Research Article


Antioxidant, antidiabetic, and anticholinesterase potential of *Chenopodium murale* L. extracts using *in vitro* and *in vivo* approaches

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**Abstract:** In this study, *Chenopodium murale* Linn. extracts have been evaluated for its *in vitro* antioxidant, enzyme inhibition, and *in vivo* neuropharmacological properties in streptozotocin (STZ)-induced memory impairment in rat model. First, the plant was subjected to extraction and fractionation, then quantitative phytochemical analysis was performed to estimate the major phytochemical groups in the extract where high amounts of phenolics and saponins were detected in crude and chloroform extract. The highest total phenolic contents, total flavonoid contents, and total tannin content were also recorded in crude extract and chloroform fraction. The *in vitro* antioxidant potential of chloroform fraction was high with IC$_{50}$ value of 41.78 and 67.33 μg/mL against DPPH and ABTS radicals, respectively, followed by ethyl acetate fraction. The chloroform fraction (ChMu-Chf) also exhibited potent activity against glucosidase with IC$_{50}$ of 89.72 ± 0.88 μg/mL followed by ethyl acetate extract (ChMu-Et; IC$_{50}$ of 140.20 ± 0.98 μg/mL). ChMu-Chf again exhibited potent activity against acetylcholinesterase (AChE) with IC$_{50}$ of 68.91 ± 0.87 μg/mL followed by ChMu-Et with IC$_{50}$ of 78.57 ± 0.95 μg/mL. *In vivo* memory impairment was assessed using the novel object discrimination task, Y-maze, and passive avoidance task. *Ex vivo* antioxidant enzyme activities and oxidative stress markers like catalase, superoxide dismutase (SOD), malondialdehyde, and glutathione were quantified, and the AChE activity was also determined in the rat brain. No significant differences were observed amongst all the groups treated with crude, chloroform, and ethyl acetate in comparison with positive control donepezil group in connection to initial latency; whereas, the STZ diabetic group displayed a significant fall in recall and retention capability. The blood glucose level was more potently lowered by chloroform extract. The crude extract also increased the SOD level significantly in the brain of the treated rat by 8.01 ± 0.51 and 8.19 ± 0.39 units/mg at 100 and 200 mg/kg body weight (*P < 0.01, n = 6), whereas the chloroform extract increased the SOD level to 9.41 ± 0.40 and 9.72 ± 0.51 units/mg, respectively, at 75 and 150 mg/kg body weight as compared to STZ group. The acetylcholine level was also elevated to greater extent by chloroform fraction that might contain a potential inhibitor of acetylcholinesterase. Treatment with *C. murale* alleviated cognitive dysfunction in behavioral study, and provided significant defense from neuronal oxidative stress in the brain of the STZ-induced diabetic rats. Thus *C. murale* Linn. could be an inspiring plant resource that needs to be further investigated for isolation of potential compounds in pure form and their evaluation as a potent neuropharmacological drug.

**Keywords:** cholinergic dysfunction, diabetes, memory impairment, discrimination index, stress biomarkers

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1 Introduction

Diabetes mellitus (DM), one of the metabolic disorders accompanied by excessive high level of blood sugar due to insufficient insulin production or insulin resistance within the body [1,2]. The International Diabetes Federation has reported 415 million diabetic cases worldwide in 2015 and has estimated that by the year 2040, this number will exceed 640 million with an increased incidence [3,4]. Type 1 diabetes mellitus is a juvenile or insulin-dependent DM in which β cells of the pancreas are destroyed by autoimmune reactions [5]. Type 2 diabetes mellitus (T2DM) is a partial insulin deficient or insulin-resistant DM, in which target receptors show improper response to the circulating insulin and eventually results in high level of insulin in the blood [6]. The subsistence high level of blood sugar ends in severe complications and could damage various organs like eyes, kidneys, heart, and nerves [4]. The complications mainly have a destructive influence on both the central and peripheral nervous systems leading to cognitive decline [5,7]. T2DM is the most common type of DM and it is more prevalent in aged population along with cognitive impairment resulting in dementia [6,8]. So, T2DM is a major culprit for cognitive decline and dementia in old age people [8]. In addition, recent studies have been reported that T2DM is associated with one of the neurodegenerative disorder, i.e. Alzheimer disease [8,9]. The cognitive decline in T2DM is associated with brain atrophy occurring in the hippocampal region, which is driven by oxidative stress, cholinergic dysfunction, and chronic hyperglycemia [5,9]. Insulin-sensitizing agents, antioxidants, acetylcholinesterase inhibitors, and antidiabetics have been documented to be effective in animal models but, at present, no established therapeutic agents are designed to prevent or manage cognitive decline in DM [10,11]. Acetylcholinesterase and butyrylcholine esterase are the enzymes responsible for the neurotransmission at the synaptic level. Enhanced activities of these enzymes lead to cholinergic deficit. Therefore, in pathological situations where activities of these enzymes are high, their antagonist are prescribed by the physicians to inhibit or reduce their activities. A number of drugs are in use and available in the market. Recently, the use of herbal remedies, more specifically botanicals or phytocannabinoids, is of major importance for scientific and pharmaceutical research communities [12,13].

Chenopodium genus belongs to Chenopodiaceae family commonly known as goosefoot family. The family includes 102 genera and 1,400 species of herbs and shrubs distributed throughout the globe and among these species, 200 species belong to the Chenopodium genus [14]. The herbs from Chenopodium genus have been used in Mexico for treating cough, hair loss, anxiety, depression, digestive discomforts, and sterility problems [15]. Other important species of the Chenopodium genus like Chenopodium album, Chenopodium ambrosioides, Chenopodium botrys, Chenopodium bonus henricus, and Chenopodium quinoa are reported to have neuroprotective properties [16,17]. The Chenopods are documented for their biologically active secondary metabolites including flavonoids, phenolics, triterpenoids, ecysteroids, and saponins [18]. Chenopodium murale Linn. is an annual erect herb and is native to Europe, Northern Africa, and some parts of Asia, but it is also distributed in tropical and subtropical regions [14,15]. The herb is known by a common name “nettle leaf goosefoot” and has demonstrated antibacterial, antifungal, analgesic, anti-inflammatory, anthelmintic, antihypertensive, antioxidant, cytotoxic, and hepatoprotective properties [19–21].

The genus and C. murale contain wide range of phytocannabinoids that are associated with therapeutic potentials. It is assumed that the herb may stabilize neurons, promote neurogenesis, and subsequently protect and amplify hippocampal function. With this aim, the present study was designed to probe the amplifying effects of C. murale on learning and memory in rat model with streptozotocin (STZ)-induced T2DM.

