In vitro study of plant extract from Chenopodium album that inhibits a key enzyme in diabetes and its role in diabetic oxidative stress

Pushpander Kumar1, Sunil Kumar81, Suresh Kumar1 and Ravindra Kumar2

1Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra
2Department of Pharmaceutical Sciences, (F.M.S.H) Gurukul Kangri University, Haridwar

ABSTRACT

Chenopodium album (CA) is traditional Indian herbal medicinal plant. It is used widely all over India and other parts of the world. The plant is traditionally used as antidiabetic, laxative, anthelmintic and cardio-tonic, snake bite, skin disease etc. In this study, in vitro antidiabetic activity antioxidant activity, phenolic content and flavonoid content of different extracts of C. album were determined. Antioxidant study was carried out by using different models i.e. DPPH scavenging, H2O2 free radicals, reducing power and phosphomolybdenum assay. In vitro antidiabetic activity was evaluated by using α-amylase inhibition assay. In the DPPH radical scavenging results are obtained in the following order: Methanolic leaves > ethyl acetate roots > methanolic leaves > ethyl acetate roots extract. From reducing power, the position of arrangement for reducing power was methanol root > methanol leaves > ethyl acetate root > ethyl acetate leaves. In the hydrogen peroxide activity of extracts from Chenopodium album was in the order of methanolic leaves extract > ethyl acetate leaves extract > ethyl acetate roots extract > methanol roots extracts respectively. From the phosphomolybdate method, antioxidant capacity of ethyl acetate root extracts of Chenopodium album was found be maximum.

Keywords: Chenopodium album, antidiabetic, antioxidant, α-amylase, DPPH.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder caused by impaired secretion of insulin from pancreatic β-cells and/or insensitivity of target organs to the insulin [1, 2]. In DM, the production of reactive oxygen species (ROS) is increased due to insulin resistance and hyperglycemia [3], very long term elevated of blood glucose causes the various diabetic complications such as ketoacidosis, nephropathy, neuropathy and retinopathy [4-5]. Due to the hyperglycemia, the increased blood level of various reducing sugars promotes protein glycation through the Maillard reaction, which consecutively produces Schiff bases, Amadori products, and advanced glycation end products, due to the production of reactive oxygen species causes the tissue damage and suppression of insulin biosynthesis [6-8]. In both, insulin dependent and non-insulin dependent diabetes there is increased level of oxidative stress [9, 10].

Antioxidants have been shown to prevent the destruction of β-cells by inhibition of peroxidation chain reaction and thus they may provide protection against beta cell destruction [11, 12]. Nowadays, synthetic drugs and insulin are used as the main means for diabetes treatment. The currently available oral hypoglycemic agents are sulfonylureas, biguanide, thiazolidinedione etc. are commonly used to control the hyperglycemia and hyperlipidemia [13]. Oral hypoglycemic agents usually show side effects, such as hypoglycemia, drug-resistance, dropsy, and weight gain [14]. The most promising therapy in DM is to maintain optimal blood glucose level after the meal. Inhibitors of alpha amylase are considered to be very effective in delaying glucose absorption. So from this point of view many researchers have focused on the intensive search of novel enzyme inhibitors and natural products [15]. Over the years, various medicinal plants have been reported to be effective in the treatment of diabetes [16]. Plants are rich
sources of phytochemicals such as flavonoids, alkaloids, glycosides, saponins and have been used as antidiabetic, antihyperlipidemic and antioxidant agents [17, 18].

Thus, the inhibitors of \(\alpha\)-amylases, which break down long chain carbohydrates, are effective in delaying glucose absorption. The inhibition of \(\alpha\)-amylase activity is considered to be an effective strategy for the control of diabetes. From this point of view, more researchers have focused on the search for more effective inhibitors of antidiabetic compounds from natural materials [19, 20].

