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To Reveal the Pharmacological Potential of Oldenlandia Corymbose

Praveen kumarDannaram, Bhuwanendra Singh*

School of Pharmaceutical Science, SVU Gajraula, Distt. Amroha (U.P) India.

ABSTRACT

The characteristic of diabetes is hyperglycemia, a complex metabolic condition brought on by inadequate or defective insulin. Type two diabetes mellitus and Alzheimer's disease are two of the most popular ailments afflicting people in their senior years; this much is certain. It is the most common significant neurocognitive disorder in the elderly, making up between 60% and 80% of dementia cases. Research from studies and clinical settings has shown that older adults are more likely to acquire type 2 diabetes mellitus (T2D) and AD concurrently because T2D is a key imperil factor for AD. The examination of the literature on Oldenlandia corymbose reveals that the medications have a range of pharmacological effects. The present investigation employed a Soxhlet apparatus to extract the material, utilising ethanol as a solvent. Subsequently, the researchers conducted pharmacological tests, including cholinesterase and glucosesidase enzyme inhibitory activities, nitric oxide scavenger activity, and Oldenlandia corymbose Reducing Power Assay, to ascertain the drug's potential pharmacological properties.

Keywords: Hyperglycemia, Neurocognitive, Alzheimer, Dementia, Pharmacological.

1. INTRODUCTION

Oldenlandiacorymbosa Linn. is a flowering plant that belongs to the Rubiaceae family. It is widely distributed in both tropical and temperate parts of the hemispheres. It is also found in the Himalayan region, at heights of up to 2000 meters. The Oldenlandia genus contains more than 240 species worldwide. During the monsoon season, fields, waste areas, lawns, and some populated areas in countries like the Philippines, Java Island, East Asia, India, and Sri Lanka are home to an annual herbaceous medicinal plant known as Oldenlandia corymbosa1,2,3,4,5. The plant is well known for its ability to flush out toxic chemicals from the body, increase blood flow, promote diuresis, and relieve obstructions in the urinary system. It possesses unique properties that protect against lymph sarcoma, digestive tract tumours, liver and laryngeal cancer. Furthermore, it has demonstrated efficacy against a range of ailments, such as cellulites, gynaecologic infections, snake bites, cholecystitis, hepatitis, pneumonia, ulcers, skin disorders, colds, coughs, and pelvic inflammation[6, 7, 8, 9].

2. METHODOLOGY

Selection, Recognition, and Validation of plant Drug

A select few plants were gathered, purchased from the neighbourhood market, and later verified by "Dr. Anju Pal, Scientist at GBPUAT Pantnagar, Uttarakhand, India."

Preparation of extracts

Alcoholic extracts were prepared by using solvents of different polarity from leaves of Oldenlandiacorymbosa will be extracted.

Alcohol soluble extractive value

Soxhlet Extraction

After mixing 50 g of the powdered material with 300 ml of 80% ethanol, the mixture was heated to 60 °C in a "Soxhlet apparatus" until the solvent's colour turned colourless. A vacuum rotary evaporator was used to evaporate the solvent after the organic layers were filtered using Whatman filter paper no. 1 to provide a gummy crude extract. To preserve it for use in later research, the concentrated ethanolic extract was placed in a beaker, covered with aluminium foil, and chilled at 4 °C.

Pharmacological Activity

Inhibitory activity of Cholinesterase Enzyme

The ethanolic extracts and their derivative fractions will be subjected to a spectrophotometric AChE and BuChE inhibition assay utilising acetylthiocholine iodide and butyryl thiocholine iod, respectively, as the substrate.A 96-well plate will be filled with 10 mL of the stock solution's enzyme (AChE, 2U/mL, and BuChE, 2U/mL), 10 mL of plant extract or fraction (15-150 g/mL), and 100 mL of phosphate buffer. After that, mix in 50 litres of DTNB solution (3.96 milligrammes of DTNB and 1.5 milligrammes of sodium bicarbonate dissolved in 10 millilitres of 200 millimetre phosphate buffer, pH 7.7), and let the mixture sit at 25°C for five minutes. In total, 10.85 mg of butyryl or acetylthiocholine iodide will be added to a 15 L amount of phosphate buffer, and the mixture will be incubated at 25 °C for an additional 5 minutes.

