BDPS
Bangladesh Pharmacological Society

Bangladesh Journal of Pharmacology

Volume: 12; Number 2; Year 2017

Cite this article as: Nile SH, Keum YS. Antioxidant, anti-inflammatory and enzyme inhibitory activities of 10 selected Unani herbs. Bangladesh J Pharmacol. 2017; 12: 162-64.

A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2017; 12: 162-164**
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088; DOI: 10.3329/bjp.v12i2.31843

Letter to the Editor

Antioxidant, anti-inflammatory and enzyme inhibitory activities of 10 selected Unani herbs

Sir,

The recognition of the Unani System of Medicine as an alternative system for health care by the World health Organization indicates its importance still present. This system uses herbal formulations that contain wide range of bioactive compounds such as phenolics, vitamins, carotenoids, and other endogenous metabolites having different biological activities. Scientific evaluation of these plants is necessary to find out the novel compounds. Among the huge number of plants, only a limited numbers are screened (Nile and Khobragade, 2011; Kchaou et al., 2015; Nile and Park, 2015). These studies mainly focus on the individual plant. Comparative studies are a few (Alam et al., 2014; Sellem et al, 2016). In the present letter, the antimicrobial, antioxidant, anti-inflammatory, and enzyme inhibitory activity as well as phytochemical analysis of 10 selected herbs (*Rubia cordifolia, Rauwolfia serpentine, Origanum vulgare, Hyssopus officinalis, Cichorium intybus, Malva sylvestris, Portulaca oleracea, Aristolochia indica, Achyranthes aspera, Symplocos racemosa)* are shown and compared.

Each herb (100 g) was extracted with 500 mL of methanol for 5-10 hours using Soxhlet apparatus. An extraction time of 8 hours showed the optimum mass yield. The extract was filtered and concentrated to dryness under vacuum at 40°C and then subjected to lyophilization until a constant weight was obtained. Resulted residue was stored at 5°C for the purpose of further *in vitro* studies. The plant extracts were screened for total phenolics, flavonoids, tannins, and saponins using previously described methods (Zengin et al., 2014). The antioxidant activity was assessed by FRAP and ORAC method (Nile and Park, 2015). Four anti-inflammatory assays were performed viz: diene-conjugate, β-glucuronidase, hyaluronidase, and lipoxidase inhibition (Nile and Khobragade, 2011). The enzyme inhibition activity checked against α-amylase, α -glucosidase, acetylcholinesterase, and butyrylcholinesterase as per methods described previously (Zengin et al., 2016).

The phytochemical study reveals the significant amount phytochemical constituents, namely phenolics, flavonoids, tannins, and saponins in all the studied plant species (Table I). The antioxidant radical scavenging results showed that *P. oleracea* exhibited highest activity (IC_{50} value by FRAP, 210.1 ± 9.1 µg/mL and by ORAC, 184.3 ± 7.4 µg/mL) followed by *H. officinalis*. All the plant extracts show an inhibitory activity against α-amylase, α-glucosidase, acetylcholinesterase and butyrylcholinesterase. The differences observed for enzyme inhibitory activity could be explained by changes in the percent inhibition respect to plant species phytochemical composition. The enzyme inhibitory activities of each herb extract and probably all herbs has justified by the highest level of phenolics and enzyme inhibition. These findings are in accordance with the observed strong relationship between antioxidant activity and phenolics in studied plant-derived extracts (Fernandez-Lopez et al., 2003; Alam et al., 2014). According to the findings for methanolic extracts of six cultivars of *P. oleracea*, the antioxidant activity is mainly attributed to the hydrophobic character of the antioxidant molecules, while the total phenolic content measures both types of antioxidants, hydrophobic and hydrophilic (Lim and Quah 2007). Fathiazad et al (2011) showed that the *H. officinalis* acts as a culinary herb and medicinal plant which may be considered as natural food ingredients to replace synthetic antioxidants due to its biologically active chemical constituents. The results obtained suggested that the herbs used in Unani System of Medicine demonstrated a significant level phytochemicals with different biological activities including antioxidant, anti-inflammatory and enzyme inhibitory activities which were utilized for future drug development.