2 Materials and methods

2.1 Chemicals

The chemical reagents like n-hexane, methanol chloroform, ethyl acetate, butanol, DPPH, ABTS, Tween-80, and donepezil were obtained from Merck (Darmstadt, Germany) and STZ powder from Sigma-Aldrich (St Louis, MO, USA).

2.2 Collection and authentication

The collection of C. murale Linn. was carried out from Lower Dir of Khyber-Pakhtunkhwa K.P.K province in July 2020. The collected specimen was identified and authenticated by Dr Gul Rahim, Herbarium curator, University of Malakand, with a voucher specimen number of BG/ChMu/20-76 and deposited in the University of Malakand, Pakistan.
2.3 Extraction and fractionation

Dried and pulverized plant (4.75 kg) was soaked at room temperature in methanol with regular stirring for 2 weeks and then filtered. The filtered content was reduced by volume to a viscous mass through rotary vacuum evaporator obtaining crude extract (ChMu-Crd, 522 g) followed by fractionating with hexane (ChMu-Hex), chloroform (ChMu-Chl), ethyl acetate (ChMu-Et), and butanol (ChMu-But) to acquire the respective fractions with last fraction of aqueous (ChMu-Aq) portion.

2.4 Preliminary phytochemical tests

Crude extract was investigated for qualitative identification of various phytochemicals like for tannins, gelatin and ferric-chloride test; flavonoids using magnesium ribbon test; sodium hydroxide by saponin emulsion; alkaloids using Dragendorff’s test and froth test; chloroform and sulfuric acid test for terpenoids; Keller Kiliani for glycosides; filter paper test for oils and fats were employed as per reported methods [22,23].

2.5 Spectrophotometric quantitative phytochemical analysis

ChMu-Crd and various fractions of C. murale were spectroscopically evaluated for the quantification of phytochemicals including total phenolics, flavonoids, and tannins.

2.6 Determination of total phenolic contents (TPC)

The TPC, were investigated by Folin–Ciocalteu method and was employed as per previous reported protocol [24]. Briefly, 0.5 mL of water and Folin–Ciocalteu reagent at volume of 125 μL were added to 125 μL of the ChMu-Crd and fractions. The mixture contents were incubated for 6 min and 1.25 mL of Na2CO3 (7%, aqueous) was added to it. Finally, the volume was raised to 3 mL by adding distilled water and was followed by incubation for 90 min. The absorbance was taken spectrophotometrically at 765 nm and was repeated thrice. TPC were taken as gallic acid equivalents (gallic acid in mg/g of extract) through the calibration curve.

2.7 Determination of total flavonoids

The flavonoid–aluminum complex method was used for the estimation of total flavonoid contents (TFC) using spectrophotometer at 420 nm. About 1 mL of 2% aluminum trichloride (AlCl3) was mixed with 1 mL of ChMu-Crd and its fractions and were then incubated for about 15 min at room temperature. Absorbance was taken and TFC (QE quercetin in mg/g of extract) was deliberated from the quercetin calibration curve [24].

2.8 Total tannin content (TTC)

The sample (500 mg) was taken in distilled water (50 mL), placed for 1 h on shaker and the contents were filtered. The volume of filtrate was raised up to 50 mL with distilled water. About 2 mL of 0.1 M FeCl3 and 0.008 M potassium ferricyanide prepared in 0.1 N HCl was added to 5 mL volume of filtrate and absorbance was taken via spectrophotometer at 200 nm. The results were calculated and quantified as mg of GAE/g (mg of gallic acid equivalents) of the dry plant extract compared to standard curve of gallic acid [25].

2.9 Screening of quantitative phytochemical by non-spectrophotometric method

Quantification of ChMu-Crd and fractions of C. murale was carried out utilizing standard non-spectrophotometric techniques for the occurrence of alkaloids, flavonoids, saponins, and terpenoids.

2.10 Quantification of alkaloids

The calculated amount of pulverized sample was macerated in acetic acid (7.5 mL, 10% in ethanol) and allowed to stand for 4 h. The contents were then filtered and reduced to one-fourth of its original volume by evaporation in a rotary evaporator. The concentrated extract was precipitated by drop wise addition of conc. NH4OH and the settled precipitates were collected by filtration. The completely washed residue obtained was dried and percent contents of alkaloid was calculated [26].

2.11 Quantification of flavonoids

About 100 mg of plant sample was repeatedly extracted at room temperature with 10 mL of methanol (80%, aqueous)
followed by filtration. The filtrate was placed in a Petri dish for complete evaporation using water bath for complete dryness and the yield was calculated [27].

2.12 Quantification of saponins

About 100 mg of pulverized sample was macerated in aqueous ethanol (15 mL, 20% in ethanol) and heated at 55°C with stirring on a water bath for 4 h. The contents were filtered and the process was repeated again. The combine contents were reduced by evaporation in a rotary evaporator. The concentrated filtrate was extracted with di-ethyl ether using separating funnel and ethereal fraction was separated. Aqueous layer was further extracted with n-butanol and repeated again. The n-butanol mixed layers were further washed with NaCl (5% aqueous). The resultant solution was evaporated to dryness and the percent of saponin content was quantified [28].

2.13 Quantification of terpenoids

Powdered sample of plant about 100 mg was soaked in methanol for 1 day at room temperature. The extracted sample was filtered and then the filtrate was extracted with hexane, dried, and the obtained extract was taken as the total terpenoids in the sample [29].

2.14 Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the extract and fractions was carried out using an Agilent USB-393752 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with an HHP-5MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness) for analysis outfitted with an Agilent HP-5973 mass selective detector (Agilent Technologies, Palo Alto, CA, USA) in the electron impact mode (ionization energy: 70 eV). The conditions used include flow rate of 1.0 mL/min, splitless injection mode of 1 μL of the sample volume, oven temperature of 70°C, injector temperature of 220°C, and detector temperature of 290°C. Mass spectra scan was recorded at ionization energy of 70 eV from 40 to 500 m/z. The components were identified using NIST database (NIST Mass Spectral Database, 2014) and the literature [30].

2.15 Determination of in vitro antioxidant activity

Antioxidant effect of the *C. murale* extract and also its fractions was screened by DPPH radical scavenging activity. Briefly, 100 μL of ChMu-Crd and fractions were treated and mixed with DPPH (100 μL, 0.1 mM) solution and was incubated for about 10 min and absorbance was noted at 517 nm. Similarly, ABTS radical scavenging assay was calculated with a slight modification. The stock solution was obtained by dissolving 10 mg of ABTS in potassium persulphate (2.45 mM) and methanol. About 100 μL of *C. murale* extract and fractions was allowed to react with 100 μL ABTS in dark for 10–15 min and the absorbance was recorded at 734 nm. Percent antioxidant activity was calculated and IC₅₀ was determined [31].

