

REPORT

Screening of selected medicinal plants for their enzyme inhibitory potential – A validation of their ethnopharmacological uses

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Abstract: In present study four medicinal plants namely *Valeriana wallichii*, *Xanthium strumarium*, *Achyranthes aspera* and *Duchesnea indica* belonging to different families were collected in Khyber Pakhtunkhwa province and crude extract and subsequent fractions were analyzed for their inhibitory potential against acetylcholinesterase, butyrylcholinesterase and α -glucosidase enzymes. *Valeriana wallichii*, *Xanthium strumarium* and *Achyranthes aspera* were significantly active against cholinesterases. Chloroform and ethylacetate fractions of *Valeriana wallichii* exhibited significant activity against acetylcholinesterase (IC₅₀: 61 μ g/ml) and butyrylcholinesterase enzymes (IC₅₀: 58 μ g/ml), respectively. Similarly ethylacetate fraction of *Achyranthes aspera* showed significant activity against acetylcholinesterase (IC₅₀: 61 μ g/ml) and butyrylcholinesterase enzymes (IC₅₀: 61 μ g/ml), respectively. In case of α -glucosidase enzyme, the chloroform fraction of *Xanthium strumarium* exhibited significant inhibitory activity (IC₅₀: 72 μ g/ml) as compared to the standard compound acarbose (IC₅₀: 483 μ g/ml). *Duchesnea indica* showed no such activities.

Keywords: Medicinal plants, enzyme inhibition, acetylcholinesterase, butyrylcholinesterase and α -glucosidase

INTRODUCTION

Xanthium strumarium L. (Compositae) also known as cocklebur, is a common weed found abundantly throughout Pakistan. In traditional Chinese medicine, the whole plant is used as sedative, diaphoretic and sialagogue (Reeta *et al.*, 2010). The hypoglycemic activity of the plant has been reported in normoglycaemic rats (Narendiran *et al.*, 2011). Reactive oxygen species play a considerable role in human diseases such as Alzheimer disease, cancer, rheumatoid, aging and inflammation. *X. strumarium* has got significant antioxidant activity and therefore should be considered for use in the treatment of these diseases (Ishwarya and Singh, 2010).

Valeriana wallichii (Valerianaceae) commonly known as Indian valerian is indigenous to the temperate region of Himalayas. In Pakistan, it occurs in Hazara, Chitral, Muree hills, Swat and Waziristan (Subhan *et al.*, 2007). In traditional medicine *V. wallichii* is used for the treatment of hysteria, neurosis, sciatica, depression and as a useful tranquilizer (Fernandez *et al.*, 2004). Recently *V. wallichii* has been reported for its enhancement in short term memory (Shalam *et al.*, 2007). This shows its potential usefulness in Alzheimer disease via augmentation of cholinergic transmission by inhibiting cholinesterases.

Achyranthes aspera (Amaranthaceae) commonly known as Prickly-chaff flower, is an erect perennial herb found throughout Pakistan and India (Priya *et al.*, 2010). In Ayurvedic medicine it is used as an abortifacient, diuretic, laxative and in the treatment of diabetes (Zambare *et al.*, 2011). Prickly-chaff leaves are reported to have strong antioxidant activities and therefore should be considered for the treatment of stress induced diseases.

Duchesnea indica (Rosaceae), an erect perennial herb and is widely distributed on shady, grassy slopes in China, India and Pakistan (Fazli *et al.*, 2012). The plant is extensively used in traditional Chinese medicine for the treatment of inflammatory disorders, diarrhea and various oxidative stress conditions (Hu *et al.*, 2011).

In the current study we have therefore screened the above mentioned plants for their enzyme inhibition properties in order to validate their ethnopharmacological uses in these diseases.

MATERIALS AND METHODS

Plant material

Roots of *D. indica* and leaves of *V. wallichii* were collected in Hazara Division, Khyber Pakhtunkhwa, Pakistan. While leaves of *A. aspera* and *X. strumarium* were collected from Charsadda, Khyber Pakhtunkhwa, Pakistan. Voucher specimens: 8708 (BOT), 9526 (BOT), 10708 (BOT) and 8708-1 (BOT) for *X. strumarium*, *V.*

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wallichii, *D. indica* and *A. aspera*, respectively were deposited in the herbarium for future reference.

