

Full Length Research Paper

Acetyl cholinesterase inhibition potential and antioxidant activities of ferulic acid isolated from *Impatiens bicolor* Linn.

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Ferulic acid was isolated from ethyl acetate extract of *Impatiens bicolor* Linn. and identified by X-ray analysis. The acetylcholine esterase inhibition, radical scavenging activity (DPPH scavenging assay) and FRAP assay were carried out on the compound. It was found that the effect of ferulic acid was strongly dose dependent i.e., 25 - 175 µg/ml in DPPH assay, 50 - 150 µg/ml in FRAP assay and 50 - 200 µg/ml in acetylcholine esterase inhibition assay. However, ferulic acid did not show significant change in acetylcholine esterase inhibition and antioxidant activity at high concentration.

Key words: Acetyl cholinesterase, FRAP, ferulic acid, *Impatiens bicolor*, medicinal plant.

INTRODUCTION

Alzheimer's disease (AD) patients present a progressive loss of cholinergic synapses in the brain regions associated with higher mental functions, mainly the hippocampus and neocortex. In the AD patients, a decrease in the acetylcholine (ACh), a neurotransmitter, appears to be critical element in the development of dementia. Hence, AD and other form of dementia could be treated by the use of agents that restore the level of acetylcholine through the inhibition of both major form of cholinesterase: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Moreover, the inhibition of AChE plays a key role not only enhancing cholinergic transmission in the brain, but also reducing the aggregation of amyloid beta peptide (A β) and the formation of the neurotoxic fibrils in AD (Loizzo et al, 2008; Candy et al., 1983).

Impatiens bicolor Linn (Balsaminaceae) is an annual herb, 45 - 60 cm tall. It is distributed throughout the Northern areas of Pakistan especially Muree, Nathia Gali and Miran Jani. The genus *Impatiens* is rich in organic acids, anthraquinones and flavonoids. Charles and Hagen (1996) have reported the isolation of 3 monoglucosides of kaempferol, quercetin and pelargonidin from the

stem of *Impatiens balsamina*. Pelargonidin and peonidin as a glucoside have been characterized from *Impatiens holstii*. Three new flavanone glycosides and six known flavonoid glycosides have been reported from the leaves of *I. bicolor* (Hassan and Tahir, 2005). Salicylic acid, sinnapic acid, caffeic acid, scopletin, 2-hydroxy, 1,4-naphthaquinone and 2-methoxy-1,4-naphthaquinone were isolated from the stem of *I. balsamina* and *I. bicolor* (Bhom and Towers, 1962; Yuan-Chuan et al., 2009). Panichayupakaranant et al. (1998) isolated a new biscoumarin, 4,4'-biisofraxidin, from the roots of *I. balsamina*. *I. balsamina* extracts have shown a long lasting skin moisturizing effect and prevents dryness, rough skin chap, dandruff and split hair ends (Toki et al., 2000). The aim of present study was to evaluate acetyl cholinesterase inhibition potential and antioxidant activities of ferulic acid from *I. bicolor*.

MATERIALS AND METHODS

Chemicals

Acetylthiocholine iodide, 5,5'-dithiobis [2-nitrobenzoic acid] (DTNB), 2,2'-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxy toluene (BHT), gallic acid and galanthamine were purchased from Sigma (St. Louis, MO, USA) while, erythrocytes (Acetylcholine esterase) obtained from the Biochemistry Lab, Mayo Hospital Lahore. All other chemicals used were of analytical grade.

