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ORIGINAL ARTICLE

Antioxidant, Acetylcholinesterase, Butyrylcholinesterase, and α-glucosidase Inhibitory Activities of *Corchorus depressus*

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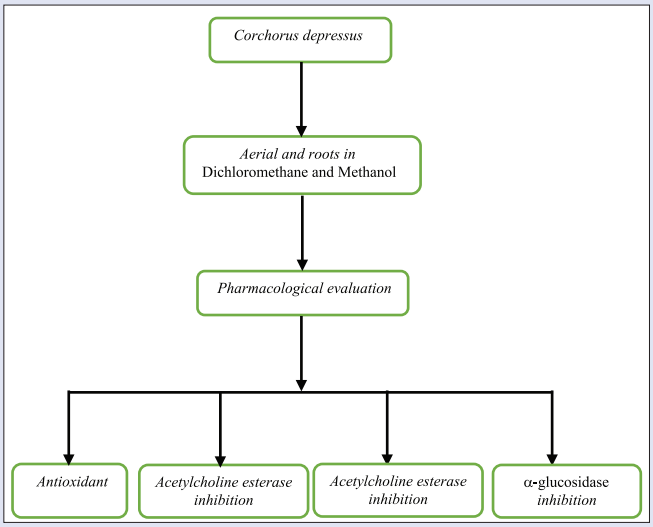
ABSTRACT

Background: *Corchorus depressus* (*Cd*) commonly known as Boa-phalee belonging to the family Tiliaceae having 50 genera and 450 species. *Cd* is not among the studied medicinal agent despite its potential in ethnopharmacology. **Objectives:** The present study investigated antioxidant, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α-glucosidase inhibitory activities of *Cd*. The dichloromethane and methanolic extracts of the *Cd* were evaluated for biological activities such as antioxidant and enzyme inhibitory activities of AChE, BChE, and α-glucosidase. **Materials and Methods:** Antioxidant activity was evaluated by measuring free radical scavenging potential of *Cd* using 1,1-diphenyl-2-picrylhydrazyl. Enzyme inhibition activities were done by measuring optical density. **Results:** The methanol extract of roots of *Cd* showed potential free radical scavenging activity 99% at concentration 16.1 μg/ml. AChE was inhibited by aerial part of dichloromethane fraction by 46.07% ± 0.45% while dichloromethane extracts of roots of *Cd* possessed significant activity against BChE with 86% inhibition compared with standard drug Eserine at concentration 0.5 mg/ml. The dichloromethane extract of roots of *Cd* showed 79% inhibition against α-glucosidase enzyme activity with IC_{50} 62.8 ± 1.5 μg/ml. **Conclusion:** These findings suggest *Cd* as useful therapeutic option as antioxidant and inhibition of AChE, BChE, and α-glucosidase activities.

Key words: 1,1-diphenyl-2-picrylhydrazyl, antioxidant, butyrylcholinesterase, dichloromethane, methanol

SUMMARY

- The aerial parts and roots of *Corchorus depressus* (*Cd*) were extracted in dichloromethane and methanol
- The extract of roots of *Cd* showed free radical scavenging activity 99% at concentration 16.1 μg/ml, Ach inhibition by aerial parts of dichloromethane fraction by 46.07%, and 79% inhibition against α-glucosidase enzyme activity with IC_{50} 62.8 ± 1.5 μg/ml
- The dichloromethane and methanolic extracts of *Cd* exhibited antioxidant inhibition of acetyl cholinesterase, butyrylcholinesterase, and α-glucosidase activities.



Abbreviations used: DPPH: 1,1-diphenyl-2-picrylhydrazyl, *Cd*: *Corchorus depressus*, AChE: Acetylcholinesterase, BChE: Butyrylcholinesterase, AD: Alzheimer's disease.

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INTRODUCTION

Nature at all times stands as a golden mark to demonstrate the exceptional phenomena of symbiosis. For a long time, natural products derived from plant and animal were used for the cure of diseases. In developing countries, approximately 80% of the people for their basic health care still depend on traditional medicine based on plant as well as animal species.[1] The current demand and popularity of herbal medicines are increasing day by day. In ancient literature, approximately 500 plants are mentioned. In indigenous medicine system, about 800 plants have been used. Since ancient times, herbal medicines have been used in medical practices as the major remedy.

