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Evaluation of anti-amnesic effect of extracts of selected *Ocimum* species using in-vitro and in-vivo models

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ABSTRACT

Ethnopharmacological relevance: *Ocimum* species are traditionally used for the treatment of anxiety, nerve pain, convulsions and a variety of neurodegenerative disorders. The present study was undertaken to evaluate the anti-amnesic effect of *O. basilicum* L., *O. sanctum* L. and *O. gratissimum* L. extracts using in-vitro and in-vivo models.

Materials and methods: *In-vitro* acetylcholinesterase (AChE) inhibitory and antioxidant activities of hydro-methanol extracts of plants were evaluated using Ellman and DPPH and FRAP assays, respectively. The most active extract i.e. *O. basilicum* extract (OBE) was further explored for the possible anti-amnesic activity in mouse model of scopolamine induced amnesia using behavioral models (elevated plus maze and passive shock avoidance task). Brain AChE activity, oxidative profile and histopathological studies were assessed to outline the anti-amnesic mechanism of the extract.

Results: Significant antioxidant and AChE inhibition activity was observed with all prepared extracts and however, OBE showed most marked free radical scavenging, reducing power and AChE inhibition (IC_{50} 0.65 ± 0.15 mg/ml) activity. Basil leaves were standardized with respect to content of 7 phenolic acids using a HPLC-PDA method. A TLC densitometric method was employed to determine the quercetin content in the leaves. The in-vivo studies showed that OBE pre-treatment (200 and 400 mg/kg, p.o.) reversed the memory deficit induced by scopolamine in mice, evident by significant ($p < 0.05$) decrease in the transfer latency time and increase in step down latency in elevated plus maze and passive shock avoidance task, respectively. Moreover, OBE significantly reduced the brain AChE activity and oxidative stress. Further, histopathological examination of brain tissues displayed decrease in vacuolated cytoplasm and increase in pyramidal cells in hippocampal and cortical regions with OBE pre-treatment.

Conclusion: OBE possesses antioxidant and AChE inhibitory activity. These biochemical changes are responsible for the anti-amnesic and neuroprotective activities of *O. basilicum* which may be attributed to the presence of phenolic and flavonoid compounds. This can be developed as an effective anti-amnesic drug.

1. Introduction

Dementia is an umbrella term that covers different types of clinical syndromes such as Alzheimer's disease (AD), vascular dementia, dementia with Lewy bodies, frontotemporal dementia, Parkinson's disease etc. AD is a most common type of adult dementia affecting approximately 24 million people globally (Awasthi et al., 2016). It is a neurodegenerative disorder characterized by memory deficits, followed by the gradual loss of other cognitive functions including behavioral changes (Huang and Mucke, 2012). Pathologically, cholinergic hypo-function and diminished central cholinergic neurotransmission resulted from hydrolysis of acetylcholine by acetylcholinesterase enzyme (AChE) are responsible for the cognitive dysfunction and memory impairment in AD (Auld et al., 2002). Thus, the acetylcholinesterase inhibitors (AChEIs) are most accepted drugs for clinical use in AD (Singh et al., 2013). However, these approved drugs are effective only in providing symptomatic treatment and the resulting non-dose related adverse effects persuade us to explore the wealth of traditional medicines.

The genus *Ocimum* (family Lamiaceae) comprises of annual and perennial herbs and shrubs that play an important part in Ayurveda and indigenous medicine system (Pullaiah, 2006). *Ocimum* species are used to treat central nervous system disorders and their anticonvulsant, analgesic and anti-inflammatory activities are well documented

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(Freire et al., 2006; Nascimento et al., 2014). *Ocimum basilicum* L., *O. gratissimum* L. and *O. sanctum* L. (commonly known as Sweet Basil, Clove Basil and Holy Basil, respectively) are used traditionally to treat diabetes, eye disorders, cardiac disorders, stress, anxiety, nerve pain, convulsions and as general tonic (Guenther and Althausen, 1949; Prakash and Gupta, 2005; Okoli et al., 2010; Bora et al., 2011a). These plants contain numerous phytoconstituents including volatile oil, phenols (Javanmardi et al., 2002; Lee and Scagel, 2010: Pattanayak et al., 2010; Igbinosa et al., 2013), flavonoids (Vieira et al., 2001; Grayer et al., 2002), sterols, terpenoids and their glycosides (Siddiqui et al., 2007).

Experimental studies showed numerous pharmacological activities of *O. basilicum*, *O. gratissimum* and *O. sanctum* including antidiabetic, antioxidant, anticancer, cardioprotective and CNS activities (Njoku et al., 1997; Jayasinghe et al., 2003; Dasgupta et al., 2004; Pattanayak et al., 2010). Our research group has established the neuroprotective role of *O. basilicum* and *O. gratissimum* plants in mice model of ischemia-reperfusion induced brain injury (Bora et al., 2011a, 2011b).

However, so far no comparative study for AChE inhibition potential of *O. basilicum*, *O. gratissimum* and *O. sanctum* has been reported. Consequently, the present study was designed with two objectives. Firstly, to compare AChE inhibition and antioxidant potential of *O. basilicum*, *O. gratissimum* and *O. sanctum* extracts using in-vitro models. Secondly, to investigate the possible cognitive enhancing and neuroprotective effect of most active extract (hydro-methanol extract of *O. basilicum*) using in-vivo model of amnesia (scopolamine induced memory deficits in mice).