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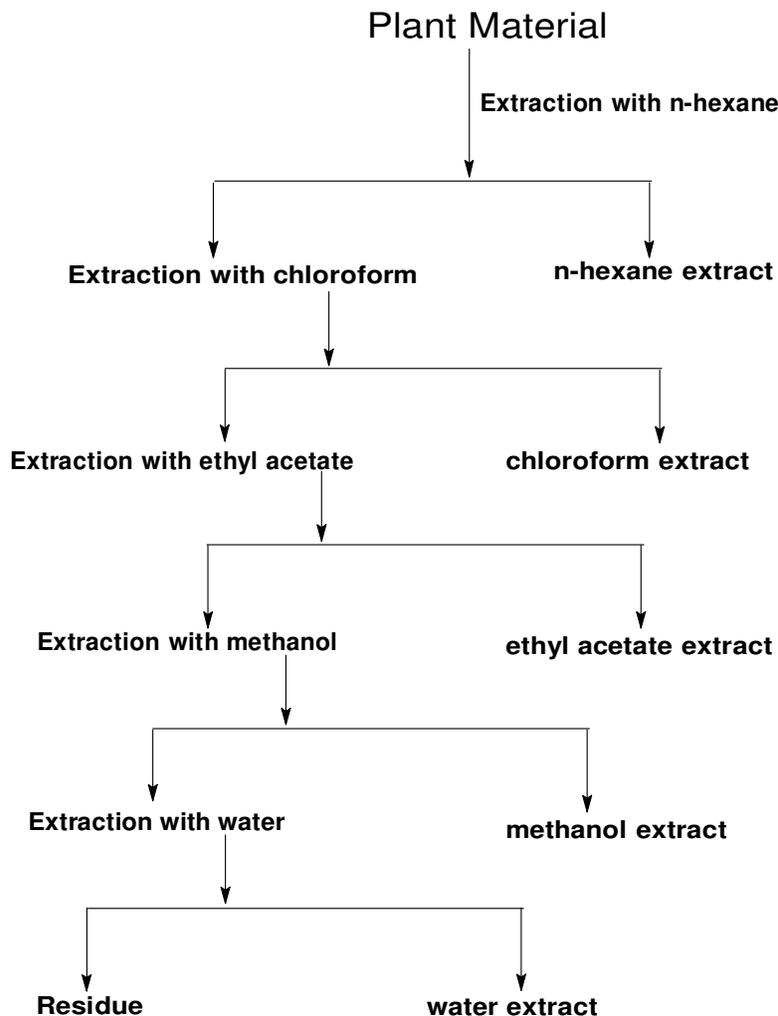


Figure 1. Extraction scheme of various extracts of *Impatiens bicolor*.

Plant material

The plant material *I. bicolor* was collected from Ayubia Park, Muree and identified at the Department of Botany (GC University, Lahore) where a voucher specimen was submitted. Air dried branches and leaves were powdered and extracted in different solvents (n-hexane, chloroform, ethyl acetate and methanol) using soxhlet apparatus. Crude extracts were filtered and concentrated at reduced temperature using rotary evaporator.

Isolation and identification of ferulic acid

The various extracts of *I. bicolor* were obtained by soaking the whole plant in different solvents with increasing order of solvent polarity, in the sequence n-hexane, chloroform, ethyl acetate, methanol (Figure 1). The ethyl acetate extract was further subjected to column chromatography in silica packed column. Ethyl acetate extract (3.0 g) of *I. bicolor* was added to the column and elution was carried out with n-hexane, n-hexane-chloroform (5:95, 10:90, 25:75, 50:50 and 75:25), chloroform, chloroform-ethyl acetate (5:95, 10:90, 25:75, 50:50 and 75:25) and ethyl acetate resulted in total 175

fractions. The fractions obtained in chloroform-ethyl acetate (25:75) were pooled, dried and rechromatographed which yielded FA-IB (ferulic acid) (9.93% yields).

The X-Ray analysis of the isolated compound (FA-IB) was carried out on Bruker KAPPA APEX II diffractometer using Mo K α X-ray (0.71073 Å) source and a graphite monochromator (Bruker, 2007) while FTIR, ^1H NMR and ^{13}C NMR were carried out at HEJ Research Institute of Chemistry Karachi, Pakistan.

Ferulic acid

Light yellow, Melting point: 169 -170 °C, Mol. Wt. 194.18
 FTIR (KBr disc, cm $^{-1}$): 3424 (OH), 2921(CH), 1620 (C=O), 1516 (C=C), 1432 (OH).

^1H NMR= (400 MHz, CDCl $_3$): δ 3.9 (3H, s, OCH $_3$), δ 6.4 (1H, d, J = 14.5 Hz, CH), δ 6.9 (1H, d, J = 8.3 Hz, CH), δ 7.15 (1H, d, J =8.3 Hz, CH), δ 7.3 (1H, s, CH), δ 7.6 (1H, d, J =14.5 Hz, CH), δ 8.3 (1H, s, -OH).

^{13}C NMR (75 MHz, CDCl $_3$): δ 55.9 (Ar.OCH $_3$), δ 112.7 (C-6), δ 115.2 (C-3), δ 115.6 (C-9), δ 119.7 (C-4), δ 126.3 (C-5), δ 145.0 (C-8), δ 147.5 (C-1), δ 148.8 (C-2), δ 168.2 (C-10).