2.16 Determination of in vitro enzyme inhibition activity

Enzyme inhibitory effect of the *C. murale* extract and its fractions was screened against α-glucosidase and anticholinesterase inhibitory activity.

2.16.1 α-Glucosidase inhibitory activity

The inhibitory activity for α-glucosidase for extract and fractions was determined. The test samples were prepared in varying concentrations by mixing 20 μL α-glucosidase (0.5 unit/mL), 120 μL of 0.1 M phosphate buffer (pH 6.9) and 10 μL of extract and fractions. For incubation of the mixture solutions, 96-well plates were used at 37°C for 15 min. To initiate the enzymatic reaction, 20 μL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to the mixture and re-incubated for another 15 min. Sodium carbonate (80 μL, 0.2 M) was added to prevent the reaction and then absorbance was taken at 405 nm using microplate reader. For positive control, sample free reaction system was used and the blank was without α-glucosidase for the purpose of background absorbance correction [32].

2.16.2 Anticholinesterase activity

To investigate the cholinesterase inhibitory activity, AChE enzyme was used. Briefly, in test tubes different dilutions
of extract and fractions (50 μL) and 0.5 mL of AChE was taken and incubated at 25°C. About 100 μL DTNB and 2.4 mL buffer was added to these test tubes, and was incubated again at 25°C for 5 min. By the addition of 40 μL of ATChI the reaction was initiated. The reaction mixture was incubated for 20 min at 25°C. Spectrophotometer was used to record the absorbance of each sample at 412 nm. Donepezil was used as standard [33].

2.17 Experimental animals

Male Balb/C mice (20–23 g) and Wistar albino male rats (170–210 g) were used in this experimental study. The animals were procured from the Veterinary Research Institute (VRI), Peshawar, Pakistan kept in standard plastic cages with standard laboratory conditions having temperature in the range of 25 ± 2°C, relative humidity of 55–65%, and light and dark cycle of 12 h, provided with a standard diet and water ad libitum. Before 2 weeks of experiment, the animals were acclimatized to the laboratory conditions. The animals were euthanized by injection of pentobarbital sodium (200 mg/kg) after the experiments. All protocols employed were accepted by the Ethical Committee vide notification Pharm/EC-ChMu/22-11/20 as per approved “Animal Bye-Laws 2008, Scientific Procedures Issue-I of the University of Malakand.”

2.18 Acute toxicity test

Acute toxicity test in mice was carried out to assess the safety profile of the sample at different dose concentrations in two phases. The animals were observed in each group for any mortality or adverse effects for the next 24 h followed by careful observation for 2 weeks [34].

2.19 Induction of DM

After acclimatization, intraperitoneal injection (i.p.) of STZ (50 mg/kg, 0.1 M citrate buffer) was administered to overnight fasted rats. In addition, to avoid death of the animals due to STZ-induced hypoglycemic shock, the rats were subsequently given 10% glucose solution for 3 days. For determination of fasting blood glucose, samples of blood were acquired from the vein of tail of the animals and glucose level was determined using SD glucometer by one touch glucometer strips (ACCU-CHECK, Active blood glucose meter, Korea). Rats having fasting glucose level in blood higher than 250 mg/dL were reflected as diabetic [35].

2.20 Animals grouping and treatment schedule

Animals were randomly divided into experimental groups (n = 6), consisting of control, diabetic, and diabetic treated with 100 and 200 mg/kg b.w. of ChMu-Crd, fractions (75 and 150 mg/kg), and donepezil positive control group. The animals in control (normal) and STZ diabetic rat groups were administered vehicle only. Treated animals received (ChMu-Crd) and fractions p.o. at their respective doses after induction of diabetes with STZ injection for 4 weeks. The positive control group was treated with standard drug donepezil (2 mg/kg).

2.21 Assessment of cognitive function

Behavioral tasks consisting of novel object discrimination (NOD), passive avoidance, and Y-maze were carried on Week 5. On Day 29 Y-maze, Day 30–31 NOD, and Day 32–33 passive avoidance task (PAT) were carried out to determine the learning and memory functions.

2.22 Novel object discrimination task (NODT)

Rats were challenged in this task for the crude extract and fractions as per previously reported protocols. After habituation and acclimatization, rats in each group had two consecutive trials for object exploration (5 min each), which was carried out with a break of 4 h in between the two trials. The open field apparatus and all of the objects were washed with 70% ethanol during the intertrial interval to prevent a confounding error due to the influence of odor. In the familiarization phase (sample), the rats were challenged to explore two objects similar to each other. In the second phase (test), any of the two objects were changed by a novel object. Time for exploration in seconds for the objects including chewing, licking, sniffing, or pointing the vibrissae of nose towards the object was recorded [36]. The discrimination ratio (% DI) was then determined using the relation:
DI\% = \frac{(T \text{ novel object} - T \text{ familiar object})}{(T \text{ novel object} + T \text{ familiar object})} \times 100. \quad (1)

2.23 Y-maze paradigm

The Y-maze task is non-invasive and a reliable behavioral test to assess the recognition memory assessment of spontaneous alternation for the crude extract and fractions as per previously reported protocols. Animals were placed at one end of the tagged arms and were allowed to move freely within the arms in a single session for about 8 min. Entry arm was calculated when the hind paws of the subject were totally inside the arm. The alternation was thus defined as a successive entry to three arms via corresponding triplet sets (i.e., B, C, A or A, B, C, etc.). Maximum number of the spontaneous alternations were then the total number of arms entered-2 and the percentage is evaluated as the ratio of actual alternations to possible alternations (that are defined as the total number of arm entries-2). The apparatus arena was cleaned with 70% ethanol during the inter-trial interval to prevent a confounding error due to the influence of odor [36].

2.24 Passive avoidance paradigm

The memory assessment of this model has been illustrated before and used with slight modifications. After habituation and acclimatization, each rat in the acquisition session was placed for 5 min in the light chamber and then the guillotine door was lifted up. Latency time in seconds was noted as the initial latency (IL) to pass through the door and enter into the dark compartment. Subsequently, an electric foot shock was given for the purpose to create a conditioned behavior in the next session. On the next day, a retention session was carried out and the step-through latency (STL) was recorded and the time required to enter the dark compartment was noted with a cut-off time of 480 s [36].

2.25 Estimation of blood glucose and insulin level

Blood samples from rats were collected after performing behavioral studies. The blood glucose levels were determined using glucometer. To evaluate the serum insulin levels, a commercial ELISA kit was used as per manufacturer’s instructions [37].