Antioxidants are nutrients in our foods which can avert or sluggish the oxidative damage to our body. Free radicals are also produced in different organs as result of metabolism of inhaled oxygen. Free radical damage may direct to cancer.[2] Antioxidants act as free radical scavengers and hence check and fix damage done by these free radicals. Now, the medicinal plants have become the aim for the hunt by cosmopolitan drug companies and study institutes for new drugs.

The inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), enzymes which breakdown acetylcholine (ACh) and butyrylcholine,

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are thought as a promising approach for the management of Alzheimer's disease (AD).[3] A potential source of AChE and BChE inhibitors is granted by the ample of plants in nature. AD is a progressive, neurodegenerative pathology that primarily affects the elderly population and is estimated to account for 50%–60% of dementia cases in persons over 65 years of age. In mammalian brain, there are two major forms of cholinesterases, namely, AChE and BChE. The most remarkable biochemical change in AD patients is a reduction of ACh levels in the hippocampus and cortex of the brain. Therefore, inhibition of AChE, the enzyme responsible for hydrolysis of ACh at the cholinergic synapse, is currently the most established approach to treating AD. While AChE is found in all excitable tissue, whether nerve or muscle, in most erythrocytes and in placental tissue, BChE is present more commonly in the body including within the central and peripheral nervous system, liver, and plasma.[4] The serious side effects caused by licensed drugs used to treat AD have forced researchers to investigate safer AChE or BChE inhibitors from natural sources. Numerous plants and their constituents are reputed in traditional practices of medicine to enhance cognitive function and to alleviate other symptoms of AD, including depression.[5]

In type 2 diabetes mellitus (DM), inhibition of α-glucosidase therapy is beneficial to delay absorption of glucose after a meal.[6] α-glucosidase plays a role in the conversion of carbohydrates into glucose. By inhibiting α-glucosidase, glucose levels in the blood can be returned within normal limits.[7] In spite of the introduction and extensive utilization of hypoglycemic agents, diabetes and the related complications continue to be a major health problem worldwide, which is affecting nearly 10% of the population worldwide and considered as a major cause of high economic loss which can in turn impede the development of nations. It is projected to become one of the world's main disablers and killers within the next 25 years.

Corchorus depressus (*Cd*) commonly known as Boa-phalee belonging to the family Tiliaceae. The Tiliaceae family has fifty genera and 450 species which are distributed in tropical and temperate regions, chiefly South Asia and South America. In Pakistan, about four genera and 24 species are found. The genus is enriched with pharmacological properties. *Corchorus capsularis* showed cardiovascular activity.[8,9] *Corchorus olitorius* also showed spasmolytic activity,[10,11] antihistaminic activity,[12] hepatobiliary, renal and hematological activity,[13-15] antibacterial activity,[16] antiestrogenic, anticonvulsant activities,[17] and antimalarial activity.[18] *Corchorus aestuans* showed anticancerous activity.[19] *Cd* showed analgesic and antipyretic activity.[20,21] Literature survey of genus revealed phytochemical constituents triterpenoids, sterols,[22] and flavonoids[23] reported from chloroform extract of *Cd*. Cardiac glycosides,[1] ionones glycosides, higher fatty acids, sterols, coumarins, and phenolics reported from leaves or seeds extraction of *C. olitorius*.[24-28] Various species of this genus have been used as folk medicine. *Corchorus* is used as a traditional medicine for the ailment of aches, dysentery, enteritis, fever, and tumors.[29] The infusion of leaves is a demulcent, laxative, carminative, stimulant, appetizer, and tonic.[30] The seeds of *Corchorus* are used for purgative, tonic, stomachic, fever, and in obstructions of abdominal problems.[31] Jute fiber is obtained from *C. capsularis* and *C. olitorius*. It is used for treating respiratory treat infections and as a nervine and tonic; constituents include volatile oil, flavonoids, and phenolic acid.[32]

Insight from literature review compelled us to investigate antioxidant and presence of possible AChE, BChE, and αglucosidase inhibitor activities of *Cd*.