The hue produced by the 5-thio-2-nitrobenzoate anion will be visible at 412 nm. We will utilise the reaction mixture without the plant sample and galantamine at concentrations of 0.12, 0.23, 0.46, 0.92, 1.84, 3.68, and 7.37 g/mL as positive and negative controls, respectively. We will compute the IC50 values and the percentage of inhibition[10].

Inhibitory activity Glucosidase enzyme

This method was adapted to evaluate the inhibition of - and -Glu activity by ethanolic extracts and its derived fractions. P-nitrophenyl D-glucopyranoside and p-nitrophenyl D-glucopyranoside, respectively, will serve as the substrates for - and. The assay solution consists of 50 L of enzyme (-Glu 0.15 unit/mL or -Glu 0.15 unit/mL), 100 L of phosphate buffer pH 6.8, and 10 L of extract or fraction at various concentrations (15, 30, 90, and 150 g/mL). It is incubated at 37°C for fifteen minutes. The experiment will

utilise 50 L of substrates (0.5 mM p-nitrophenyl-D-glucopyranosideorp-nitrophenyl-D-glucopyranoside). A 50 mL container containing 200 mM Na2CO3 was added to halt the process. We will measure the enzyme's spectrophotometric activity at 415 nm to compute the Ic 50 values and inhibition %age.

DPPH radical scavenging assay

The ability of the active "fractions of the ethanolic extract" of the specified plant to scavenge DPPH radicals will be used to measure the "antioxidant activity" of these fractions using a modified version of the method. A "fraction" (1.5 mL) of each plant will be mixed with 9 mL of the DPPH solution at concentrations of 50, 200, and 400 g/mL (60 mM). The reaction mixes will be prepared under low light. After giving the mixtures a good shake, they will be left in the dark for 30 minutes. "A 96-well microplate reader" will gauge the decrease in purple hue at 517 nm. The positive and negative controls will be ascorbic acid and ethanol, respectively. The ascorbic acid equivalents will represent the DPPH radical scavenging capacity[12].

3. RESULTS

Ethanol soluble extractive Value was found Mean±SD (%) = 6.7±0.12

Cholinesterase Inhibitory Activity

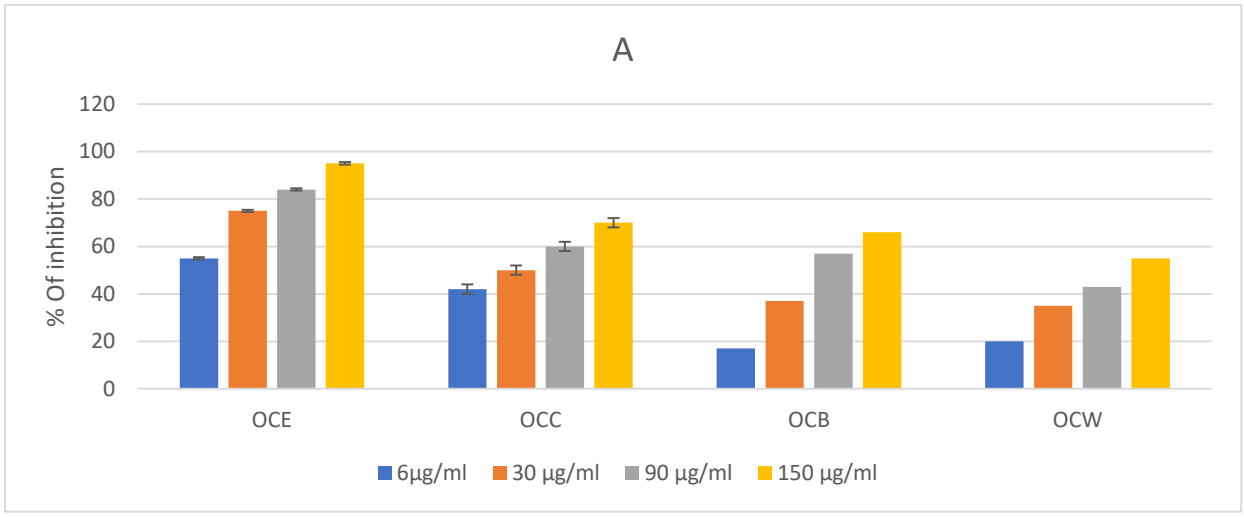
The ethanolic plant extracts (OCE) and its derived fractions (OCC, OCB, and OCW) were assessed for their inhibitory activity against the enzymes AChE and BuChE using Ellman's colorimetric method. A common drug called galactamine was used as the "positive control". The plant extracts and fractions that were evaluated at different dosages (6, 30, 90, and 150 µg/mL) had dose-dependent inhibitory effects on the "enzymes" AChE and BuChE. (Picture 1). With an IC50 range of 4.96 µg/mL, the OCE extracts were effective in inhibiting both AChE and BuChE, as shown in Table 1. The ethanolic fraction was shown to be the most effective among the fractions, exhibiting more activity than the other fractions against the AChE and BuChE enzymes, with IC50 ranging of 4.96 to 7.5µg/mL.