Extraction

Air dried and powdered leaves were extracted using methanol at room temperature for three days. After filtration the dark green extract was concentrated to dryness under vacuo at low temperature (40°C) using rotary evaporator, until 25g of the crude extract was obtained. The extract was then dissolved in distilled water and sequentially partitioned with various solvents to obtain n-hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions.

Cholinesterase inhibition assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibiting activities were measured by method previously developed (Begum *et al.*, 2012). Electric-eel AChE (type VI-S, Sigma) and horse serum BChE (Sigma) were used as source of the cholinesterases. While acetylthiocholine iodide and butyrylthiocholine chloride (Sigma), respectively, were used as substrates in the reaction. 5, 5 - Dithiobis (2-nitrobenzoic acid) (DTNB, Sigma) was used for the measurement of cholinesterase activity. 140 µL of sodium phosphate buffer 100mM, (pH 8.0), 10 µL of DTNB, 20 µL of the test samples solutions and 20 µL of acetylcholinesterase/butyrylcholinesterase solution were mixed and incubated for 15 min at 25°C. The reactions were then initiated by the addition of 10 µL acetylthiocholine/butyrylthiocholine, respectively. The hydrolysis of acetylthiocholine and butyrylthiocholine was monitored at a wavelength of 412 nm by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine and butyrylthiocholine, respectively. The samples and the control were dissolved in 50% ethanol. Percent (%) inhibition was calculated according to Michaelis–Menten model by using “EZ-Fit. Software (EZ-Fit: Enzyme Kinetics, Perrella Scientific, Inc., USA).

Alpha-glucosidase Inhibition Assay

Alpha-glucosidase inhibition assay was evaluated according to the method previously reported (Zia-Ul-Haq *et al.*, 2012). Alpha-Glucosidase (E.C.3.2.1.20) from *Saccharomyces* species was purchased from Sigma Aldrich. The enzyme inhibition was measured spectrophotometrically through continuous monitoring of the nitrophenyl produced by the hydrolysis of the substrate *p* nitrophenyl α -D-glucopyranoside (PNP-G) (0.7mM) and 500 milli units/ml of the enzyme used. Whole enzymatic reaction was performed at 37°C for 30 min. The increment in absorption at 400 nm, due to the hydrolysis of PNP-G by α -glycosidase, was monitored continuously on microplate spectrophotometer (Spectra Max, Molecular Devices and U.S.A.). Phosphate saline buffer at pH 6.9 was used, which contains 50 mM sodium

phosphate containing 100 mM sodium chloride. Acarbose (0.78 mM) was used as positive control.

RESULTS

The results of acetylcholinesterase inhibition assay employing the crude extract and various fractions of all the selected plants in comparison to Galanthamine (standard drug) are presented in table 1. The crude extracts of *V. wallichii* and *A. aspera* displayed 73 % and 65% inhibition at 100 µg/ml and the IC₅₀ values were 68 and 79 µg/ml, respectively, as compared to that of standard. The chloroform fraction of *V. wallichii* showed the highest inhibition (76%) with IC₅₀ as 61µg/ml, followed by the ethylacetate fraction of *A. aspera* having (75%) inhibition and IC₅₀ was 61µg/ml. The *n*-Hexane fraction of *X. strumarium* also showed considerable inhibitory activity (73%) with IC₅₀ value of 63 µg/ml.

Similarly the crude extract of *V. wallichii* exhibited 82% inhibition against butyrylcholinesterase and the IC₅₀ was 89 µg/ml, as compared to standard (table 2). Among various fractions, ethylacetate fraction of *V. wallichii* revealed highest inhibition (86%) with the IC₅₀ value of 58 µg/ml followed by ethylacetate fraction (75% inhibition) of *A. aspera* having the IC₅₀ value of 61 µg/ml. However in case of *X. Strumarium* the most active fraction was chloroform fraction (69% inhibition) followed by *n*- Hexane fraction (66% inhibition) having the IC₅₀ value of 77 and 83 µg/ml, respectively (table 2).

Similarly the crude extracts of *V. wallichii* and *X. Strumarium* showed significant inhibition against α -glucosidase enzyme (table 3). Their percent inhibitions were 69% and 74%, respectively, while the IC₅₀ values were 89 and 76 µg/ml. The chloroform fraction of *X. Strumarium* was found to be the most active with 76% inhibition and its IC₅₀ value was 72 µg/ml (table 3). The *n*-butanol fraction of *V. wallichii* also showed significant activity (71% inhibition) with the IC₅₀ value of 71µg/ml.