*Chenopodium album* is an annual shrub widely grown in Asia, Europe, North America and Africa. It is commonly known as pigweed, fathen or lamb-quarters [21, 22]. *C. album* is commonly used for food and medicinal values. It is also known by various vernacular names Bathu Sag(Hindi), Chandan bethu (Bengali) and grows in waste places as weed in wheat or other crops in almost all place of the world [23]. The herb is laxative, anthelmintic and cardiotonic. The powdered plant when mixed with normal food, was reported to suppress oestrus cycle. The plant is used in dysentery, diabetes diarrhea, headache, wound healing, hepatic disorder, skin diseases etc. [24-29].

**MATERIALS AND METHODS**

2.1 Plant material
The plant material was collected in the month of March -April from Kaithal and Kurukshetra, Haryana (India) and was authenticated by Senior Scientist Raw material and herbarium Dr. Neelam Singh from NISCAIR with the specimen Ref. No. NISCAIR/RHMD/consult/2014/2523/102-1.

2.2 Chemicals
\(\alpha\)-amylase (Sigma-Aldrich), Di-Nitro Salicylic Acid (Sigma-Aldrich), acarbose, starch, quercerin (Sigma aldrich), DPPH(Loba), potassium molybdate, Hydrogen Peroxide (\(H_2O_2\)), Folin Ciocalteu’s reagent, sodium carbonate(Na\(_2\)CO\(_3\)), gallic acid, sodium nitrite, Aluminium chloride(AlCl\(_3\)), sodium hydroxide(NaOH), Tri chloro acetic acid(TCA), ferric chloride, ammonium molybdate, disodium hydrogen orthophosphate(Na\(_2\)HPO\(_4\)), Methanol, Ethyl acetate reagents etc. All the chemicals were used of analytical grade.

2.3 Preparation of extract
Leaves and roots were dried in shade; powdered part was extracted with petroleum ether (60-80° C) in Soxhlet’s apparatus at a temperature not exceeding 60° C. The defatted plant material was then extracted with methanol and ethyl acetate at a temperature not exceeding 50° C. The extract was dried at 45\(^\circ\)C in rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10\(^\circ\)C.

2.4 Total phenolic content
Total phenolic content was estimated by Singleton method with slight modification [30]. 1 mL of extract(500 \(\mu\)g/mL) and standard gallic acid (25, 50, 75, 100, 120 \(\mu\)g/mL) was put into the test tubes and 5 mL of distilled water and 0.5 mL of Folin Ciocalteu’s reagent was added and shaken. After 5 minutes, 1.5 mL of 20 % sodium carbonate was added and volume made up to 10 mL with distilled water. It was allowed to incubate for 2 hours at room temperature. Intense blue color was developed. After incubation, absorbance was measured at 750 nm spectrophotometer using UV visible instrument. The absorbance of extracts was performed in triplicates. Gallic acid was used as standard. The calibration curve was plotted using standard Gallic acid.

2.5 Total flavonoid content
Total flavonoid content was measured with the aluminium chloride colorimetric assay [31]. 1mL of extract (500 \(\mu\)g/mL) and 1 mL of standard quercetin solution (25, 50, 75, 100, 120 \(\mu\)g/mL) was positioned into test tubes and 4 mL of distilled water and 0.3 mL of 5 % sodium nitrite solution was added into each. After 5 minutes, 0.3 mL of 10 % aluminum chloride was added. At 6th minute, 2 mL of 1M sodium hydroxide was added. Finally, volume was making up to 10 mL with distilled water and mix well. Orange yellowish color was developed. The absorbance was measured at 510 nm spectrophotometer using UV-visible instrument. The blank was performed using distilled water. Quercetin was used as standard.

2.6 In vitro antioxidant activity
Antioxidant properties of different extracts can be evaluated by using various in vitro models.

2.6.1 DPPH scavenging activity:
DPPH scavenging activity was carried out by method of Blois [32] with a slight modification. Briefly, a 0.1 mM solution of DPPH radical solution in methanol/water (8:2, v/v) was prepared and then 1 mL of this solution was mixed with 3 mL of sample solution in different concentrations (100-500 \(\mu\)g/mL) of plant extract. Finally, after 30
min, the absorbance was measured at 517 nm. Decreasing the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. The DPPH solution without sample solution was used as a control. Ascorbic acid was used as standard.