2.26 Measurement of antioxidant enzyme activities and oxidative stress markers

The brain was extracted and a homogenate (10% w/v) was made with 0.1 M phosphate buffer having pH of 7.4. This was made by centrifugation for the assessment of brain oxidative status and estimating the brain acetylcholinesterase activity. Lipid peroxidation (LPO) was quantified by evaluating glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA), as oxidative stress markers. Acetylcholinesterase activities were quantified in homogenate as reported in the method illustrated by Ellman et al. (1961) [38].

2.27 Statistical analysis

Data are tabulated as mean ± SEM and are statistically manipulated with statistical software (GraphPad Prism 5.01 version) using analysis of variance and Dunnett’s test. P-value of P < 0.05 is considered statistically significant.

3 Results

3.1 Phytochemical screening

Results of initial phytochemical screening of the crude extract of C. murale (ChMu-Crd) are summed up and revealed the existence of saponins, phenolics, alkaloids, flavonoids, glycosides, tannins, and terpenoids (Table 1).

3.2 Quantitative phytochemical analysis for TFC, TTC, and TPC by spectrophotometric method

Results of phytochemical analysis TPC, TFC, and TTC spectrophotometrically in the ChMu-Crd and fractions of C. murale are illustrated in Table 2. ChMu-Chl and ethyl ChMu-Et fractions demonstrated highest contents of
3.3 Quantitative non-spectrophotometric phytochemical analysis of terpenoid flavonoids, saponins, and alkaloids

*C. murale* and fractions were quantified for phytochemicals by non-spectrophotometric methods and the results of flavonoids, alkaloids, terpenoids, and saponins are presented as percent yield/g in Table 3.

### 3.4 GC-MS analysis

The GC-MS analysis of pharmacologically active fractions (ChMu-Chl) of *C. murale* was performed for the identification of major phytochemical components that has been compiled in Table 4. A total number of 26 compounds have been identified and among them 2-((4-amino-3-methylphenyl)(ethyl)amino)ethanol, *n*-hexadecanoic acid, 9-octadecenoic acid, diisooctyl phthalate, oleic acid, stigmasterol, rhodopin, and 12-O-acetylgligol 8-tiglate have been detected.

### 3.5 *In vitro* antioxidant activity of ChMu-Crd and fractions

To estimate the radical scavenging potential of extract, DPPH assay was used and the results are given in Table 5. The test samples showed DPPH radical scavenging activity with *IC*₅₀ values ranging from 41.78 to 612.13 μg/mL. The ChMu-Chl of *C. murale* exhibited potent antioxidant activity against DPPH with *IC*₅₀ of 41.78 ± 0.49 μg/mL followed by ChMu-Et with *IC*₅₀ of 53.59 ± 0.51 μg/mL. The crude extract against DPPH produced radical scavenging potential with *IC*₅₀ of 422.45 ± 0.95 μg/mL. Standard ascorbic acid produced significant response of antioxidant effect with *IC*₅₀ of 2.78 ± 0.39 μg/mL (Table 5).

#### Table 1: Preliminary phytochemical evaluation of crude extract of *C. murale*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test performed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>Froth/emulsion</td>
<td>++ +/+</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Ferric chloride</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>NaOH/Mg ribbon</td>
<td>+/-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Keller Kiliani</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Gelatin/ferric chloride</td>
<td>+/-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Sulfuric acid, chloroform</td>
<td>++ +</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>Filter paper</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>Xanthoproteic/biuret</td>
<td>+/-</td>
</tr>
</tbody>
</table>

All the values are expressed as mean ± SEM, *n* = 3, TPC: total flavonoid contents, TFC: total phenolic contents, TTC: total tannin contents, ChMu-Crd: crude extract, ChMu-Chl: chloroform fraction, ChMu-Hex: n-hexane fraction, ChMu-But: butanol fraction, ChMu-Et: ethyl acetate fraction, ChMu-Aq: aqueous fraction.

Flavonoids with 59.33 ± 0.77 and 46.12 ± 0.89 of mg GAE/gm of dry sample.

TFC was assessed against calibration cure and results revealed that the ChMu-Chl and ChMu-Et fractions revealed highest flavonoid contents with 74.22 ± 1.08 and 65.59 ± 1.01 mean values. Similarly, the results of TTC are also shown in Table 2. On the other hand, n-hexane, butanol, and aqueous fractions were found with lowest contents of phenolics, flavonoids, and tannins.

#### Table 2: Quantitative spectrophotometric analysis of phytochemical in crude extract and fractions of *C. murale*

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPC (mg GAE/g)</th>
<th>TFC (mg QE/g)</th>
<th>TTC (mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChMu-Crd</td>
<td>34.56 ± 0.67</td>
<td>40.88 ± 0.93</td>
<td>72.16 ± 0.97</td>
</tr>
<tr>
<td>ChMu-Hex</td>
<td>27.03 ± 0.89</td>
<td>32.12 ± 0.98</td>
<td>42.98 ± 0.98</td>
</tr>
<tr>
<td>ChMu-Chl</td>
<td>59.33 ± 0.77</td>
<td>74.22 ± 1.08</td>
<td>47.84 ± 1.07</td>
</tr>
<tr>
<td>ChMu-Et</td>
<td>46.12 ± 0.89</td>
<td>65.59 ± 1.01</td>
<td>58.36 ± 0.81</td>
</tr>
<tr>
<td>ChMu-But</td>
<td>40.45 ± 1.01</td>
<td>49.88 ± 0.89</td>
<td>51.11 ± 0.93</td>
</tr>
<tr>
<td>ChMu-Aq</td>
<td>20.09 ± 0.71</td>
<td>27.81 ± 0.93</td>
<td>49.71 ± 0.97</td>
</tr>
</tbody>
</table>

All the values are expressed as mean ± SEM, *n* = 3, TPC: total flavonoid contents, TFC: total phenolic contents, TTC: total tannin contents, ChMu-Crd: crude extract, ChMu-Chl: chloroform fraction, ChMu-Hex: n-hexane fraction, ChMu-But: butanol fraction, ChMu-Et: ethyl acetate fraction, ChMu-Aq: aqueous fraction.