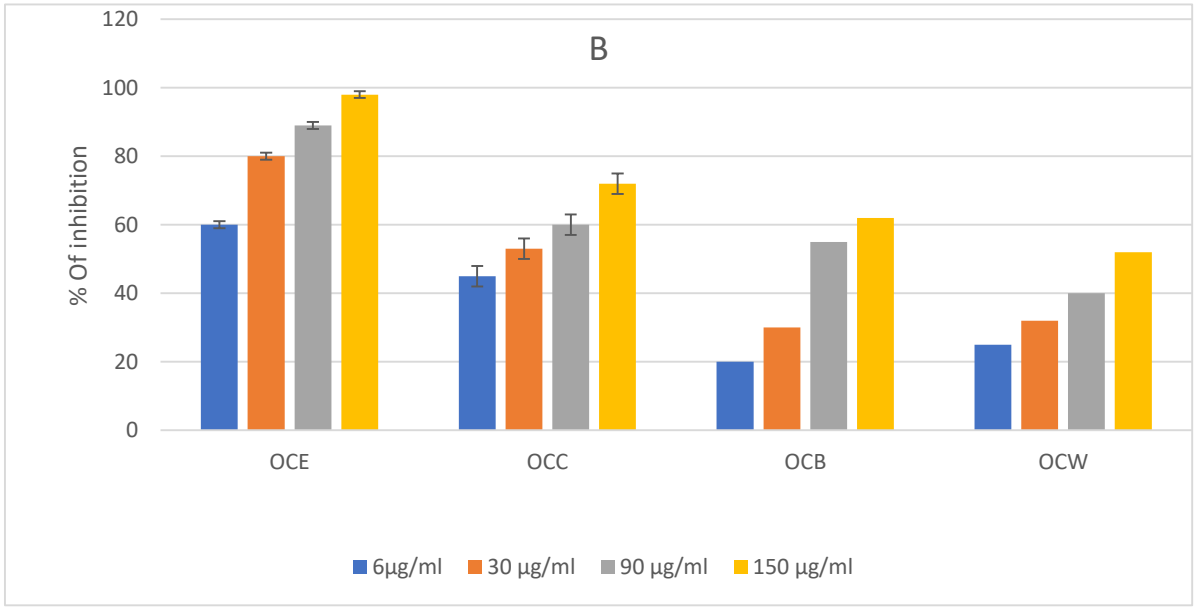
Thus, plant can be considered dual inhibitors of AChE and BuChE enzymes the IC50 value of "galactamine oil" table 1. The plants can be considered as dual inhibitors of AChE and BuChE enzymes. The IC50 values of galantamine were 0.77 ± 0.09 and 8.1± 0.02 against AChE and BuChE, respectively. In case of chloroform and butanol and water fraction chloroform fraction displayed moderate activity aganisyt Buche &Ache.