2.6.2 Reducing power assay
The reducing power of *C. album* was determined by the method of Oyaizu [33] with slight modification. Substance which have reduction potential react with potential react with potassium ferricyanide (Fe$^{3+}$) to form potassium ferrocyanide (Fe$^{2+}$), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. Increase in reduction of ferric to ferrous ion increases the absorbance indicating the reducing ability of *C. album* varying concentration of *C. album* (100-500 µg/mL) in double distilled water was mixed 2.5 mL of phosphate buffer incubated at 50˚C for 20 min after which, 1.5 mL of TCA was added and centrifuged at 3000×g for 10 min. From all the tubes, 0.5 mL of ferric chloride was taken. The absorbance was measured at 700 nm in a spectrophotometer [34]. Ascorbic acid was used as a standard for comparison. Increased absorbance of the reaction mixture indicates increasing reducing power. Incubation with water in place of additives was used as blank.

\[
\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100
\]

2.6.3 Hydrogen peroxide scavenging activity
The ability of *C. album* to scavenge H$_2$O$_2$ was determined according to the method of Ilavarasa [35] with modification. A solution of H$_2$O$_2$ (40 mmol/L) was prepared in phosphate buffer (pH 7.4). H$_2$O$_2$ concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer in distill water were added to a H$_2$O$_2$ solution (0.6 mL, 40 mmol/L). Absorbance of H$_2$O$_2$ at 230 nm was determined after 10 min against blank solution containing phosphate buffer without H$_2$O$_2$. Different concentrations (100, 200, 300, 400, 500 µg/mL) of *C. album* extracts were used for hydrogen peroxide scavenging activity. The percentage of inhibition of H$_2$O$_2$ of *C. album* extract was calculated using the following equation.

\[
\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100
\]

2.6.4 Phosphomolybdate assay
Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Total antioxidant capacity can be calculated by the method described by Umamaheswari [36] with slight modification. 1 mL of sample solution is combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube is capped and incubated in a boiling water bath at 95˚C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution is measured at 695 nm against blank in UV spectrophotometer. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it is incubated under same conditions as rest of the sample. The percentage of inhibition was calculated by using the following equation.

\[
\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100
\]

2.7 Alpha amylase activity assay
The α-amylase inhibitory activity for *C. album* extracts (CAE) was determined based on the spectrophotometric assay using acarbose as the reference compound [37]. The CAE was dissolved in DMSO to give concentrations from and 100, 200, 300, 400, 500 µg/mL. The enzyme α-amylase solution (0.5 unit/mL) was prepared by mixing 3.246 mg of α-amylase in 100 mL of 40 mM phosphate buffer pH 6.9. Add 1 mL of 40 mM phosphate buffer (pH 6.9)/acarbose/ CAE and 1mL of α-amylase enzyme are pre-incubated at 37˚C for 10 min and then 1 mL of Dinitro salislyic acid (DNSA) was added, mixed and incubated at 37˚C for 8 min. The absorbance was measured at 405 nm and control reaction was carried out without the extract. Percentage inhibition was calculated by expression:

\[
\% \text{ Inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Control}}} \times 100
\]
RESULTS

3.1 DPPH scavenging activity
DPPH radical scavenging effects of various extract were at different concentrations (100-500 µg/mL) is shown in table 1. Though, it was found that the antioxidant potential of leaves and root extracts was found to be lower than those of ascorbic acid (Figure 1&2). The study revealed that leaves extracts have prominent antioxidant activity; the presence of phenolic compounds are mainly found in leaves extract and could be attributable to the observed high antiradical properties of these extracts.

3.2 Reducing power activity
Figures 3 and 4 show the dose response curves for the reducing powers of extracts (100-500 µg/mL) from C. album. It was found that the reducing power increased with concentration of each sample. Significantly higher percentage inhibition (80.59 ± 0.12) was evident in methanol roots extract (Table 2).