Acetylcholine esterase assay

Acetylcholine esterase inhibitory activity of ferulic acid was measured by the spectrophotometric method developed by Ellman et al. (1961). Acetylthiocholine iodide was used as substrate in the assay. The reaction mixture contained 1500 μ l of (100 mM) tris buffer (pH 7.8), 1000 μ l of DTNB, 200 μ l (50, 100, 150, 200, 250 μ g/ml) of test-compound solution and 200 μ l of acetyl cholinesterase solution (erythrocytes), which were mixed and incubated for 15 min (25°C). The reaction was initiated by the addition of 200 μ l acetylthiocholine. The hydrolysis of acetylthiocholine was monitored at 412 nm after 30 min. Galanthamine was used as positive control. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows:

$$\% \text{ age inhibition} = (E - S) / E \times 100$$

Where; E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound.

DPPH scavenging assay

Free radical scavenging activity was measured by using DPPH method (Erasto et al., 2004). Different concentrations of ferulic acid (25 - 250 μ g/mL) solution were added to 1 ml methanolic solution of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) (2 mg/ml). The absorbance was measured at 517 nm after 30 min. Results were evaluated as percentage scavenging of radical (% scavenging of DPPH = $(\text{Abs. of blank} - \text{Abs. of sample}) / \text{Abs. of blank} \times 100$). IC₅₀ value (concentration of sample where absorbance of DPPH decreases 50 % with respect to absorbance of blank) of the sample was determined.

FRAP assay

Ferric reducing antioxidant power assay was carried out using diluted sample of ferulic acid in the range of 25 - 200 μ g/ml following the Benzie and strain's protocol (Benzie and Strain, 1999). 150 μ L of FRAP reagent and 20 μ L of sample were mixed. Before mixing with the FRAP reagent, all the samples were diluted so that ferulic acid concentration were equal to 25, 50, 100, 150, 200 μ g/ml in 20 μ l sample volume respectively. A time interval of eight minute was chosen between addition of the sample to the FRAP reagent and the second absorbance reading to allow the samples to react with ferric tripyridyltriazine (Fe^{III}-TPTZ) complex in the reagent.

RESULTS

Characterization of compound

Column chromatography of the ethyl acetate extract of dried powdered plant of *I. bicolor* resulted in the isolation of compound FA-IB as a light yellow crystalline solid (298 mg, 9.93%). The X-ray structure (Figure 2) of the isolated compound (FA-IB) was determined by X-ray diffraction technique while structure was confirmed through FTIR, ¹HNMR and ¹³CNMR spectral studies as well (Table 1).

Acetylcholine esterase inhibition potential

Acetylcholine esterase potential of FA-IB was determined in a range of concentration (50, 100, 150, 200, 250 μ g/ml)

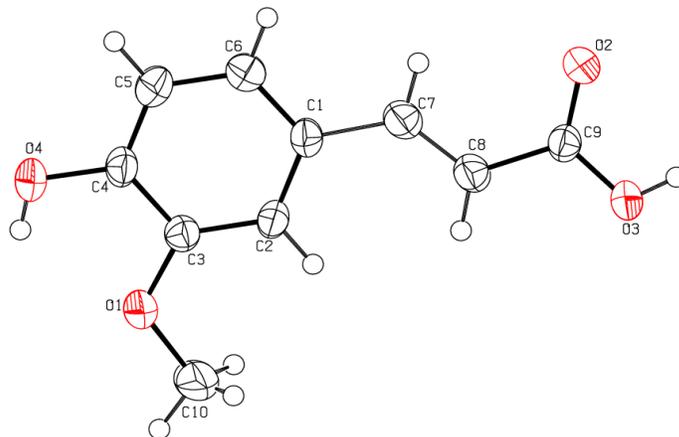


Figure 2. X-ray structure of FA-IB.

by Ellman's method (1961). The inhibitory activity of different concentrations is summarized in Figure 3. The inhibition of AChE was increased rapidly from 12.38 - 42.86% with the increase in the concentration of ferulic acid from 50 - 200 μ g/ml followed by slow change till 250 μ g/ml. At this concentration a steady state was achieved.

