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Full Length Research Paper

Acetylcholine esterase and antioxidant potential of some members of Asteraceae and Euphorbiaceae

Durre Shahwar[1]*, Sami Ullah[1], Muhammad Asam Raza[2], Uzma Sana[1], Asma Yasmeen[1], Sadia Ghafoor[1] and Naeem Ahmad[1]

[1]Research Laboratory II, Department of Chemistry, Government College University, Lahore-54000, Pakistan.
[2]Department of Chemistry, University of Gujrat, Hafiz Hayat Campus, Gujrat, Pakistan.

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Asteraceae and Euphorbiaceae have great medicinal importance. Keeping this in view, the present study was conducted to evaluate the enzyme inhibition and antioxidant potential of some members of both families. *Bidens biternata* (Family: Asteraceae), *Senecio macrophylla* (Family: Asteraceae) and *Croton sparsiflorus* (Family: Euphorbiaceae) were extracted in methanol and partitioned with n-hexane, chloroform and n-butanol successively. Acetylcholine esterase and antioxidant activities were carried using reported methods. It was concluded that butanolic extract of *C. sparsiflorus* exhibited maximum enzyme inhibition activity (95.0 ± 2.0% with IC_{50} = 7 ± 1 µg/ml), while all other extracts showed significant activity except n-hexane extract of *S. macrophylla* which remained inactive. Total phenolic contents in the extracts ranged from 20 ± 1 to 720 ± 6 mg GAE/g of extract and highest phenols were present in the butanol extract of *S. macrophylla*. The butanolic extract of *B. biternata* showed highest antiradical activity (88.9 ± 2.2%, IC_{50} = 55 ± 3 µg/ml) and FRAP activity 8.98 ± 0.11 µM/g of the extract. The n-hexane extract of *B. biternata* and *S. macrophylla* showed very low response in 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP) activity respectively.

Key words: Asteraceae, Euphorbiaceae, antioxidant, enzyme inhibition.

INTRODUCTION

Asteraceae with its approximately 1,620 genera and more than 23,600 species is the largest family of flowering plants (Bekir et al., 2011). The family is distributed worldwide, except for Antarctica but is especially diverse in the tropical and subtropical regions of North America, eastern Brazil, southern Africa, central Asia, and southwestern China. The family contains several species that are important sources of cooking oils, sweetening agents, and tea infusions (Rieseberg et al., 2003). Most of the members of Asteraceae are used as phytomedicines. In some species of Asteraceae, sesquiterpene lactones have been isolated and found to have cytotoxic, mutagenic and anti-tumor properties (Picman, 1986).

Bidens biternata (Family: Asteraceae), commonly known as spanish needle, is located in Himalayan regions. The genus *Bidens* has been used in traditional medicine as anti-inflammatory, anti-malarial, anti-allergic, anti-ulcer, anti-diabetic, anti-cancer and antibacterial agent (Sandra et al., 2000; Parimalakrishnan et al., 2006; Masako and Yoshiyuki 2006; Maicon et al., 2008). Phytochemical studies of *Bidens* showed that it is a rich source of bioactive compounds such as polyacetylenic glycosides, aurons, auron glycosides, *p*-coumeric acid derivatives, flavonoids and flavonoid glycosides, sesquiterpenes, phenylpropanoid glucosides, diterpenes (Khemraj et al., 2010; Yutaka et al., 1991; Carmelita et al., 1995).

Senecioneae is one of the largest tribes of Asteraceae, comprised of about 150 genera and 3000 species. *Senecio macrophylla* also known as *Ligularia macrophylla* a herb of family Asteraceae, occurs in damp places. Several chemical constituents of this plant are reported; kaempferol, 2,4'-dihydroxy-5-methoxychalcone, 5-hydroxy-3,4',7-trimethoxyflavone, isobutyl ester terephthalic acid, lupeol, *β*-sitosterol and bisesquiterpene (Tian et al., 2010). Traditionally the roots of *S.*

*Corresponding author. E-mail: drdshahwar@yahoo.com. Tel: +92-42-9213340, Ext. 266. Fax: +92-42-9213341.

macrophylla are used as antiasthmatic, anticancer and antibacterial agents (Tong et al., 2005).

Euphorbiaceae comprises of 300 genera and 5,000 species including *Croton sparsiflorus* Morong, a monoecious woody herb, is widely distributed in plan areas of Asia, South America and Brazil. Some of the species of this genus are reported to possess analgesic, purgative, and anti-inflammatory activities (Nadkarni and Nadkarni, 1992). The genus *Croton* is well known for its diterpenoid content such as phorbol esters, clerodane, labdane, kaurane, trachylobane, pimarane. Several bioactive aporphine alkaloids have also been isolated from this genus (Bhakuni et al., 1970; Helda et al., 1996).