#### Table 3: Non-spectrophotometric quantitative phytochemical analysis of crude extract and fractions of *C. murale*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChMu-Crd</td>
<td>5.63 ± 0.51</td>
<td>9.01 ± 0.91</td>
<td>4.67 ± 0.69</td>
<td>7.8 ± 0.88</td>
</tr>
<tr>
<td>ChMu-Hex</td>
<td>0.90 ± 0.29</td>
<td>2.98 ± 0.57</td>
<td>3.61 ± 0.56</td>
<td>6.11 ± 0.73</td>
</tr>
<tr>
<td>ChMu-Chl</td>
<td>3.77 ± 0.33</td>
<td>8.91 ± 1.01</td>
<td>4.09 ± 0.71</td>
<td>7.07 ± 0.81</td>
</tr>
<tr>
<td>ChMu-Et</td>
<td>2.09 ± 0.41</td>
<td>7.96 ± 0.71</td>
<td>3.88 ± 0.76</td>
<td>7.13 ± 0.79</td>
</tr>
<tr>
<td>ChMu-But</td>
<td>1.88 ± 0.67</td>
<td>4.67 ± 0.66</td>
<td>3.01 ± 0.69</td>
<td>5.72 ± 0.66</td>
</tr>
<tr>
<td>ChMu-Aq</td>
<td>1.73 ± 0.73</td>
<td>2.72 ± 0.56</td>
<td>2.12 ± 0.49</td>
<td>6.01 ± 0.69</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM, *n* = 3.
The IC$_{50}$ values of the investigated ChMu-Crd and fractions using ABTS method were in the range of 67.33–689.10 µg/mL. Among all the test samples, ChMu-Chf of C. murale exhibited potent antioxidant activity against ABTS with IC$_{50}$ of 67.33 ± 0.56 µg/mL followed by ChMu-Et with IC$_{50}$ of 69.93 ± 0.47 µg/mL. Standard ascorbic acid produced significant response of antioxidant effect with IC$_{50}$ of 4.09 ± 0.31 µg/mL.

### 3.6 In vitro enzyme inhibition activity

To determine the enzyme inhibition potential of extract and fractions, glucosidase and cholinesterase assay were used and the results are given in Table 6. The extract and fractions showed in vitro glucosidase activity with IC$_{50}$ values ranging from 340.61 to 89.72 µg/mL. The ChMu-Chf of C. murale exhibited potent activity against glucosidase with IC$_{50}$ of 89.72 ± 0.88 µg/mL followed by ChMu-Et with IC$_{50}$ of 140.20 ± 0.98 µg/mL. Standard acarbose produced significant response with IC$_{50}$ of 93.21 ± 1.05 µg/mL (Table 6).

### 3.7 Evaluation of learning behaviors

The discrimination index in NODT was significantly lesser in the STZ diabetic rats that was found to be 37.58 ± 2.01 (P < 0.001) versus the normal control group (74.47 ± 2.11). Administration of crude extract (ChMu-Crd) at a dose of

---

### Table 4: List of compounds in the chloroform fraction of C. murale

<table>
<thead>
<tr>
<th>S. no.</th>
<th>RT</th>
<th>Name of compound</th>
<th>CAS</th>
<th>Mol Wt</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.73</td>
<td>Trichloromethane</td>
<td>67-66-3</td>
<td>118</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>4.43</td>
<td>m-Cymene</td>
<td>535-77-3</td>
<td>134</td>
<td>0.46</td>
</tr>
<tr>
<td>3</td>
<td>8.58</td>
<td>Pyrazole-4-carboxylic acid</td>
<td>24447-68-5</td>
<td>127</td>
<td>0.32</td>
</tr>
<tr>
<td>4</td>
<td>9.13</td>
<td>2-((4-Amino-3-methylphenyl)(ethyl)amino)ethanol</td>
<td>359-51-5</td>
<td>194</td>
<td>8.65</td>
</tr>
<tr>
<td>5</td>
<td>10.78</td>
<td>3-Methyl-4,7-dioxo-oct-2-enal</td>
<td>NA</td>
<td>168</td>
<td>4.40</td>
</tr>
<tr>
<td>6</td>
<td>12.02</td>
<td>tert-Hexadecanethiol</td>
<td>25360-09-2</td>
<td>258</td>
<td>0.30</td>
</tr>
<tr>
<td>7</td>
<td>12.59</td>
<td>Methyl 1-allyl-2-hydroxy-6-methylcyclohexanecarboxylate</td>
<td>124899-20-3</td>
<td>212</td>
<td>0.34</td>
</tr>
<tr>
<td>8</td>
<td>13.45</td>
<td>n-Octacosane</td>
<td>630-02-4</td>
<td>394</td>
<td>1.85</td>
</tr>
<tr>
<td>9</td>
<td>14.16</td>
<td>Dihydroxanthin</td>
<td>NA</td>
<td>308</td>
<td>0.38</td>
</tr>
<tr>
<td>10</td>
<td>14.77</td>
<td>2-Dodecen-1-yl(-)sucinic anhydride</td>
<td>19780-11-1</td>
<td>266</td>
<td>0.17</td>
</tr>
<tr>
<td>11</td>
<td>15.22</td>
<td>9-Octylethyldecane</td>
<td>7225-64-1</td>
<td>352</td>
<td>0.49</td>
</tr>
<tr>
<td>12</td>
<td>16.91</td>
<td>9-n-Hexylethyldecane</td>
<td>55124-79-3</td>
<td>324</td>
<td>1.56</td>
</tr>
<tr>
<td>13</td>
<td>17.40</td>
<td>Hexahydrofarnesyl acetone</td>
<td>502-69-2</td>
<td>268</td>
<td>2.63</td>
</tr>
<tr>
<td>14</td>
<td>17.79</td>
<td>Methyl 8-(2-((2-pentylcyclopropyl)methyl)cyclopropyl)octanoate</td>
<td>10152-66-6</td>
<td>322</td>
<td>1.49</td>
</tr>
<tr>
<td>15</td>
<td>18.26</td>
<td>Methyl 14-methylpentadecanolate</td>
<td>5129-60-2</td>
<td>270</td>
<td>2.52</td>
</tr>
<tr>
<td>16</td>
<td>18.79</td>
<td>n-Hexadecanoic acid</td>
<td>57-10-3</td>
<td>256</td>
<td>9.64</td>
</tr>
<tr>
<td>17</td>
<td>19.60</td>
<td>Ethyl linoleate</td>
<td>44-35-4</td>
<td>308</td>
<td>4.24</td>
</tr>
<tr>
<td>18</td>
<td>20.05</td>
<td>9-Octadecenoic acid</td>
<td>112-80-1</td>
<td>282</td>
<td>8.47</td>
</tr>
<tr>
<td>19</td>
<td>20.70</td>
<td>12-O-Acetylglycol 8-tiglate</td>
<td>51906-13-9</td>
<td>490</td>
<td>1.32</td>
</tr>
<tr>
<td>20</td>
<td>21.07</td>
<td>5H-Cyclopenta[3,4]benz[1,2-e]azulen-5-one,9,9a-bis (acetoxy)-1,1a,1b,2,4a,7b,8,9,9a-decahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-1(1aa,1bb,2a,4ab,7aa,7ba,8a,9b,9aa)]:(-9CI)</td>
<td>77573-19-4</td>
<td>464</td>
<td>4.04</td>
</tr>
<tr>
<td>21</td>
<td>22.29</td>
<td>Diphenyl ether</td>
<td>27554-26-3</td>
<td>390</td>
<td>22.58</td>
</tr>
<tr>
<td>22</td>
<td>23.03</td>
<td>2-([9Z]-9-Octadecone)(vinyl)ethyl stearate</td>
<td>29027-97-2</td>
<td>578</td>
<td>2.15</td>
</tr>
<tr>
<td>23</td>
<td>23.88</td>
<td>8-[(2-Aminoethylthio)(guanosine-3,5’-cyclic monophosphate</td>
<td>115974-70-4</td>
<td>419</td>
<td>2.64</td>
</tr>
<tr>
<td>24</td>
<td>25.02</td>
<td>3-(Methoxymethoxy)cholest-4-ene</td>
<td>4707-85-1</td>
<td>430</td>
<td>0.33</td>
</tr>
<tr>
<td>25</td>
<td>25.92</td>
<td>Stigmasterol</td>
<td>83-49-7</td>
<td>412</td>
<td>17.74</td>
</tr>
<tr>
<td>26</td>
<td>27.18</td>
<td>Rhodopin</td>
<td>105-92-0</td>
<td>554</td>
<td>0.88</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM, n = 3.