DPPH assay

The results of DPPH assay as shown in Figure 4 indicated significant free radical scavenging potentials of ferulic acid at low concentration. The rate of reaction measured as the colour change was observed to be very fast up to 175 μ g/ml. No significant change was observed beyond this concentration (94.32% at 200 μ g/ml).

FRAP assay

Antioxidants in the sample reduce Fe^{III}-TPTZ to form a blue coloured Fe^{II}-TPTZ complex, which results in increase of absorbance at 593 nm (Benzie and Strain, 1996, 1999). The calibration curve revealed a high positive linear ($R^2 = 0.993$) relationship between mean FRAP value and concentration of FeSO₄·7H₂O standards (Figure 5). This curve was therefore, employed to reliably estimate antioxidant potential of the test sample. The mean FRAP value of ferulic acid ranges from 40.98 ± 4.23 to 145.6 ± 3.23 of FeSO₄·7H₂O equivalent (Figure 6).

DISCUSSION

Ferulic acid is used in the food industry to inhibit oxidation of fats. It is also used as starting material for microbial conversion to vanillin. It shows antineoplastic properties and gyrostatic activity. Choleric agent and antifungal agent used to prevent fruit spoilage (Graf, 1992). Repeated column chromatography of the ethyl

Table 1. X-ray crystal data of FA-IB.

Empirical formula	C ₁₀ H ₁₀ O ₄
Formula Weight	194.18
Temperature/K	296(2)
Wavelength / Å	0.71073
Crystal System, Space group	Monoclinic, P 1 21/n 1
Unit cell dimensions	
a/ (Å)	4.6351(8)
β / (°)	90.00
b/ (Å)	16.797(2)
γ / (°)	90.155(7)
c/ (Å)	11.9958(17)
γ _o / (°)	90.00
Volume/ Å ³	933.9(2)
Z, Calculated density/ (g.cm ⁻³)	4, 1.38
Absorption coefficient/ mm ⁻¹	0.108
F (000)	408
Crystal size/ mm	0.38×0.22×0.14
Range for data collection/(°)	2.4 to 28.4
Limiting indices	-3 ≤ h ≤ 6, -22 ≤ k ≤ 21, -15 ≤ l ≤ 15
Reflection collected/unique	6611/2123 [R(int) = 0.063]
Completeness to θ	90.1%(28.32)
Absorption correction	multi-scan
Refinement method	Full-matrix least- squares on F²
Data/restraints/parameters	2123/0/134
Goodness-of-fit on F ²	0.968
Final R indices [I > 2 σ (I)]	R1 = 0.067, wR2 = 0.105
R indices (all data)	R1 = 0.188, wR2 = 0.132
Largest diff. peak and hole/(e.Å ⁻³)	0.176 and -0.213

acetate extract of *Impatiens bicolor* resulted in the isolation of white crystalline compound in 9.93% yield (298 mg). The sample was found to be analytically pure through thin layer chromatography analysis. The sample was identified as ferulic acid using X-ray analysis. The assignments of the structure were in consonance with the previously observed structure (Nethaji et al., 1988).

Acetylcholine (ACh), a neurotransmitter, is widely distributed in the nervous system and has been implicated to play an important role in cerebral cortical development, cortical activity, cerebral blood flow control, modulation of cognitive performance, and a signal transfer in the synapses. Loss of cholinergic innervations, as demonstrated by reduced choline acetyltransferase (ChAT) and increased AChE activity, is correlated with the degree of dementia and the severity of the neuropathological hallmarks of Alzheimer's disease (AD). AChE inhibitors decrease the hydrolysis of ACh to elevate the endogenous level of ACh in the brain and to boost cholinergic

neurotransmission. Therefore, it was considered that elevating the level of ACh might be helpful in attenuating the symptoms of neuronal deficits and treating cognitive dysfunction in the mild to moderate cases of AD (Ogawa et al., 2006; Scliebs and Arendt, 2006; Ferreira et al., 2006).

In order to search for an effective AChE inhibitor from natural sources, different concentrations of ferulic acid were screened by using Ellman's method (1961). The inhibitory activity of different concentration of FA-IB is summarized in Figure 3. Highest inhibitory activity against the AChE was observed at the concentration of 250 μ g/ml.