EXPERIMENTAL PROCEDURES

Plant collection

The *Cd* was collected from Peruwal (District Khanewal) and identified by Professor Dr. Altaf Ahmed Dasti, plant Taxonomist, Institute of pure and applied biology, Bahauddin Zakariya University, Multan, Pakistan, whereas voucher specimen fl.p. 472/4 for *Cd* was deposited.

Extraction

Extraction method was followed as reported.[33] The aerial parts and roots of *Cd* were cleaned, shade dried for 14 days, and pulverized to fine powdered in a mechanical grinder. The 1000 g of plant materials were subjected for extraction procedure using solvents dichloromethane and methanol at room temperature occasionally shaking for 24 h. Extracts were filtered by Buchner funnel. The filtrate extracts were concentrated by Rotavapor – R200 at 35°C. The dichloromethane and methanolic extracts of *Cd* were collected in separate sample bottles with designated different codes. The final extracts were obtained and used for *in vitro* antioxidant activity and AChE, BChE, and αglucosidase inhibitor activities. The results of the extraction along with the abbreviations used for different extracts are given in Table 1.

Phytochemical analysis

The dried and powdered aerial parts of *Cd* were investigated for the presence of alkaloids, anthraquinones, cardiac glycosides, tannins, and saponins as reported.[33] The results of phytochemical analysis are given in Table 2.

Detection of alkaloids

Tem grams of the grinded plant material was boiled with 10 ml of acidified water in test tube for 1 min, cooled, and allowed the debris to settle. Filtered the supernatant liquid into another test tube. 1 ml of this filtrate was taken and 3 drops of Dragendorff's reagent were added; there was no precipitate. The remainder of filtrate was made alkaline by addition of dilute ammonia solution. A volume of 5 ml of chloroform was added to the solution in separating funnel; two layers were observed. The lower chloroform layer was pipetted out into another test tube. Chloroform layer was extracted by the addition of 10 ml of acetic acid and then discarded the chloroform. Then, extracts were divided into three portions, to one portion, few drops of Dragendorffs reagent were added and to the second portion, few drops of Mayer's reagent were added. Turbidity or precipitate was compared with the third untreated control portion.

Detection of anthraquinones glycosides

Two gram of powdered plant material was taken and extracted with 10 ml of hot water for 5 min, allowed it to cool and filtered; filtrate was extracted with 10 ml of carbon tetrachloride. Then, carbon tetrachloride layer was taken off, washed it with 5 ml water, and then, 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as the absence of the appearance of pink to cherry red color in the ammoniacal layer.

Two gram of second sample of the plant was extracted with 10 ml of ferric

Table 1: Results of the extraction of the plant *Corchorus depressus*

Plant name	Part used	Solvent	Weight of extract (g)	Abbreviation for the extracts
Cd	Aerial parts (1000 g)	Dichloromethane	39.85	CDAD
		Methanol	7.95	CDAM
Cd	Roots (1000 g)	Dichloromethane	9.4	CDRD
		Methanol	17.84	CDRM

Table 2: Results of phytochemical screening of *Corchorus depressus*

Name of plants	Alkaloid	Anthraquinone	Cardiac glycosides	Saponins
Cd	+	+	+	+
Cd (root part)	+	+	+	+

Cd: *Corchorus depressus*

chloride solution and 5 ml of hydrochloric acid; then, it was heated on water bath for 10 min and filtered. Filtrate was cooled and treated as above.

Detection of cardioactive glycosides

One gram of ground plant material was taken in a test tube, and 10 ml of 70% alcohol was added. It was then boiled for 2 min and filtered. Filtrate was diluted twice of its volume with water, and then, 1 ml of strong lead subacetate solution was added. This treatment leads to the precipitation of chlorophyll and other pigments, which were then filtered off. Filtrate was extracted with an equal volume of chloroform. Chloroform layer was pipetted out and evaporated to dryness in a dish over a water hath. Residue was dissolved in 3 ml of 3.5% ferric chloride in glacial acetic acid and was transferred to test tube after leaving for 1 min. A volume of 1.5 ml of sulfuric acid was then added, which formed a separate layer at the bottom. Cardioactive glycosides were revealed the appearance of brown color at interface (due to deoxy sugar) on standing and appearance of pale green color in the upper layer (due to the steroidal nucleus).