DISCUSSION

Plants have been used as a source of new bioactive compounds for drug discovery since ancient time. Numerous medicinal plants have received much attention to improve cognitive function. However the search for potent long-acting anti-cholinesterase (acetyl cholinesterase and butyrylcholinesterase) inhibitors is still ongoing. Based on cholinergic hypothesis and ethnopharmacological uses we screened the above mentioned plants for dual anti-cholinesterase activity against the BChE and AChE enzymes which are considered to be related to the prognosis of cognitive diseases. Our results, although preliminary, are interestingly significant and highlight their potential therapeutic role and can be made as a base for the

Table 1: Acetyl cholinesterase inhibition activity of crude extract and various fractions

Plant species	IC ₅₀ ±SEM (µg/mL)						St
	Cr	Ch	He	Bu	Et	Aq	
<i>V. wallichii</i>	68±0.41 (73) ^a	61±0.19 (76)	92±0.28 (63)	94±0.17 (55)	59±0.21 (58)	114±0.62 (39)	0.18±0.01 (85)
<i>X. strumarium</i>	114±0.23 (43)	79±0.38 (66)	63±0.16 (73)	151±0.41 (40)	141±0.27 (38)	124±0.36 (46)	
<i>A. Aspera</i>	79±0.15 (65)	142±0.34 (38)	113±0.26 (46)	89±0.16 (54)	61±0.36 (75)	151±0.24 (35)	
<i>D. indica</i>	226±0.26 (25)	137±0.16 (39)	184±0.22 (31)	237±0.21 (24)	351±0.32 (21)	148±0.41 (37)	

Standard: Galanthamine

Cr: Crude, Ch: Chloroform, He: *n*-Hexane, Bu: *n*-Butanol, Et: Ethylacetate, Aq: Aqueous, St: Standard^aEach value in parentheses indicates the % inhibition**Table 2:** Butyrylcholinesterase inhibition activity of crude extract and various fractions

Plant species	IC ₅₀ ±SEM (µg/mL)						St
	Cr	Ch	He	Bu	Et	Aq	
<i>V. wallichii</i>	89±0.17 (82) ^a	76±0.29 (72)	97±0.36 (68)	113±0.27 (38)	58±0.13 (86)	146±0.42 (36)	3.12±0.01 (81)
<i>X. strumarium</i>	106±0.29 (40)	77±0.21 (69)	83±0.18 (66)	133±0.48 (36)	109±0.34 (40)	149±0.13 (30)	
<i>A. aspera</i>	79±0.15 (65)	142±0.34 (38)	113±0.26 (46)	89±0.16 (54)	61±0.36 (75)	151±0.24 (35)	
<i>D. indica</i>	278±0.14 (22)	124±0.27 (39)	204±0.23 (27)	197±0.42 (28)	174±0.31 (29)	109±0.19 (44)	

Standard: Galanthamine

^aEach value in parentheses indicates the % inhibition**Table 3:** α-glucosidase inhibition activity of crude extract and various fractions

Plant species	IC ₅₀ ±SEM (µg/mL)						St
	Cr	Ch	He	Bu	Et	Aq	
<i>V. wallichii</i>	89±0.11 (69) ^a	119±0.24 (55)	184±0.31 (58)	71±0.37 (71)	93±0.19 (62)	86±0.18 (60)	483.25±0.03 (70)
<i>X. strumarium</i>	76±0.29 (74)	72±0.21 (76)	102±0.18 (69)	113±0.48 (60)	64±0.34 (67)	146±0.13 (64)	
<i>A. aspera</i>	167±0.34 (33)	189±0.31 (29)	258±0.19 (22)	152±0.24 (36)	97±0.14 (53)	241±0.26 (23)	
<i>D. indica</i>	281±0.17 (20)	136±0.21 (34)	194±0.24 (28)	341±0.26 (15)	234±0.34 (23)	173±0.19 (31)	

Standard: Acarbose

^aEach value in parentheses indicates the % inhibition

investigation of new lead compounds for the treatment of cognitive dysfunctions such as Alzheimer's disease.

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