Radical scavenging action is an important attribute of antioxidants, which is measured by DPPH radical scavenging assay. DPPH[•], a protonated radical, has a characteristic maximum absorbance at 517 nm, which decreases with the scavenging of the protonated radical (Jao and Ko, 2002). Ferulic acid exhibited a marked

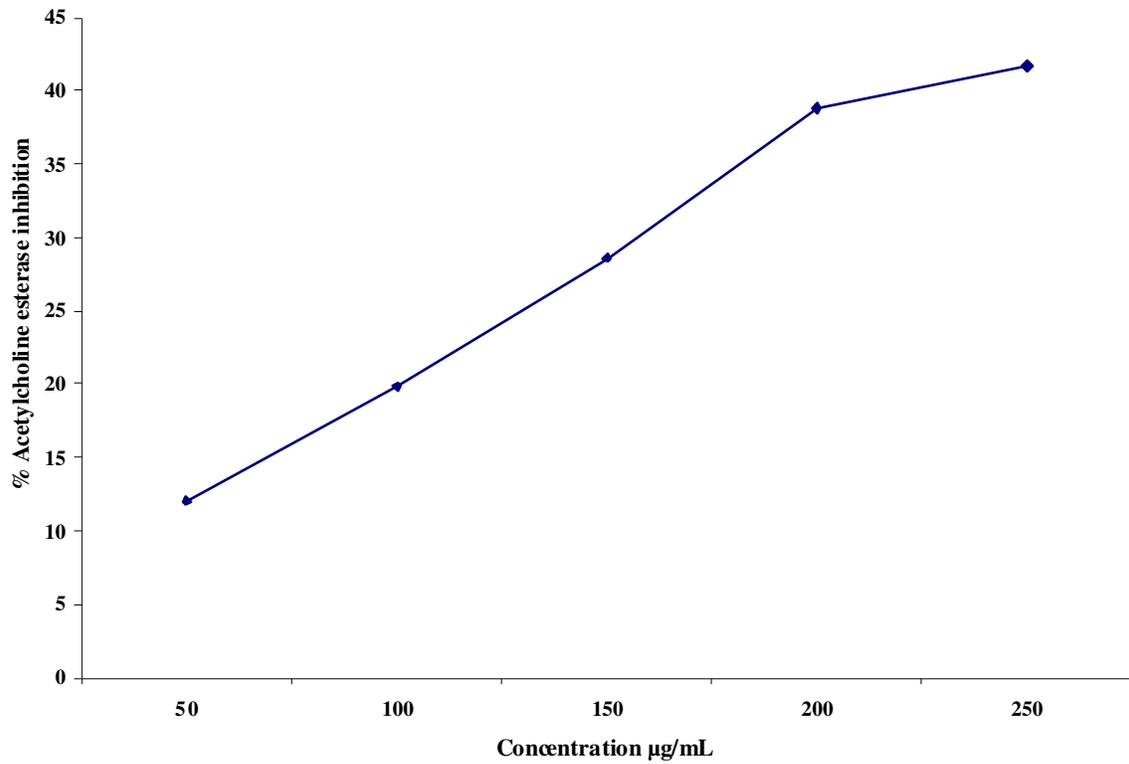


Figure 3. AChE inhibition potential of FA-IB.