3.3 Hydrogen peroxide radical scavenging activity
The scavenging effect of extracts of C. album on hydrogen peroxide was concentration-dependent (100-500 µg/mL) as shown in figures 5 and 6. Methanolic extracts of leaves displayed strong H$_2$O$_2$ scavenging activity (67.22 ± 0.00) whereas that of the standard, ascorbic acid exhibited 91.37 percentage inhibitions (Table 3). The scavenging activity for hydrogen peroxide of extracts from C. album was in the order of Methanolic leaves extract (MLE) > Ethyl acetate leaves extract (ELE) > Ethyl acetate roots extract (ERE) > Methanol roots extract (MRE) respectively.

3.4 Phosphomolybdate assay
In the phosphomolybdate method, antioxidant capacity of extracts of C. album is shown in figure 7 and 8. It was found to decrease in this order: ERE<ELE < MRE< MLE (Table 4). All results showed antioxidant activity in dose dependent manner at the concentration 100 -500 µg/mL.

3.5 Total phenolic content and flavonoid content
Total phenolic content was estimated by using Folin- Ciocalteu reagent. Total phenolic content of the different fractions of C. album extract was concentration dependent and expressed as percentage of Gallic acid equivalents (GAE) equivalent. Table 8 summarizes that total phenolic compounds in fractions varied widely, ranging from 12.60 and 8.2 expressed as percentage of phenolic content of leaves and 10.17, 6.9 of roots. MLE exhibited the highest total phenolic content, And as a result from table 8 flavonoid content was found to be 11.62 and 7.2 as percentage of flavonoid content of leaves and roots are 11.73, 5.58. MRE contains the highest flavonoid content.

3.6 Alpha amylase assay
C. album extracts showed in vitro antidiabetic activity by using α-amylase inhibition assay. The percentage of inhibition was found in the order: MLE> MRE >ERE> ELE. Antidiabetic activity may be due to the phenolic compound present in the extract.

DISCUSSION

In the present study, antioxidant activity was evaluated by using the in vitro assay such as DPPH, H$_2$O$_2$, Phosphomolybdate and Reducing power assay. In vitro antidiabetic activity was demonstrated by using α-amylase inhibition assay of the C. album plant extracts. A significant relationship between antioxidant activity of the plant and diabetes was found out in the present study.

C. album is the most widely used plant in the all over India. It contains flavonoids, phenols, saponin, glycosides [23, 38-40]. Quercetin, kaempherol, and rutin were isolated from the C. album are reported to have antidiabetic activity [41-44]. All the three compounds are also having antioxidant activity [45, 46]. The other species, C. quinoa has also antioxidant activity [47-49]. Chenopodium abrosides demonstrated the antidiabetic activity by using streptozotocin induced diabetes model. C. murale is having antioxidant and antimicrobial activity [50].

In both insulin dependent (type 1) and non-insulin-dependent diabetes (type 2), there is increased oxidative stress [51, 52]. Now, it is reported that free radicals play a major role in the diabetes and its complications such as Neuropathy, retinopathy and nephropathy [53]. Apart from the potential of polyphenols to lower oxidative stress and thus protect pancreatic β-cells they may also have direct antidiabetic activity by influencing glucose homeostasis through various mechanisms [54].

The DPPH assay is one of the most popular and frequently employed methods to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant capacity. The DPPH radical is long-
lived organic nitrogen radical with a deep purple color. When a solution of DPPH radical is mixed with an antioxidant/reducing compound, its color turns from purple to yellow of the corresponding hydrazine. The reducing ability of antioxidants towards DPPH can be evaluated by monitoring the decrease of its absorbance at 515–528 nm as the formed corresponding hydrazine DPPH₂ yields a yellow [31]. In the present study among all the extracts methanol and ethyl acetate, methanol extract showed significantly higher percentage inhibition and positively correlated with total phenolic content.

The antioxidant capacity of the extracts was measured by using phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 765 nm. The present study demonstrated that methanolic leaves extract exhibited the highest antioxidant capacity for phosphomolybdate reduction. Recent studies also have shown that flavonoid content and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [55, 56]

Hydrogen peroxide is non reactive, but sometimes it can be toxic to living cells, because in living cell it is converted into free radical called hydroxyl radicals (•OH), react with biomolecules, cause tissue damage and cell death [57]. The hydroxyl radical is regarded as a detrimental species in pathophysiological processes and capable of damaging almost every molecule of biological system and contributes to carcinogenesis, mutagenesis and cytotoxicity [58]. Methanol leaves extract of C. album efficiently scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide.