Acetylcholine, the first neurotransmitter to be identified is the principal neurotransmitter in the peripheral, central, somatic and the autonomic nervous system. It relays the information across the gap (synapse) between the neuron and its neighboring cells. In the absence of acetylcholine estrase enzyme, acetylcholine accumulates at the synapse, thus paralysis occurs and it also stops the heart beat (Campbell and Reece, 2002). Acetylcholine esterase enzyme hydrolyzes the acetylcholine (neurotransmitters) into choline and acetate group and blocks its signaling effect (Luis et al., 2006). It is mainly found in the neuromuscular junction. Cholinesterase inhibitors have crucial role for the treatment of Alzheimer s disease (AD), which is due to low AChE neuromuscular junction. Inhibition of AChE increases the neurotransmitter concentrations within the synaptic cleft, which have positive influence on AD patients (Aleksandro et al., 2011).

Antioxidants, being non enzymatic defenses of the biological systems against free radicals are important for health. Free radicals are present in living physiologies in the form of reactive oxygen and nitrogen species (Wong et al., 2006). Oxidative stress causes an over production of free radicals. These reactive species, interacting the biological systems causes cell death, thus leading to chronic diseases like cancer, cardio and cerebrovascular systems. Free radicals causes neural disorders such as Alzheimer's and Parkinson's diseases. Phytochemicals such as phenolic compounds terminates the action of free radicals, so beneficial for health (Kubola et al., 2008). Polyphenolics decrease the oxidative stress and reduces the risk of degenerative diseases. These polyphenols are widely distributed in plants, so they are the main contributors of antioxidants. Flavanoids being the most potent antioxidants effectively quenches free radicals and reduce damages (Picinelli et al., 2009).

Recently studies have demonstrated the importance of medicinal plants as an abundant source of biologically active compounds, which can provide basis for the development of new lead compounds for pharmaceuticals. Therefore, present study was carried out to assess enzyme inhibition and antioxidant potential of various extracts of *B. biternata, S. macrophylla* and *C. sparsiflorus*.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu (FC) reagent, DPPH, butylated hydroxy toluene (BHT), acetylthiocholine iodide, 5,5´-dithiobis [2-nitrobenzoic acid] (DTNB) were purchased from Sigma-Aldrich (USA) while, erythrocytes (acetylcholine esterase) obtained from the Biochemistry Laboratory, Mayo Hospital Lahore. All other chemicals and solvents used were analytical grade from Merck.

Collection of plant material

B. biternata and *S. macrophylla* were collected from Northern Areas of Pakistan, while *C. sparsiflorus* was collected from Lahore regions. The plant materials were identified at Department of Botany (GC University, Lahore), where a voucher specimen was deposited (*B. biternata* = GCU-BOT-192, *S. macrophylla* = GCU-BOT-196, *C. sparsiflorus* = GCU-BOT-202).

Preparation of plant extract

Aerial parts of all plants were dried under shade, powdered and soaked in methanol, crude extract was filtered and concentrated on rotary evaporator at reduced pressure. The crude extracts were dissolved in water and fractionated with n-hexane, chloroform and n-butanol successively.

In-vitro AChE inhibition assay

Acetylcholine esterase inhibitory (AChE) activity was measured by Ellman's (1961) method with minor modification (Shahwar et al., 2010a, 2011). Acetylthiocholine iodide was used as substrate in the assay. The reaction mixture contained 2.0 ml of 100 mM tris buffer (pH 7.8), 100 µl of acetylcholine esterase (erythrocytes), 200 µl of extract/fractions, 100 µl of DTNB and incubated for 15 min (25℃). The reaction was initiated by the addition of 200 µl acetylthiocholine. The hydrolysis of acetylthiocholine was monitored at 412 nm after 30 min. The percentage inhibition was calculated as follows:

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

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

Determination of antioxidant activity

Scavenging assay of DPPH free radical

Radical scavenging activity of extracts/fractions was assayed according to the method of (Shahwar et al., 2010b) using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical. DPPH solution was prepared as 0.0025 g/ml in methanol. 200 µl of sample (1 mg/ml) was mixed with 1.0 ml DPPH solution, test tubes were kept in dark for half an hour and noted the absorbance at 517 nm. The scavenging of free radical was calculated using following formula.

$$\% \text{ Inhibition of DPPH} = \frac{A - B}{A} \times 100$$

Where A is the absorbance of blank and B is the absorbance of sample.

