhibitors as well as prevention of neuron degeneration by using antioxidants like polyphenols, flavonoids, glucosinolates and berberine (Ravi et al. 2018; Ribaudo et al. 2018; Venditti and Bianco 2020).

The potential clinical benefits of herbal products in the management of neurodegenerative disorders, like PD, have been earlier established. Among these herbs are Withania somnifera root, commonly called Ashwagandha (Vegh et al. 2021), Angelica sinensis root, commonly called angelica (Li et al. 2019), Glycyrrhiza glabra root, commonly called licorice (Ravanfar et al. 2018), and Simmondsia chinensis berries, commonly called jojoba (Manoharan et al. 2016).

Studies revealed that Withania somnifera (L.) Dunal (family Solanaceae) methanolic extract induced axon and dendrite outgrowth (Kuboyama et al. 2002; Zhao et al. 2002), increase in the number of dopaminergic neurons (Ahmad et al. 2005), reduction in the oxidative stress and regulation of the catecholamine content in mid brain of
rats (Sankar et al. 2007). The main bioactive phthalide compounds in *Angelica sinensis* (Oliv.) Diels (family Apiaceae) exerted significant neuroprotective effects (Gong et al. 2016). Liquiritigenin and iso-liquiritigenin in *Glycyrrhiza glabra* L. (family Leguminosae/Fabaceae) were reported as potential inhibitors of MAO-B (Jeong et al. 2020) with anti-inflammatory, antioxidant, anti-apoptotic and anti-amyloid-β toxicity (Hwang and Chun 2012). The antioxidant and anti-apoptotic neuroprotective effects of the flavonoid liquiritin were also proven (Kim et al. 2012). *Simmondsia chinensis* (Link) Schneider (family buxaceae) methanolic extract inhibited with high potency both lipoxygenase (LOX) and cyclooxygenase (COX), the key enzymes in inflammation cascade (Abdel-Mageed et al. 2014).

In this work, we have identified 30 secondary metabolites in the four herbs under study and in a prepared mixture of the methanol extracts of the four herbs using HPLC-MS/MS technology. Moreover, the individual extracts of the four herbs and their prepared mixtures have proven strong activity when subjected to *in-vitro* screening of the total antioxidant activity and presented outstanding results when evaluated for their *in-vitro* monoamine oxidase-B (MAO-B) inhibitory activity.

2. Results and discussion

2.1. Chemical composition of the dried extracts

HPLC-MS/MS analysis of the methanolic dried extracts allowed characterization of selected bioactive metabolic components including phenolic acids, derivatives of phenolic acids, flavonoids, iridoids and xanthones. Table S1 displays the 30 bioactive components, expressed in μg/g of dried weight extract, monitored in the five different samples. Figure S1 shows the HPLC-MS/MS chromatogram of standard mixture of 30 bioactive compounds plotted as overlapped MRM transition. All identified metabolites in the different four plants extracts (*A. sinensis*, *S. chinensis*, *W. somnifera* and *G. glabra*) were detected in their prepared mixture with a proportional concentration to their percentage in the mixture (Table S2). 5-caffeoylquinic acid amounted to 578.05, 40.67 and 189.70 μg/g; 3-caffeoylquinic acid amounted to 80.71, 4.94 and 12.92 μg/g in *A. sinensis*, *W. somnifera* and the prepared formula, respectively. The amount of 3,5-dicaffeoylquinic acid reached 251.10 and 80.82 μg/g in *A. sinensis* extract and the prepared mixture, respectively.

The main constituents of the identified compounds in *G. glabra* extract were phenolic acids and their derivatives, with vanillic acid (28.78 μg/g), 5-caffeoylquinic acid (8.44 μg/g), syringic acid (4.32 μg/g) and p-coumaric acid (1.53 μg/g) being the most prevalent.

*S. chinensis* extract was the richest extract in terms of flavonoids (identified flavonoid content, 18.46 μg/g). In particular, quercetin (7.82 μg/g) and hyperoside (6.69 μg/g) were the most abundant secondary metabolites having MAO-B inhibitory activity in jojoba berries (Abdel-Mageed et al. 2014; Dhiman et al. 2019).

2.2. In-vitro MAO-B inhibitory activity

As depicted from Figure S2 and Table S3, the inhibition potencies of the methanolic extracts of the four tested plants and their prepared mixture against MAO-B enzyme
were evaluated using an in-vitro fluorometric assay. The prepared mixture exhibited a significantly higher MAO-B inhibitory activity (IC50 of 712.19 ± 13.90 ng/mL) compared to the other individual herbal extracts except Glycyrrhiza glabra which didn’t show any significant change from the mixture. This could be attributed to the synergistic potential interaction between the bioactive metabolites of the four different herbs like ferulic acid, caffeic acid, chlorogenic acid, quercetin, isoquercitrin, naringin, hyperoside, liquiritigenin, iso-liquiritigenin, catechin and epicatechin. The inhibitory activity of the prepared mixture was equivalent to that of the standard MAO-B inhibitor, selegiline.

2.3. Total phenolic content

Determination of the total phenolic content was carried out by Folin–Ciocalteu method (Saboo et al. 2010). There was a statistically significant difference between all the tested extracts and the mixture prepared as well. The total phenolic content and hence the antioxidant capacity of the mixture was higher than that of the Withania somnifera and Simmondsia chinensis extracts, but still lower than that of the Glycyrrhiza glabra and Angelica sinensis methanolic extracts. The highest total phenolic content was recorded in Angelica sinensis extract (76.15 ± 0.1 mg/g) followed by Glycyrrhiza glabra extract (65.74 ± 0.1 mg/g), then the extract of the mixture of the four herbs (37.04 ± 0.1 mg/g). The lowest content of the total phenolic compounds was determined in Withania somnifera extract (24.11 ± 0.1 mg/g), then that of Simmondsia chinensis (16.15 ± 0.1 mg/g). The results of this determination were presented in Figure S3 and Table S4.

3. Conclusion

Thirty secondary metabolites were identified using HPLC-MS/MS in methanolic extracts of four herbs acquired from the Egyptian market (Withania somnifera, Angelica sinensis, Glycyrrhiza glabra, and Simmondsia chinensis). Due to the synergetic effect of the phenolic acids and flavonoid content in the four herbs, the methanolic extract of the formula excreted the most potent MAO-B inhibitory action (IC50 of 712.19 ± 13.90 ng/mL) among the five tested samples. Angelica sinensis methanolic extract had the highest phenolic content (76.15 ± 0.1 mg/g), followed by Glycyrrhiza glabra extract (65.74 ± 0.1 mg/g), and finally the extract of the herbal mixture (37.04 ± 0.1 mg/g).

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