Acknowledgment: This research was supported by KU-Research Professor Program-2017, Konkuk University, Seoul, Republic of Korea

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Table I

Phytochemical analysis, anti-oxidant and enzyme inhibitory activities of the extracts of selected herbs

Botanical name	*Rubia cordifolia*	*Rauwolfia serpentina*	*Origanum vulgare*	*Hyssopus officinalis*	*Cichorium intybus*	*Malva sylvestris*	*Portulaca oleracea*	*Aristolochia indica*	*Achyranthes aspera*	*Symplocos racemosa*
Unani name	Majeeth	Asrol	Mirzanjosha	Zufa	Hindaba	Khubazi	Khurfah	Zaravand	Chirchita	Lodh Pathani
Phytochemical analysis										
Phenolics (mg GAEs/g extract)	41.4 (2.0)	28.6 (1.2)	56.2 (2.2)	52.3 (2.0)	25.3 (1.3)	40.4 (2.1)	50.6 (2.0)	32.9 (1.2)	22.9 (1.3)	35.2 (1.0)
Flavonoids (mg REs/g extract)	52.6 (1.1)	39.6 (1.3)	71.6 (2.1)	66.5 (2.5)	36.1 (2.0)	50.4 (1.5)	65.4 (2.4)	42.5 (1.3)	33.8 (1.1)	45.7 (1.1)
Tannins (mg CEs/g extract)	36.2 (1.9)	24.9 (1.0)	52.1 (1.1)	48.5 (2.0)	24.3 (1.1)	36.5 (1.1)	45.9 (1.3)	30.1 (1.0)	20.2 (1.0)	30.1 (0.9)
Saponins (mg QAEs/g extract)	2.8 (0.3)	3.1 (0.2)	15.8 (0.9)	18.6 (0.1)	8.6 (0.1)	10.3 (0.2)	14.9 (0.2)	5.6 (0.9)	5.2 (0.1)	8.9 (0.5)
Anti-oxidant activity IC_{50} (µg/mL)										
FRAP	125.6 (5.1)	80.2 (3.4)	152.8 (6.7)	205.8 (8.3)	74.6 (3.5)	110.5 (7.1)	210.1 (9.1)	91.5 (3.1)	75.1 (2.6)	105.2 (4.1)
ORAC	96.3 (2.7)	60.8 (1.8)	120.5 (9.7)	175.1 (8.1)	56.2 (1.8)	89.8 (5.4)	184.3 (7.4)	71.6 (2.7)	58.6 (2.9)	80.1 (3.1)
Anti-inflammatory activity inhibition (%)										
β-Glucuronidase	58.5 (1.2)	31.3 (1.1)	68.9 (1.2)	78.2 (1.2)	25.5 (1.1)	52.4 (1.2)	80.1 (1.2)	42.6 (1.1)	31.2 (1.2)	48.1 (1.2)
Diene-conjugate	61.3 (0.3)	35.2 (0.1)	71.6 (0.2)	80.1 (0.3)	30.8 (0.1)	58.5 (0.2)	83.8 (0.3)	46.8 (0.1)	34.8 (0.2)	53.4 (0.3)
Hyaluronidase	40.8 (2.1)	32.8 (1.0)	64.8 (1.4)	70.2 (2.1)	20.7 (1.0)	45.6 (1.4)	73.6 (2.1)	25.9 (1.0)	22.6 (1.4)	41.6 (2.1)
Lipoxidase inhibition	52.7 (1.5)	38.4 (1.3)	71.2 (1.1)	76.1 (1.5)	28.2 (1.3)	52.2 (1.1)	74.8 (1.5)	30.5 (1.3)	27.4 (1.1)	46.9 (1.5)
Enzyme inhibitory activities										
α-Amylase (mmol ACEs/g extract)	4.7 (0.1)	3.5 (0.2)	6.6 (0.3)	7.2 (0.2)	2.6 (0.2)	4.8 (0.3)	7.8 (0.3)	4.3 (0.2)	3.2 (0.1)	4.5 (0.2)
α-Glucosidase (mmol ACEs/g extract)	7.2 (0.1)	4.3 (0.3)	8.1 (0.2)	8.6 (0.3)	3.2 (0.3)	5.8 (0.2)	8.5 (0.2)	4.6 (0.3)	3.5 (0.2)	5.2 (0.6)
Acetylcholinesterase (mg GALAEs/g extract)	4.5 (0.1)	1.3 (0.1)	3.8 (0.2)	4.4 (0.2)	2.1 (0.3)	2.9 (0.2)	4.2 (0.1)	2.7 (0.1)	1.9 (0.2)	3.2 (0.1)
Butyrylcholinesterase (mg GALAEs/g extract)	2.5 (0.2)	0.8 (0.2)	1.0 (0.2)	2.5 (0.2)	0.9 (0.3)	1.0 (0.3)	2.5 (0.2)	0.7 (0.1)	0.6 (0.2)	1.2 (0.3)

Data are the mean and SD (within the parenthesis)

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