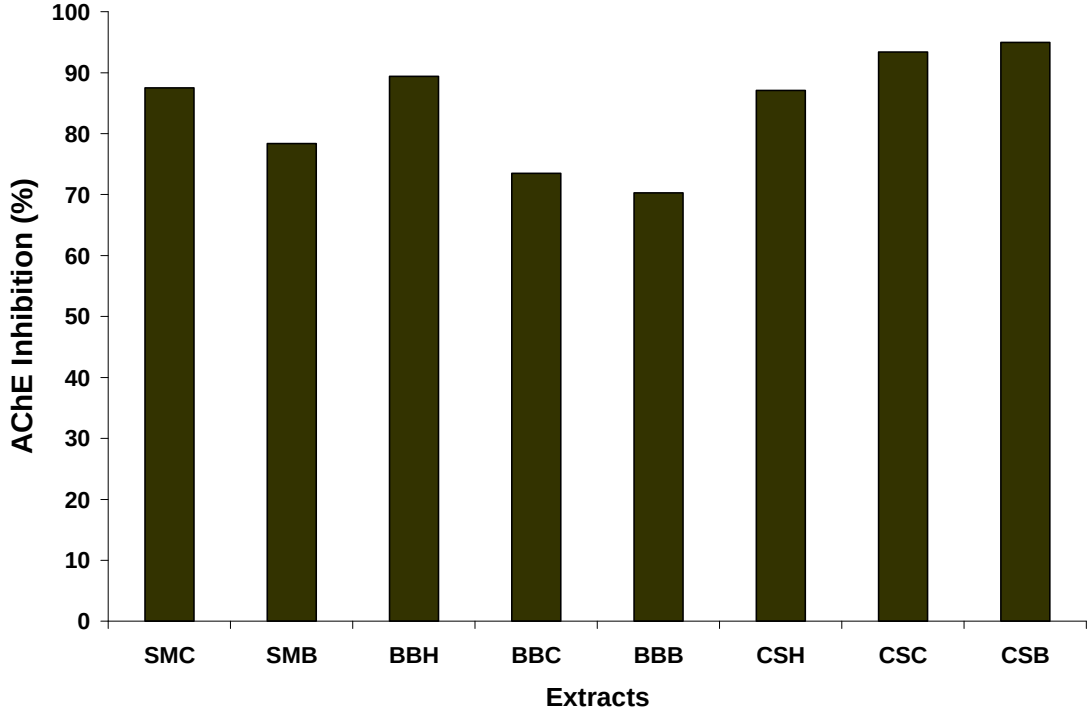

Figure 1. AChE inhibition potential of *B. biternata*, *S. macrophylla* and *C. sparsiflorus.*

Reducing ability (FRAP assay)

Reducing ability of extracts/fractions was determined according to method of Benzie and Strain (1999). The TPTZ reagent consist of 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) and 20 mM $FeCl_3.6H_2O$ solution. 150 µl plant extracts/fractions were mixed to FRAP reagent, allowed to stand for six minutes and absorbance was noted at 593 nm.

Determination of total phenolic contents (TP)

Total phenolic contents of extracts were determined using Folin-Ciocalteu reagent (Qureshi et al., 2011; Shahwar et al., 2010c). 50 µl of sample combined with 1.0 ml 7.5% Na_2CO_3 solution and 50 µl FC reagent. Absorbance was measured 725 nm after 40 min using UV/VIS spectrophotometer. Total phenolic contents were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract by computing with standard calibration curve obtained at different concentrations of gallic acid.

RESULTS

Acetylcholine esterase assay

Acetylcholine esterase (AChE) inhibitory potential of the extracts was determined by colorimetric method (Ellman et al., 1961; Shahwar et al., 2011) based upon the measurement of yellow anion of thionitrobenzoic acid formed by the hydrolysis of DTNB, measured at λ = 412 nm. The results of AChE inhibition potential are shown in Figure 1. The extracts of *C. sparsiflorus* remarkably inhibited the activity of AChE with IC_{50} values in the range of 7 to 10 µg/ml, in the order of % inhibition CSB > CSC > CSH. Similarly, all the extracts of *B. biternata* showed inhibitory activity above 50% at 1.0 mg/ml. The hexane extract was the most active extract with IC_{50} 23 ± 3 µg/ml followed by butanol extract (BBB) and chloroform extract (BBC) with IC_{50} value 30 ± 2 and 47 ± 3 µg/ml, respectively. SMC and SMB of *S. macrophylla* exhibited IC_{50} values 22 ± 1 and 32 ± 2 µg/ml, respectively, while SMH did not show positive result (Table 1).