Detection of tannins

Prepare 10% w/v aqueous extract of grinded drug by boiling it with distilled water for about 10–20 min. Filtered the extract and performed the chemical tests with clear solution.

Ferric chloride test

A volume of 2 ml of ferric chloride solution was mixed to 1–2 ml clear solution of extract. A blueback precipitate indicated the presence of hydrolysable tannin.

Catechin test

Dip the match stick in plant extract, dry, and then, moist it with concentrated hydrochloric acid. Warm near flame, a red or pink wood is produced which shows the presence of catechin.

Determination of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

The free radical scavenging activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH). The DPPH stock solution was prepared by dissolving 20 mg DPPH in 100 ml 95% methanol. This stock solution was stored at 20°C until needed not >10 days. DPPH working solution was prepared by diluting the stock of DPPH solution by adding methanol, and absorbance was adjusted about 0.980 ± 0.02 at wavelength 517 nm using the spectrophotometer. A volume of 3 ml aliquot of this working solution mixed with 100 µl of the plant samples at five different varying concentrations (4–322 µg/ml). The solutions in the test tubes were shacked well and put in dark for 15 min at room temperature. Then, again the absorbance was measured at 517 nm. The percentage scavenging activity was determined based on the percentage of DPPH radical scavenged by using the following equation.[34]

Scavenging effect (%) = {control absorbance − sample absorbance/ control absorbance} × 100.

Determination of acetylcholinesterase inhibitory activity

The evaluation of AChE inhibitory activity should be performed using thin layer chromatography (TLC) plates. These plates were treated with acetone. These plates were dried completely before their use. The solvents were removed from TLC plates completely by using hairdryer. Enzyme stock solution was then sprayed on the plates and again dried. The plates were put in flat position using plastic plugs in a tank with some water. The plates were covered and subjected to incubation at 37C for 20 min. The mixture containing 1-napththyl acetate (250 mg in 100 ml of ethyl alcohol) and Fast Blue B salt amounting to 400 mg in distilled water was prepared and used for detection of the enzyme. After incubation process, the mixture of the naphthyl acetate 10 ml and 40 ml of the Fast Blue B salt was prepared and sprayed on the plate so that there is purple color after a few minutes.[35]

In vitro α-glucosidase inhibitory activity

A volume 135 µl of 50 mM phosphate saline buffer pH (6.8) was dispensed in the 96 well plate. 20 µl of the test sample in 70% dimethyl sulfoxide dispensed into the wells. 20 µl of the enzyme was added into the wells and incubate the plate for 15 min. After incubation, preread of the plate was taken by the spectra max. After the preread, 25 µl of the substrate was added and readings were taken on spectra max at 400 nm for 30 min. In the end, normal read is taken and the percent inhibition was calculated.

RESULTS AND DISCUSSION

Phytochemical screening showed the presence of alkaloids, anthraquinones, cardiac glycosides, tannins, and saponins as shown in Table 2.

In DPPH radical scavenging assay, the methanolic extract of roots of *Cd* showed maximum radical scavenging activity 99% at concentration 161 µg/ml and 80% at concentration 16 µg/ml. The methanolic extract of aerial parts of *Cd* showed 80% radical scavenging activity at high concentration 161 µg/ml as shown in Table 3.

DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extract. The methanolic extracts of the aerial and roots of the plant showed the strongest DPPH radical scavenging activity as compared to dichloromethane extracts of *Cd* as shown in Figure 1. This finding indicates that active constituents of plants are in methanolic fraction and mostly in root parts as compared to aerial parts of same fraction. The therapeutic potential of natural medicinal plants

Table 3: 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of different extracts of *Corchorus depressus*

Plant extracts	Percentage radical scavenging at concentration				IC_{50} (µg/ml)
	161 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	
CDRD	23	10	5	2	Nil
CDRM	99	80	40	16	41.23±6.34
CDAM	80	30	18	10	20±6.60
CDAD	33	10	6	4	Nil
P control	97	93	89	56	