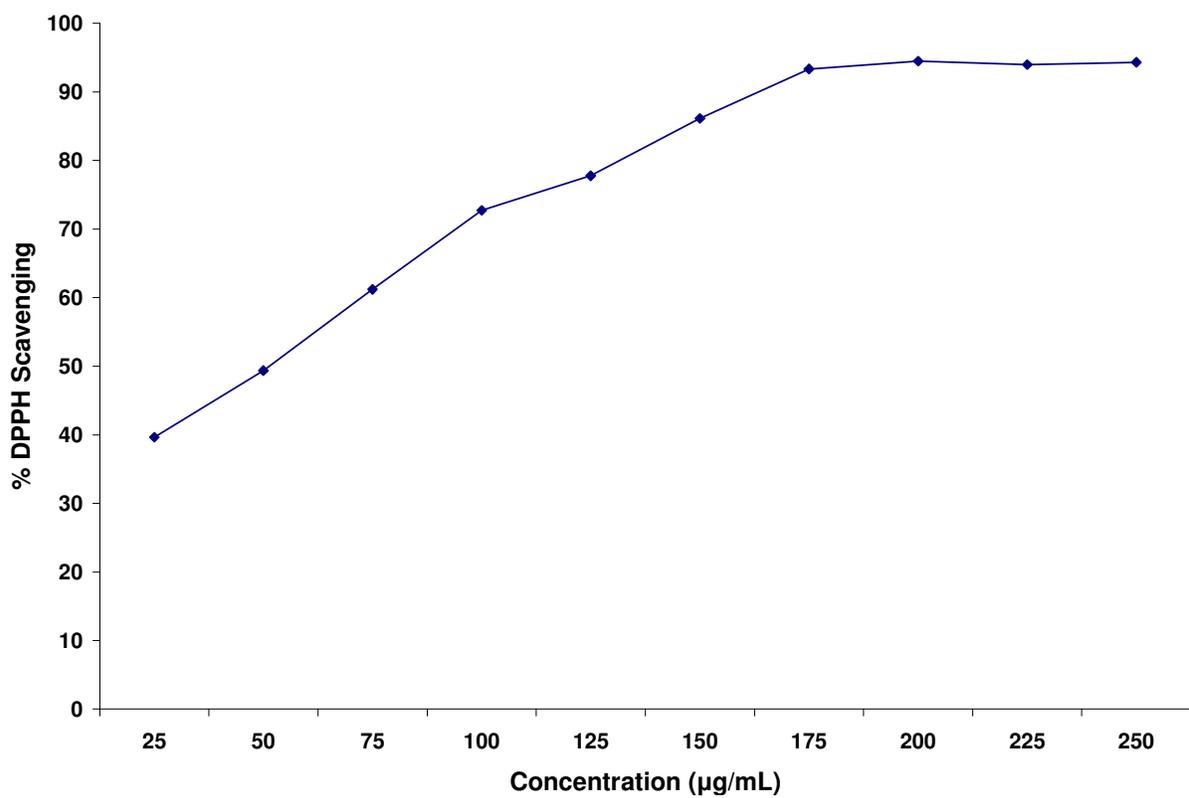


Figure 4. Scavenging potential of FA-IB in DPPH assay.

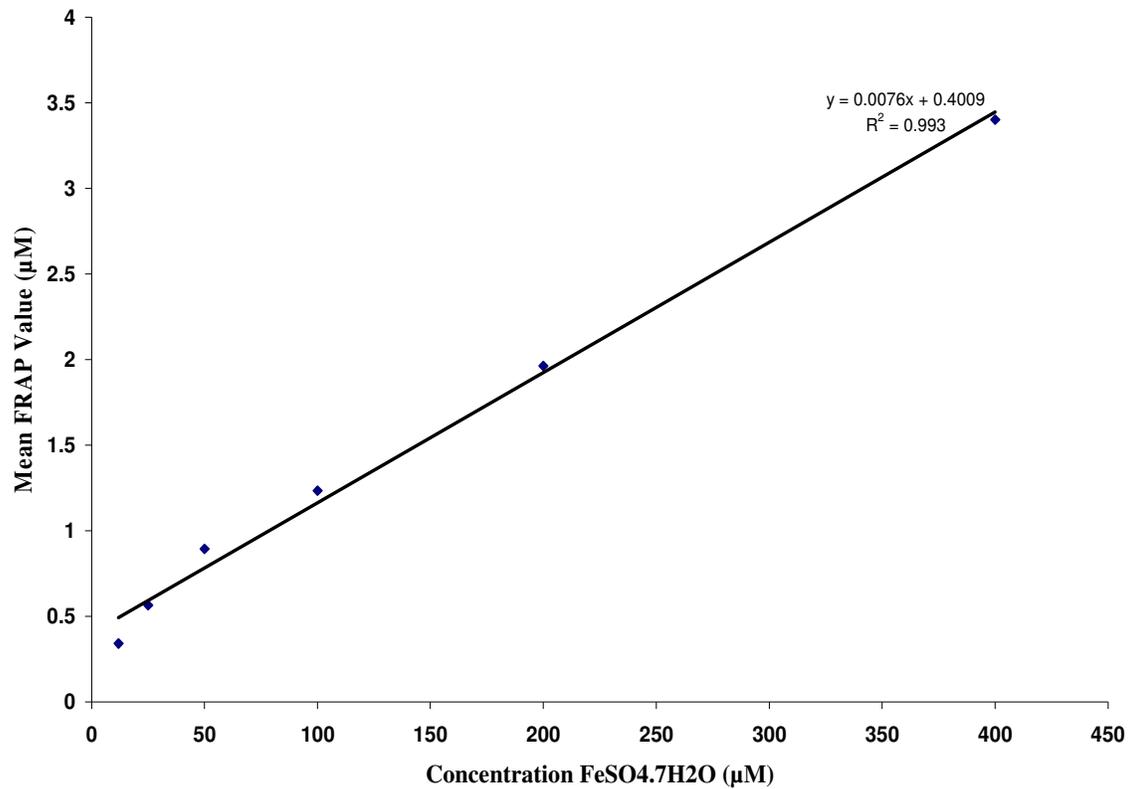


Figure 5. A representative calibration curve for the determination of mean FRAP value using FeSO₄.7H₂O standards (12.5, 25, 50, 100, 200, 400 µM).