DPPH inhibition activity

The solvent extracts of *C. sparsiflorus* showed moderate results in the DPPH assay in the order CSB > CSC > CSH. The most significant result was obtained for BBB of *B. biternata* with lowest IC_{50} = 55 ± 3 µg/ml. However, BBC and BBH of the same plant exhibited very close results of % inhibition with significantly different IC_{50} values of 290 ± 4 and 524 ± 2 µg/ml, respectively. Butanolic and chloroform extracts of *S. macrophylla* were also found to have close values of % inhibition (66.5 ± 1.3 and 62.4 ± 3.8, respectively) in DPPH assay (Table 2 and Figure 2).

Table 1. Acetylcholine esterase inhibitory potential of *B. biternata*, *S. macrophylla* and *C. sparsiflorus.*

Extracts	AChE (%)[a]	IC_{50} (µg/ml)
SMH	-	-
SMC	87.5 ± 2.6	22 ± 1
SMB	78.4 ± 2.3	32 ± 2
BBH	89.4 ± 2.3	23 ± 3
BBC	73.5 ± 1.5	47 ± 3
BBB	70.3 ± 1.7	30 ± 2
CSH	87.1 ± 1.9	10 ± 0
CSC	93.4 ± 2.3	8 ± 1
CSB	95.0 ± 2.0	7 ± 1

[a] 1000 µg/ml, - = not calculated.

Table 2. Total phenol and total flavonoids of the extracts of *B. biternata*, *S. macrophylla* and *C. sparsiflorus.*

Extracts	Total phenols [a]	Antioxidant activities		
		DPPH [b] (%)	IC_{50} (µg/ml)	FRAP [c]
SMH	190 ± 2	-	-	0.97 ± 0.03
SMC	640 ± 4	62.4 ± 3.8	186 ± 3	1.18 ± 0.15
SMB	720 ± 6	66.5 ± 1.3	171 ± 4	4.24 ± 0.12
BBH	-	54.5 ± 1.5	524 ± 2	1.52 ± 0.12
BBC	31 ± 1	56.1 ± 1.1	290 ± 4	8.19 ± 0.16
BBB	29 ± 1	88.9 ± 2.2	55 ± 3	8.98 ± 0.11
CSH	20 ± 1	16.7 ± 0.5	-	2.95 ± 0.15
CSC	113 ± 1	49.6 ± 0.1	-	3.21 ± 0.06
CSB	521 ± 4	58.2 ± 0.1	-	3.73 ± 0.13

[a] mg equivalent to gallic acid/g of sample, [b] 1000 µg/ml, [c] equivalent to $FeSO_4.7H_2O$ µM/g of extract, - = not calculated.

FRAP assay

Ferric reducing antioxidant power assay has been extensively used to determine reducing ability of the plant extracts (Shahwar et al., 2010). The results of FRAP assay shown in Table 2 are expressed in terms of $FeSO_4.7H_2O$ equivalent (R^2 = 0.9902). Among all the extracts butanolic and chloroform extract of *B. biternata* exhibited highest FRAP value, in the range of 8.5 µM/g of extract, followed by the butanolic extract of *S. macrophylla*. FRAP value of *C. sparsiflorus* extracts CSB, CSC and CSH was determined as 3.73 ± 0.13, 3.21 ± 0.06 and 2.95 ± 0.15 µM/g of extract respectively. The other extracts, BBH, SMC and SMH showed mild reducing ability towards Fe^{+3} ions in the FRAP assay.

Total phenols

The results shown in Table 2 revealed that the polar solvent extracts of *S. macrophylla* and *C. sparsiflorus* (SMB, SMC and CSB) possess significant quantity of phenols, while SMH, CSH and CSC showed moderate results. Poor results were obtained for the total phenols of *B. biternata* extracts.

DISCUSSION

Alzheimer s disease (AD) is a neurologic disorder resulting in loss of memory. One of the ways to treat AD is to control the activity of acetylcholine esterase (AChE) through acetylcholine esterase inhibitors (AChEIs). In our study nine extracts of three medicinal plants were tested for their AChE inhibitory potential by Ellman's colometric method. The extracts exhibited significant inhibition, IC_{50} values were also determined. The results showed AChE strong inhibitory potential by all the extracts except SMH of *S. macrophylla*, *C. sparsiflorus* showed most significant results with lowest IC_{50} values. Moreover, a literature survey of *C. sparsiflorus* revealed the presence of polyphenols and alkaloids (Subramanian et al., 1971).

Therefore, activity of the extracts (CSH, CSC and CSB) can be attributed to the synergistic effect of these compounds.