### Table 5: Antioxidant activity of extract and fractions of C. murale

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH (IC$_{50}$ µg/mL)</th>
<th>ABTS (IC$_{50}$ µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChMu-Crd</td>
<td>422.45 ± 0.95</td>
<td>497.08 ± 0.93</td>
</tr>
<tr>
<td>ChMu-Nhx</td>
<td>461.09 ± 0.81</td>
<td>508.61 ± 0.68</td>
</tr>
<tr>
<td>ChMu-Chf</td>
<td>417.8 ± 0.49</td>
<td>67.33 ± 0.56</td>
</tr>
<tr>
<td>ChMu-Et</td>
<td>53.59 ± 0.51</td>
<td>69.93 ± 0.47</td>
</tr>
<tr>
<td>ChMu-But</td>
<td>95.38 ± 0.77</td>
<td>106.03 ± 0.44</td>
</tr>
<tr>
<td>ChMu-Aqs</td>
<td>612.13 ± 0.96</td>
<td>689.10 ± 0.69</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>2.78 ± 0.39</td>
<td>4.09 ± 0.31</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM, n = 3.
Table 6: Enzyme inhibition of crude extract and fractions against IC₅₀ values

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucosidase</th>
<th>AChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChMu-Crd</td>
<td>197.20 ± 1.03</td>
<td>121.15 ± 1.33</td>
</tr>
<tr>
<td>ChMu-Nhx</td>
<td>340.61 ± 1.39</td>
<td>197.01 ± 1.13</td>
</tr>
<tr>
<td>ChMu-Chf</td>
<td>89.72 ± 0.88</td>
<td>68.91 ± 0.87</td>
</tr>
<tr>
<td>ChMu-Et</td>
<td>140.20 ± 0.98</td>
<td>78.57 ± 0.95</td>
</tr>
<tr>
<td>ChMu-But</td>
<td>178.16 ± 1.01</td>
<td>121.21 ± 1.09</td>
</tr>
<tr>
<td>ChMu-Aqs</td>
<td>156.80 ± 0.79</td>
<td>133.12 ± 0.98</td>
</tr>
<tr>
<td>Acarbose</td>
<td>93.21 ± 1.05</td>
<td>—</td>
</tr>
<tr>
<td>Donepezil</td>
<td>—</td>
<td>6.98 ± 0.49</td>
</tr>
</tbody>
</table>

The IC₅₀ values of the investigated ChMu-Crd and fractions using AChE that were in the range of 68.91–197.01 µg/mL. Among all test samples, ChMu-Chf of C. murale exhibited potent activity against AChE with IC₅₀ of 68.91 ± 0.87 µg/mL followed by ChMu-Et with IC₅₀ of 78.57 ± 0.95 µg/mL. Standard donepezil produced significant response with IC₅₀ of 6.98 ± 0.49 µg/mL.

100 and 200 mg/kg b.w. fractions (75 and 150 mg/kg b.w.) significantly prevented this reduction and enhanced (P < 0.01 and P < 0.001) the index when compared to diabetic control (STZ) group as shown in Figure 1a. The administration of 100 mg/kg b.w. crude extract of C. murale (ChMu-Crd) exhibited 57.36 ± 2.09 of %DI and 200 mg/kg produced 59.44 ± 1.99 of %DI response. The ChMu-Chl produced a significant %DI of 63.22 ± 2.01 and 64.51 ± 1.95 (P < 0.001) versus the STZ diabetic group (37.58 ± 2.01). Similarly, the ChMu-Et displayed similar results. The %DI in NODT was significantly higher for standard donepezil in the diabetic rats (67.09 ± 2.09%, P < 0.001) versus the normal control group. Appraisal of behaviors in the Y-maze model is a marker of spatial recognition memory (Figure 1b) and signifies that the percent spontaneous alternations was significantly lesser in the STZ diabetic rats in comparison to the normal control rats (P < 0.001), and ChMu-Crd, ChMu-Chl, ChMu-Et, and positive control donepezil administration to the diabetic rats significantly ameliorated this alteration (P < 0.01 and P < 0.001). Figure 1c displays the results acquired from the PAT as designated by the variables IL and STL.

3.8 Estimation of blood glucose and insulin level

Blood glucose and insulin levels of the various groups estimated in the blood samples of rats collected just after completion of behavioral studies are summarized in Table 7. As compared to the normal control group (98.36 ± 1.69, n = 6), mean blood glucose level of STZ diabetic group was higher to a level of 379.24 ± 2.03, P < 0.001, and their mean circulating insulin levels was found to be lower to 5.11 ± 0.48 versus normal control group (19.06 ± 0.61, n = 6). In comparison to the STZ diabetic group, the ChMu-Crd, ChMu-Chl, and ChMu-Et-treated groups had lower blood glucose levels significantly (P < 0.01 and P < 0.001) and higher blood insulin level. Administration of ChMu-Crd at 100 and 200 mg/kg b.w. lowers the blood glucose levels significantly to 157.31 ± 1.61 and 142.45 ± 1.51 (P < 0.01), respectively. ChMu-Chl at 75 and 150 mg/kg b.w. significantly lowers the blood glucose levels to 128.75 ± 1.79 and 103.89 ± 1.88, respectively, when compared to STZ diabetic group.