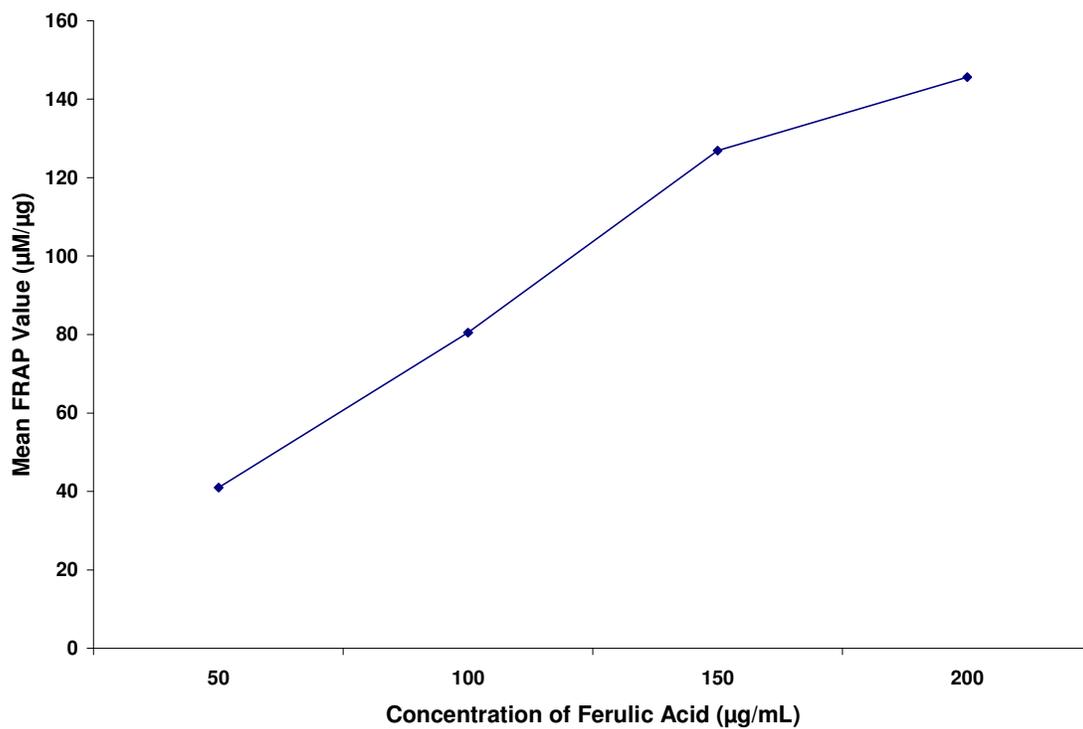


Figure 6. FRAP assay at different concentration of FA-IB.

increase in radical scavenging activity with increase in concentration up to 175 µg/ml and then become almost steady at high concentrations. These results can be attributed to the presence of -OH group at the para position in ferulic acid as reported by Karamac et al. (2005), Masashi et al. (2002).

Total antioxidant power of ferulic acid was measured by using the ferric reducing/antioxidant power (FRAP) assay. In the FRAP assay, reductants (antioxidants) in the sample reduce Fe³⁺/tripyridyltriazine complex, present in stoichiometric excess, to the blue colored ferrous form, with a resultant increase in absorbance at 593 nm. The change in absorbance (ΔA) is thus theorized to be proportional to the combined (total) ferric reducing/antioxidant power (FRAP value) of the antioxidants in the sample. The Mean FRAP values of ferulic acid indicated the strong antioxidant tendency of this compound for Fe³⁺ present in the human blood plasma. Our results were in agreement with the finding of Jeffrey et al. (2007).