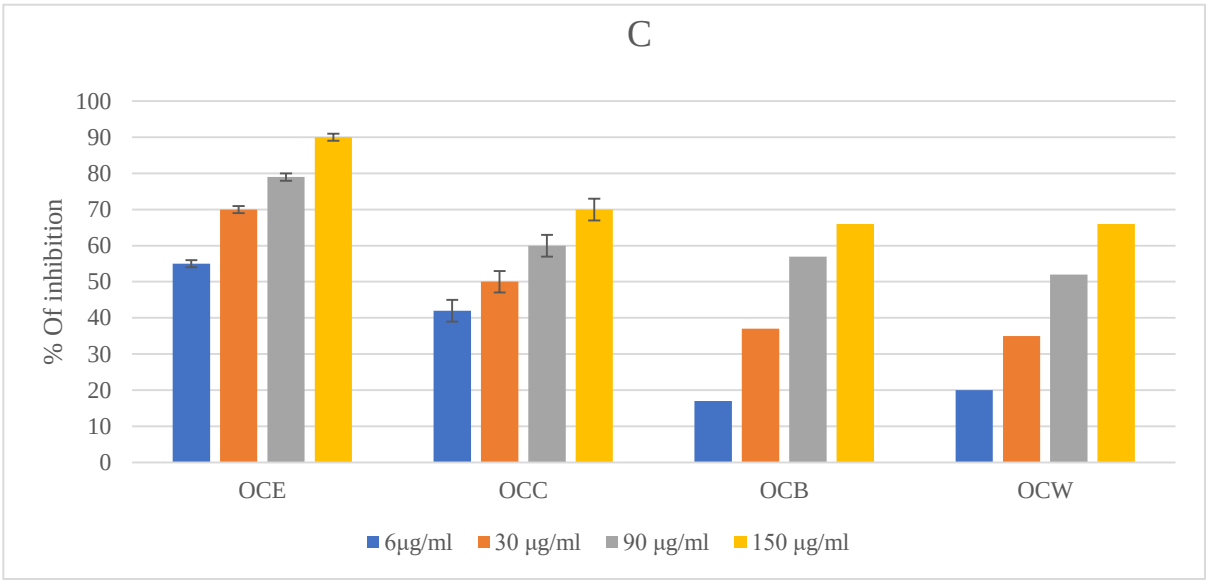
Inhibitionofα–andβ–glucosidaseenzymes

To ascertain the antidiabetic potency of the designated plants, the α- and β-Glu inhibitory activity of the extracts and produced "fractions" was assessed by in vitro enzyme assay using previously described methodology. The IC50 values of the examined extracts and fractions on α– and β–Glu are shown in the table (IC50 plots). The IC50 values of acarbose and D-Glucono-δ-lactone, which

were used as reference drugs, varied between 17.20 and 25.94 for these two compounds against "α– and β–Glu". The ethanolic extracts (OCE) that were evaluated demonstrated different degrees of inhibition against α- and β-Glu, with IC50 values ranging from 17.20 to 25.94. Every plant with a title showed greater effectiveness against α-Glu than the ordinary acarbose. These results indicate that the fractions OCC had the strongest inhibitory effects on α- and β-Glu activity. "The activity of α- and β-Glu was somewhat inhibited by OCW"; however, the OCB and OCW fractions showed lower activity against both enzymes.