Some of the species of genus *Bidens* are known for their traditional use in treating various diseases and thus studied for their biological activity and phytochemicals (Brandao et al., 1997), but no significant work regarding

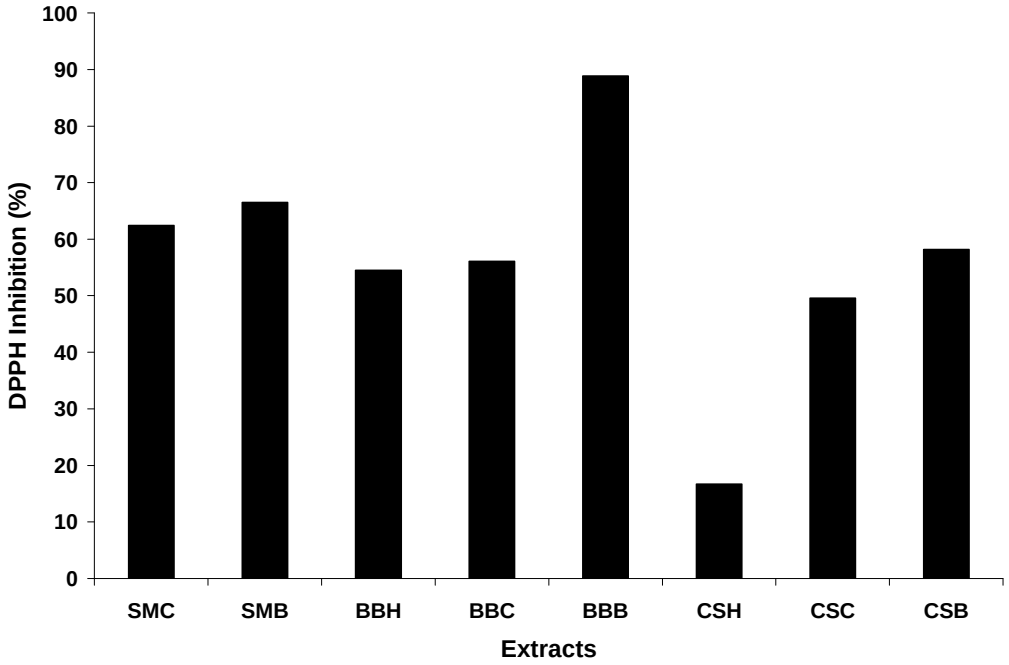

Figure 2. % DPPH Inhibition activity of *B. biternata*, *S. macrophylla* and *C. sparsiflorus.*

bioactivity and phytochemistry is reported on *B. biternata*. All the extracts of *B. biternata* significantly inhibited AChE. Therefore, it can be proposed that this plant could be a better candidate for AChE inhibitors. Antimicrobial activity of *S. macrophylla* has been reported by Loizzo et al. (2004). Only the polar extracts of this plant (SMB, SMC) showed AChE inhibitory potential, while hexane extract remained inactive. There are several reports of the presence of pyrolizidine alkaloids (Ahmad et al., 1994) in the genus *Senecio*. Our studies of the polar extracts indicated polyphenols in SMB and SMC, therefore a synergestic action of both alkaloids and polyphenols may be involved in the AChE inhibitory activity.

Antioxidant activity and health benefits of plants are usually found correlated with their total polyphenols and flavonoids (Zainol et al., 2003). Therefore, antioxidant activity of the extracts of *S. macrophylla* and *C. sparsiflorus* can be attributed to the polyphenols. The results were linearly correlated with the quantity of total phenols (Table 1) for *S. macrophylla* and *C. sparsiflorus*. Highest % inhibition of the butanol extract (BBB) with lowest IC_{50} (IC_{50} = 55 ± 3 µg/ml) was not consistent with the presence of high phenolics because the total phenols were negligible in the active extracts (BBB = 29 ± 1 mg GAE/g of extract, BBC = 31 ± 1 mg GAE/g of extract). Therefore, the high antioxidant activity and significant reducing ability of BBB as indicated in the FRAP assay (8.98 ± 0.11 µM/g of extract) may be due to some other phytochemicals.

Conclusions

Inhibition of AChE activity is an effective strategy to control Alzhemer's disease (AD). There is also a growing consensus of opinion that the free radicals are the major contributors to the aetiology of age related neurodegenerative diseases such as AD. Synthetic choline esterase inhibitors, approved for AD patients such as THA causes severe side effects such as nausea and liver damage. Therefore, the polar extracts of the selected plants possessing significant antioxidant and AChE inhibition activities may prove of novel values in clinical trials for the treatment of AD patients. Further work on the identification of active principals is proposed.

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