Conclusion

Ferulic acid (FA-IB) purified from the ethyl acetate extract of *I. bicolor* has been reported for the first time in this species. It showed a significant AChE inhibitory potential and radical scavenging activity in different *in vitro* assays which provide scientific validation of this compound to be used as a herbal remedy.

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REFERENCES

- Benzie IFF, Strain JJ (1999). Ferric reducing/antioxidant power assay: Direct measure of the total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* 299: 15-27.
- Bohm BA, Towers GHN (1962). A study of phenolic compounds in *Impatiens*. *Can. J. Bot.* 40: 677-683.
- Bruker C (2007). APEX2SAINT. Bruker AXS Inc., Madison, Wisconsin, USA.
- Candy JW, Perry RH, Perry EK, Irving D, Blessed G, Fairbairn AF (1983). Pathological changes in the nucleus of Meynert in Alzheimer's and Parkinson's diseases. *J. Neurol. Sci.* 59: 277-289.
- Charles W, Hagen JE (1996). Isolation of antimicrobial compounds from *Impatiens balsamina* L. *Am. J. Bot.* 53: 46.
- Ellman GL, Courtney KD, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7: 88-95.
- Erasto P, Bojase-Moleta G, Majinda RRT (2004). Antimicrobial and antioxidant flavonoids from the root wood of *Bolusanthus speciosus*. *Phytochem.* 65: 875-880.
- Ferreira A, Proenca C, Serralheiro ML, Araujo ME, (2006). The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *J. Ethnopharmacol.* 108: 31-37.
- Graf E (1992). Antioxidant potential of ferulic acid. *Free-Radic-Biol-Med.* 13: 435-448.
- Hassan A, Tahir N (2005). Flavonoids from the Leaves of *Impatiens bicolor*. *Turk. J. Chem.* 29: 65-70.
- Jaoo CH, Ko WC (2002). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. *Fish. Sci.* 68: 430-435.
- Jeffrey G, Krzysztof K, Maciej K, Piotr N, Boguslaw K, Dariusz N (2007). Ferric-Reducing Ability Power of Selected Plant Polyphenols and Their Metabolites: Implications for Clinical Studies on the Antioxidant Effects of Fruits and Vegetable Consumption. *Basic Clin. Pharmacol. Toxicol.* 5: 345-352.
- Karamac M, Aadam B, Ronald BP, Ryszard A (2005). Antioxidant and Antiradical Activity of Ferulates. *Czech J. Food Sci.* 23: 64-68.
- Loizzo MR, Tundis R, Menichini F, Menichini F (2008). Natural Products and their Derivatives as Cholinesterase Inhibitors in the Treatment of Neurodegenerative Disorders: An Update. *Curr. Med. Chem.* 12: 1209-1228.
- Masashi H, Kanae H, Kayo A, Hisaji T. (2002). Antioxidant Properties of Ferulic Acid and Its Related Compounds Hiroe Kikuzaki. *J. Agr. Food. Chem.* 50: 2161-2168.
- Nethaji M, Ratrabhi V, Desiraju GR (1988). Structure of 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid (ferulic acid). *Acta Cryst. C.* 44: 275-277.
- Ogawa M, Iida Y, Nakagawa M, Kuge Y, Kawashima H, Tominaga A, Ueda M, Magata Y, Saji H (2006). Change of central cholinergic receptors following lesions of nucleus basalis magnocellularis in rats: search for an imaging index suitable for the early detection of Alzheimer's disease. *Nucl. Med. Biol.* 33: 249-254.
- Panichayupakaranant P, Noguchi H, De-Eknamkul W (1998). A New Biscoumarin from *Impatiens balsamina* Root Cultures. *Planta Med.* 64: 774-775.
- Sciebs R, Arendt (2006). The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. *J. Neural Transm.* 113: 1625-1644.
- Toki M, Kondo M, Maeda M (2000). Flavonoids from the Leaves of *Impatiens bicolor*. *Jpn. Kokai Tokkyo Koho. J.* 143: 488.
- Yuan-Chuen W, Wan-Yu L, Deng-Chyang W, Jeh-Jeng W, Cheng-Hsun W, Jyun-Ji L, Cheng-Kun L (2009). *In vitro* activity of 2-methoxy-1, 4-naphthoquinone and stigmasta-7,22-diene-3b-ol from *Impatiens balsamina* L. against multiple antibiotic-resistant *Helicobacter pylori*. *eCAM.* pp.1-8.