3.9 Assessment of antioxidant enzyme activities and oxidative stress markers

Administration of STZ causes substantial elevation of MDA and AChE level, decreases the contents of ACh, and augmented oxidative stress in rats as evidenced from decrease in the CAT and SOD level in the rat brain. The crude extract, chloroform, and ethyl acetate fractions showed distinct effect on these alterations by declining the content of MDA and AChE. Likewise, it also enhances the level of ACh, CAT, and SOD contents signifying the possible role as antioxidant on oxidative stress.

STZ administration resulted in a significant increase in the level of MDA (↑ 2.12-folds) that was reverted by administration of donepezil and the level fell to ↓ 2.01-folds (Figure 2a). However, ChMu-Crd decreased the level by ↓ 1.54 and 1.56-folds at 100 and 200 mg/kg b.w., respectively, ChMu-Chl decreased the level by ↓ 1.71 and 1.81-folds, and ChMu-Et decreased the level by ↓ 1.60 and 1.65-folds, respectively, at 150 and 75 mg/kg b.w. in comparison to diabetic amnesic group (↑ 2.12-folds). Similar type of results was observed when the brain was processed for the determination of GSH level (Figure 2b).

The results from Figure 2c produced the significant output of ChMu-Crd and fractions on CAT level in the brains of the studied animals. In contrast with control treated level of CAT 17.89 ± 0.61, STZ administration caused a significant fall of 5.87 ± 0.57, P < 0.001 (↓ 3.04-folds) in level of CAT enzyme. Donepezil increased the level significantly to 16.98 ± 0.59, P < 0.001 (↑ 2.89-folds). ChMu-Crd produced a similar response to standard and significantly increased the level of CAT (↑ 2.48 and 2.52-folds) at dose of 200 and 100 mg/kg b.w. in
comparison to diabetic amnesic group. ChMu-Chl significantly increased the level of CAT (↑2.63 and 2.72-folds) at 75 and 150 mg/kg. The STZ administration resulted in significant decline in the level of SOD by 6.19 ± 0.41 units/mg of protein, ↓2.18-folds, *P < 0.001, n = 6 in the brain homogenate compared to control (13.47 ± 0.49 units/mg protein, *P < 0.05, **P < 0.01, and ***P < 0.001 as comparison of STZ-treated diabetic (amnesic) group vs donepezil, crude extract, and fraction-treated groups, using one-way ANOVA followed by Dunnett comparison. Crude extract, chloroform, and ethyl acetate fraction.

3.10 Effect on AChE activity

A considerable rise in the level of AChE in the brain homogenate was observed after STZ administration (Table 8), and was effectively reversed by the donepezil, crude
Table 7: Effects of crude extract and fractions on blood glucose and insulin level

<table>
<thead>
<tr>
<th>Treatment/dose (mg)</th>
<th>Blood glucose level (mg/dL)</th>
<th>Blood insulin level (µU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>98.36 ± 1.69</td>
<td>19.06 ± 0.61</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>379.24 ± 2.03</td>
<td>5.11 ± 0.48</td>
</tr>
<tr>
<td>Donepezil 2</td>
<td>319.39 ± 1.49</td>
<td>7.21 ± 0.55</td>
</tr>
<tr>
<td>Metformin 50</td>
<td>161.37 ± 1.69***</td>
<td>15.37 ± 0.58***</td>
</tr>
<tr>
<td>ChMu-Crd 100</td>
<td>157.31 ± 1.61**</td>
<td>10.06 ± 0.51**</td>
</tr>
<tr>
<td></td>
<td>142.45 ± 1.51***</td>
<td>10.88 ± 0.53***</td>
</tr>
<tr>
<td>ChMu-Chl 75</td>
<td>128.75 ± 1.79***</td>
<td>12.40 ± 0.69***</td>
</tr>
<tr>
<td></td>
<td>103.89 ± 1.88***</td>
<td>13.78 ± 0.58***</td>
</tr>
<tr>
<td>ChMu-Et 75</td>
<td>134.73 ± 1.71***</td>
<td>11.18 ± 0.49***</td>
</tr>
<tr>
<td></td>
<td>114.61 ± 1.69***</td>
<td>11.96 ± 0.63***</td>
</tr>
</tbody>
</table>

Mean ± SEM (n = 6). One-way ANOVA after which Dunnett’s post hoc multiple comparison test to ascertain the P values. !!!P < 0.001 comparison of STZ-treated diabetic (amnesic) group vs normal control, *P < 0.05, **P < 0.01, and ***P < 0.001 as comparison of STZ-treated diabetic (amnesic) group vs donepezil, crude extract, and fraction-treated groups, using one-way ANOVA followed by Dunnet comparison. Crude extract (ChMu-Crd), chloroform (ChMu-Chl), and ethyl acetate (ChMu-Et).

extract (ChMu-Crd), and fractions (P < 0.05, P < 0.01, and P < 0.001) signifying its function in the treatment of memory impairment probably via ChE inhibition. Simultaneously, a significant descent in the ACh content was also noted in STZ amnesic group that was reverted back by analyzing the data of the groups treated with samples and standard.