2. Methods

2.1. Chemicals

Acetylcholinesterase, acetylthiocholine iodide, DPPH (2,2-diphenyl-1-picrylhydrazyl), reduced glutathione (GSH), scopolamine hydrobromide, 1,1,3,3-tetraethoxypropane, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), tacrine (Amino-1,2,3,4-tetrahydroacridine hydrochloride hydrate), benzoic acid, *p*-hydroxy benzoic acid, caffeic acid, chlorogenic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, gallic acid and vanillic acid, were obtained from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile (Sigma Aldrich), methanol (JT Baker), ultrapure water (Elga®), acetic acid were used in HPLC studies. All other chemicals and reagents were of analytical grade.

2.2. Collection and authentication of plant materials

Leaves of *Ocimum basilicum*, and *O. gratissimum* were procured from the nursery of National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India whereas, leaves of *O. sanctum* were procured from botanical garden, Department of Botany, Punjabi University, Patiala, Punjab India in August 2013. The identity of *O. basilicum* was confirmed by Dr. Sunita Garg, Chief Scientist, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (voucher number-NISCAIR/RHMD/Consult/2013/2337-117-1) and of *O. gratissimum* and *O. sanctum* were authenticated by from Department of Botany, Punjabi University, Patiala, Punjab, India (voucher number - PUN-2700/1982).

2.3. Preparation of extracts

The procured leaves were shade dried and powdered. The dried powdered (100 g) of each plant, separately, was defatted with petroleum ether (60–80 °C) and then hydro-methanol extract (methanol: water, 70:30) was prepared by maceration as described in Fig. 1. The yield of *O. basilicum* (OBE), *O. gratissimum* (OGE) and *O. sanctum* (OSE) extracts were calculated on dry weight basis.

2.4. Standardization of bioactive extract

Phytochemical screening of the *O. basilicum* extract (OBE) showed the presence of phenols and flavonoids. Hence, it was standardised with respect to total phenol and total flavonoid contents. Seven phenolics acids and quercetin were quantified in OBE using HPLC-PDA and TLC densitometric methods.

2.4.1. Determination of total phenolic content (TPC)

TPC of OBE was determined by Folin-Ciocalteu method (Hagerman et al., 2000). The amount of TPC was calculated as gallic acid equivalent from the calibration curve of standard gallic acid solutions and expressed as milligrams gallic acid equivalent (mg GAE) per gram dry plant extract. All measurements were done in triplicate.

2.4.2. Determination of total flavonoid content (TFC)

TFC of OBE was determined by aluminium chloride method as described by Lin and Tang (2007). The amount of TFC was determined as quercetin equivalent from the calibration curve of standard quercetin solutions and expressed as milligrams quercetin equivalent (mg QE) per gram dry plant extract. All measurements were done in triplicate.

2.4.3. Estimation of phenolic acids in O. basilicum leaves using HPLC-PDA method

The quantification of phenolic acids in *O. basilicum* was performed as described by Jayasinghe et al., (2003). HPLC-PDA system (Shimadzu Corporation, Kyoto, Japan) fitted with a SIL-20 AC HT autosampler and SPD-M20A photodiode-array detector was used for the quantification of nine phenolic compounds viz. Benzoic acid (1), p-OH benzoic acid (2), Caffeic acid (3), Chlorogenic acid (4), Cinnamic acid (5), p-Coumaric acid (6), Ferulic acid (7), Gallic acid (8) and Vanillic acid (9). The separation of compounds was achieved on reversed-phased C_{18} column (InertSustain®, 4.6×250 mm; 5 µm) operating at 40 °C with a linear gradient of solvent A (acetonitrile/water with 1% acetic acid, 15:85; v/v) and solvent B (methanol) at a flow rate of 0.5 ml/min. The gradient conditions were as follows: 0 min, 2% B; 15 min, 25% B; 30 min, 35% B; 50 min, 55% B. Post run time was

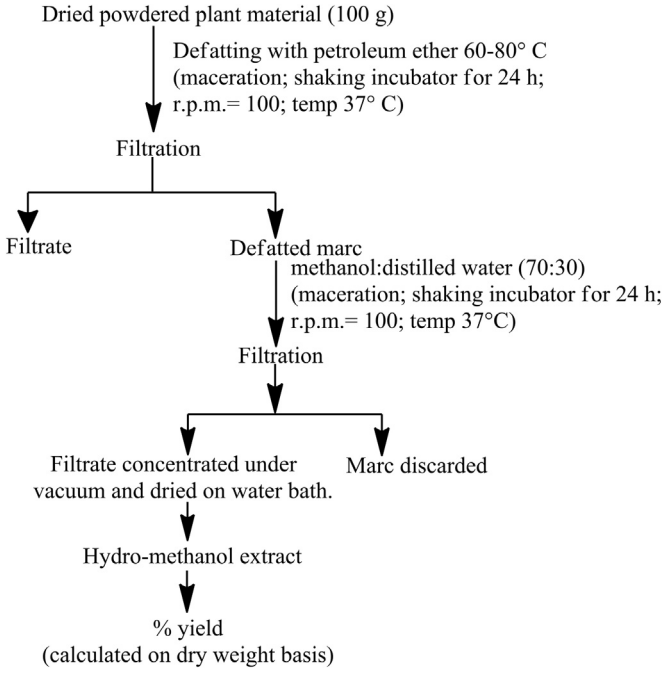

Fig. 1. Scheme for preparation of extracts.