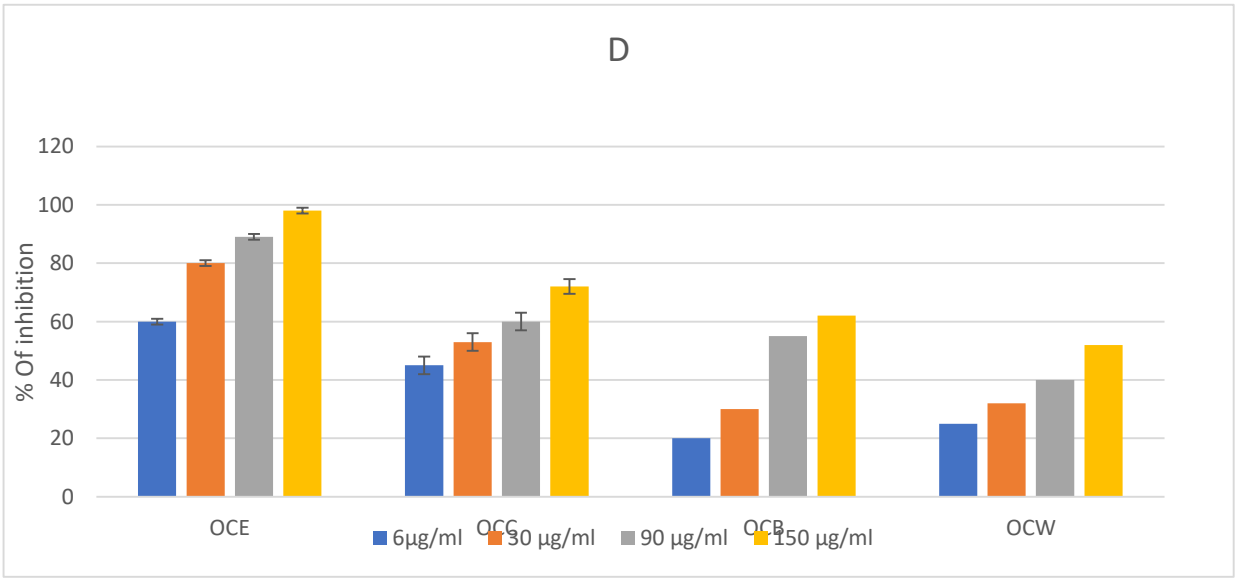


Fig 1: Fractions against A AChE; B BuChE; C α–Glucosidase; D β–Glucosidase

Table 1: IC50 Values of 90% ethanolic extracts and its derived fractions for AChE, BuChE, α– and β–Glucosidase inhibition assays

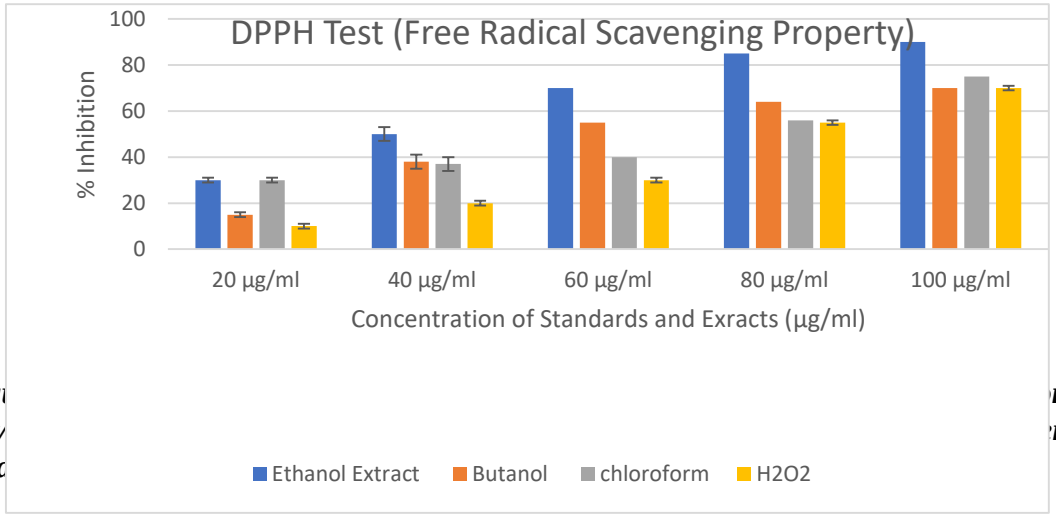
Plant	Extract	IC_{50} Values (µg/mL)			
		AChE	BuChE	α–Glc	β–Glc
O. corymbosa	90% EeOH	4.96 ± 0.96	7.5 ± 0.49	17.14 ± 1.9	25.94 ± 0.4

	$CHCl_3$	12.29 ± 2.14	9.94 ± 2.14	16.65 ± 1.99	27.38 ± 1.24
	n-BuOH	67.51 ± 4.81	168.62 ± 39.5	86.61 ± 5.32	270.95 ± 34.09
	H_2O	136.21 ± 8.2	245.1 ± 35.2	244.66 ± 12.8	387.59 ± 37.8
Galactamine	—	0.77±0.09	8.1±0.02	—	—
Acarbose	—	—	—	117.20±0.017	—

DPPH Test (Free Radical Scavenging Property)

When compared to extracts of ethanol, butanol, chloroform, and water ether, the hydro-"ethanolic extract exhibited a significantly "($p<0.05$)" higher capacity for scavenging free radicals". In comparison to the OCB standard IC50 of 41.11µg/ml, the IC50 values of hydro-ethanol, ethanol, ethyl acetate, and "petroleum ether extracts were found" to be "58.26µg/ml, 59.89µg/ml, 78.96µg/ml, and 108.06µg/ml, respectively".

Comparing the hydro-ethanolic extract to ethanol, butanol, chloroform, and water extract, a notable increase in free radical scavenging activitywasobserved.



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