4 Discussion

Ample and umpteen number of natural products from microorganisms, fungi, animals, plants, and other origins from nature provides a unique and a rich resources in the discovery of new drugs [39] and have been assessed and analyzed in-depth in relation with therapeutical applications in different pathologies. From Table 1, it is clear that almost all the major phytochemical groups are present in the extracts with greater quantities of saponins and phenolics. Phenolics being benzene ring containing compounds can easily accommodate the singlet electron of the free radicals and thus act as good antioxidants. The observed results on DPPH and ABTS assays may therefore be attributed to the phenolic contents. In Table 2 the enlisted TPF, TFC, and TTC contents are high in crude extracts followed by chloroform fraction. As these components are polar in nature they have been detected in more quantities in the tested amounts in polar solvents like methanol, chloroform, etc. as compared to hexane fraction. The non-spectroscopic estimation of phytochemical groups revealed the high contents of alkaloids, flavonoids, saponins, and terpenoids in crude extract followed by chloroform fraction (Table 3). The in vitro antioxidant potential of chloroform fraction is high with IC50 value of 41.78 and 67.33 µg/mL against DPPH and ABTS radicals, respectively, as shown in Table 4 followed by ethyl acetate fraction. The high antioxidant potential of chloroform extract may be due to high TPC, TFC, and TTC contents. The effect of extract on memory enhancement observed in this study is similarly dependent on active phytoconstituents. No significant differences were observed amongst all the groups of ChMu-Crd, ChMu-Chl, ChMu-Et, and positive control donepezil in connection to IL. On the other hand, the STZ diabetic group displayed a significant fall in recall and retention capability, which is evident by a significant drop (P < 0.001) in STL. The administration of ChMu-Crd, ChMu-Chl, ChMu-Et, and positive control donepezil to the STZ diabetic rats significantly attenuated this drop (P < 0.01 and P < 0.001). Similarly, the blood glucose level has been more potently lowered by chloroform extract. Pretreatment of rats with ChMu-Crd and fractions significantly ascend the SOD level in the brain. ChMu-Crd increased the SOD level significantly in the brain by 8.01 ± 0.51 and 8.19 ± 0.39 units/mg protein (1.9 and 1.32-folds), respectively, at 100 and 200 mg/kg b.w. P < 0.01, n = 6 in comparison with STZ-treated group. SOD level in the brain by ChMu-Chl significantly increased to 9.41 ± 0.40 and 9.72 ± 0.51 units/mg protein (1.52 and 1.57-folds), respectively, at 75 and 150 mg/kg. The ACh level was also elevated by chloroform fraction indicating that this extract contains phytoconstituent, which most probably be a potential inhibitor of AChE whose identification and isolation needs further investigations.

Considerable amount of epidemiological data supports that cognitive dysfunction is a common complication associated with diabetes and has been expected that 20–70% of patients show cognitive decline with diabetes, and 60% present at higher risk of dementia [40]. Various stages of memory dysfunction have been linked with diabetes, relying on age or prognosis, affected cognitive characters, and most likely the participation of underlying mechanisms [41]. These reports are also attested by cognitive impairment that is experiential in diabetic animal model studies [42,43]. The underlying mechanisms of action of extracts and natural products of medicinal plants remain fundamentally elusive, and it is possible that different positive effects in combination, including antioxidant, vascular protection, anti-inflammatory, antioxidant, anti-apoptotic, and cholinergic activities are responsible for...
Pragmatic beneficial effects in cognitive alterations associated with DM. Concretely, mangiferin and its rich content extract from *Mangifera indica* has been shown to counteract and significantly improve the impairments of learning and memory in STZ diabetic rats, when evaluated in Morris water maze test [44,45]. Quercetin, chrysin, and *Andrographis paniculata* extract also ameliorates STZ-induced memory impairment by minimizing the time spent in target quadrant in the test trial in the Morris water maze and increasing escape latency in the elevated plus maze [11,46–48]. Administration of *Hedera nepalensis* extract to STZ-aluminum trichloride rat model also produce similar outcomes [49]. In addition, extract of grape seed, kola nuts, and *Garcinia kola* also improves the cognitive impairment in STZ diabetic rat models [50–52]. Studies reported the beneficial and positive effects of *Brassica juncea* extract and resveratrol [53,54].
on learning and memory in diabetic rats. Beside these, *Rosa canina*, *Ludwigia octovalvis*, *Bacopa monnieri*, and *Urtica dioica* ameliorated cognitive impairment in mice models after administration and restore memory deficits in diabetic mouse models [55–59]. Andrographolide-enriched extract of *Andrographis paniculata*, ameliorate cognitive function in STZ-induced diabetic rats and the effect appears to be mediated by acetylcholinesterase activity and reducing oxidative stress [48]. Similar underlying mechanisms have been documented for *Clitorea ternatea* extract that improve memory in diabetic rats in the Morris water maze, Y maze, and radial arm maze [60]. The nootropic potentials from hydroalcoholic extract of *Teucrium polium* has been documented to alleviate cognitive impairment using passive avoidance test as behavioral model while reducing the markers associated with oxidative stress in diabetic rats [61]. Extract of *Flos puerariae* also ameliorates cognitive impairment in diabetic mice (STZ), by restoring cholinergic activity and lowering oxidative stress (enhancing CAT, SOD, and lowering the LPO) in the cortex [62], and similar findings have been reported with *Aloe vera* and *Withania somnifera* extracts [63]. The *C. murale* itself consists of saponins, terpenoids, flavonoids, which are known antioxidants [19,20]. *C. murale* is also a rich source of alkaloids, coumarins, oxalic acid, calcium, and vitamins A and C [14,18].

In addition to this, flavonol glycosides have been extracted from *C. murale* in which kaempferol glycoside is a novel one. Besides kaempferol, other well-known botanicals isolated from this herb are quercetin, scopoletin, and herbacetin [14]. Alkaloids are reported as neuropharmaceuticals, while kaempferol is reported for restoring neurogenesis, reducing oxidative stress and proinflammatory cytokines, and cognition enhancement [64–67]. Flavonoids, saponins, and terpenoids have been proved to be neuroprotectants in neurodegenerative disorders like Alzheimer’s disease and Parkinson’s disease [68–71].

### 5 Conclusions

In the current study *C. murale* Linn. in the form of extracts was evaluated for its *in vitro* antioxidant and *in vivo* neuropharmacological properties in STZ-induced memory impairment in rat model. The preliminary phytochemical investigations showed that this plant could be a potential medicinal plant to cure memory impairments as it contains almost all major phytochemical groups. Chloroform fraction has shown significant free radical scavenging potential against DPPH and ABTS radicals. The results of NODT, Y-maze, and PAT tests indicated that the extracts might contain a potential remedy of neurodegenerative diseases as CAT, SOD, MDA, and GSH levels were brought nearly to normal level by extracts as compared to STZ group. The acetylcholine level was considerably enhanced by chloroform extract showing that the most potent inhibitor of acetylcholinesterases might be present in this extract. It can be concluded from the results that *C. murale* could be a promising candidate to be used in the designing of a potent neuropharmacological drug in treating neurodegenerative diseases. However, these findings need further exploration to isolate pharmacologically active compound responsible for the observed potential in this study.

### Abbreviations

- **ChMu-Aq**: aqueous fraction
- **ChMu-But**: butanol fraction
- **ChMu-Chl**: chloroform fraction
- **ChMu-Crd**: crude extract
- **ChMu-Et**: ethyl acetate fraction
- **ChMu-nHex**: n-hexane fraction

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Conflict of interest: The authors declare no conflict of interest.

Ethical approval: All of the protocols that were employed in the study were in agreement with the Departmental Ethical Committee of the University (Pharm/EC-ChM22-11/20) as per approved “Animal Bye-Laws 2008, Scientific Procedures Issue-I of the University of Malakand”.

Data availability statement: All data generated or analyzed during this study are included in this published article.

References