Ascorbic acid was used as standard

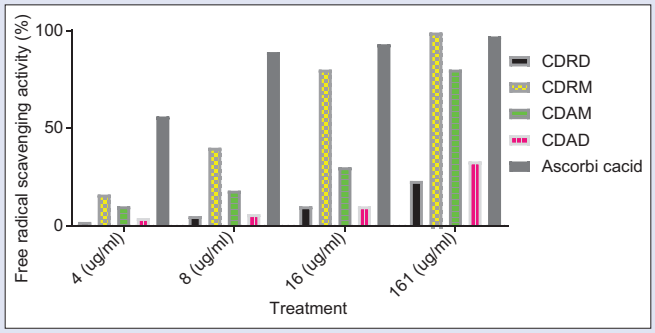

Figure 1: 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity of different fractions of different parts of *Corchorus depressus*

as an antioxidant in free radical-induced tissue injury, suggests that antioxidant activities of medicinal plant can be therapeutically useful for treatment.

IC50 values of different fractions and different parts of the plant *Cd* (concentration of the samples) required to inhibit the 50% population of the enzymes ACh esterase, and BChE were calculated as shown in Table 4. *Cd* did not show any promising activity against enzyme ACh esterase except aerial parts of dichloromethane fraction which showed 46.07% ± 0.45% inhibition. At the same time, dichloromethane fraction of root part of *Cd* showed 86.36 ± 0.65 inhibition of BChE with IC_{50} value 132.8 ± 0.87 µg as shown Table 4.

The result showed that dichloromethane extracts of aerial and roots of *Cd* possessed significant inhibition against ACh esterase and BChE inhibitory activities, respectively, as among other extracts compared with standard drug. Although it is indicating the effects of different fractions of *Cd* on CNS, these effects have not been observed in situation of enhanced cholinergic activity. These results are important because of the growing body of evidence suggesting that anticholinergic medications contribute to memory impairment in older adults.[36-40] *Cd* like rivastigmine can be used as therapeutic option for the treatment of AD for having dual inhibitory effects on AChE and BChE esterase. Although *Cd* dominates against BChE esterase activity when compared to AChE, multifarious role can help to treat AD and problem associated with dementia. In case of AD, the ratio between BChE and AChE in the cortical region of brain changes from 0.5 to as high as 11.[41] This indicates the supporting role of BChE in the regulation of AChE making functional importance of this enzyme in AD. Inhibiting both enzymes using *Cd* increased the ethnopharmacological importance of *Cd*.

The dichloromethane extract of roots of *Cd* showed 79% inhibition against α-glucosidase enzyme activity with IC_{50} 62.8 ± 1.5 µg/ml while others are inactive as shown in Table 5. The glucosidase inhibition enzyme activity of dichloromethane extract of roots of *Cd* showed better responses when compared to standard drug with lower IC50 values. This indicates the potential use of *Cd* in diabetes can be a future therapeutic outcome. The α-glucosidase enzyme inhibitor has been used in DM.[42,43]

Table 4: Results of acetylcholinesterase and butyrylcholinesterase inhibitory activities of *Corchorus depressus*

Code	AChE (%) at 0.5 mg/ml	AChE (IC_{50}) ug/ml	BChE (%) at 0.5 mg/ml	BChE (IC_{50}) ug/ml
CDAD	46.07±0.45	Nil	37.72±0.39	Nil
CDAM	23.93±0.63	Nil	60.49±0.89	Nil
CDRD	4.64±0.75	Nil	86.36±0.65	132.8±0.87 µg
CDRM	15.00±0.85	Nil	67.72±0.99	
Control	Eserine	0.04±0.001	Eserine	0.85±0.001

AChE: Acetylcholinesterase; BChE: Butyrylcholinesterase

Table 5: Results of α-glucosidase against inhibition of dichloromethane and methanol extracts of *Corchorus depressus*

Sample code	α-glucosidase		
	Concentration (mg/ml)	Percentage inhibition	IC_{50}±SEM (µg/ml)
CDRD	0.5	79.2	62.8±1.5
CDRM	0.5	44	Inactive
Standard drug (control)			
Acarbose	0.64	59.1	83.33±0.34

SEM: Standard error of mean

CONCLUSION

Aerial and root parts of methanolic and dichloromethane fractions of *Cd* possess antioxidant activity, AChE, BChE, and α-glucosidase inhibitory activities. However, the dichloromethane extract of roots showed better BChE and α-glucosidase inhibitory activities than the other fractions.

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Conflicts of interest

There are no conflicts of interest.

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