Several Epidemiological studies supports that dietary intake of antioxidant they have protective effect against the development of diabetes by inhibiting the peroxidation chain reaction [59]. Relationship between the consumption of phenolic rich food products and a low incidence of diabetes, myocardial heart disease, and certain forms of cancer [60-62].These include, amongst others, inhibiting the key carbohydrate digestive enzymes, α-amylase and α-glucosidase, DPPIV stimulation of insulin secretion from pancreatic cells, modulation of glucose release from the liver and increasing glucose uptake in peripheral tissues [63, 64].

So, free radical scavenging and antioxidant effect may be responsible for its antidiabetic effect. It is possible that extract exert its effect by causing hypoglycemia. The exact mechanism is still unclear but it may be due antioxidant and free radical scavenging effect of the plant and presence of flavanoids, tannins and other phenolic compounds in the extracts.

### Table: 1 DPPH radical scavenging activity of methanolic and ethyl acetate extract of C. album

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>%DPPH radical scavenging</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MLE</td>
<td>ELE</td>
</tr>
<tr>
<td>1</td>
<td>500 µg/ml</td>
<td>87.87±0.03</td>
</tr>
<tr>
<td>2</td>
<td>400 µg/ml</td>
<td>76.09±0.00</td>
</tr>
<tr>
<td>3</td>
<td>300 µg/ml</td>
<td>59.01±0.05</td>
</tr>
<tr>
<td>4</td>
<td>200 µg/ml</td>
<td>41.48±0.06</td>
</tr>
<tr>
<td>5</td>
<td>100 µg/ml</td>
<td>27.42±0.00</td>
</tr>
</tbody>
</table>

(N=3), Data represents: mean ± SEM; MLE=methanol leaves; ELE= Ethyl acetate leaves extract; MRE=methanol root extracts; ERE= Ethyl acetate root extracts

### Table: 2 Reducing power radical scavenging activity of methanolic and ethyl acetate extract of C. album

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Ferric ion reducing activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MLE</td>
<td>ELE</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>79.80±0.28</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>66.98±0.03</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>54.02±0.00</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>38.84±0.06</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>23.83±0.74</td>
</tr>
</tbody>
</table>

(N=3), Data represents: mean ± SEM; MLE=methanol leaves; ELE= Ethyl acetate leaves extract; MRE=methanol root extracts; ERE= Ethyl acetate root extracts
Table: 3 H₂O₂ radical scavenging activity of methanolic and ethyl acetate extract of C. album

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (µg/mL)</th>
<th>H₂O₂ Radical scavenging activity of extracts</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MLE</td>
<td>ELE</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>67.03±0.18</td>
<td>40.89±0.73</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>58.26±0.27</td>
<td>33.05±0.27</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>34.34±1.30</td>
<td>24.36±0.00</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>24.36±0.46</td>
<td>16.27±0.30</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>16.80±0.17</td>
<td>07.24±0.70</td>
</tr>
</tbody>
</table>

(N=3), Data represents: mean ± SEM; MLE= methanol leaves; ELE= Ethyl acetate leaves extract; MRE= methanol root extracts; ERE= Ethyl acetate root extracts

Table: 4 Phosphomolybdate activity of methanolic and ethyl acetate extract of C. album

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (µg/ml)</th>
<th>Phosphomolybdate assay of extract</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MLE</td>
<td>ELE</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>45.63±0.51</td>
<td>43.84±0.76</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>35.89±1.28</td>
<td>38.97±0.50</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>24.03±0.91</td>
<td>27.69±0.76</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>12.28±0.13</td>
<td>19.46±0.26</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>3.58±0.25</td>
<td>9.22±0.88</td>
</tr>
</tbody>
</table>

(N=3), Data represents: mean ± SEM; MLE= methanol leaves; ELE= Ethyl acetate leaves extract; MRE= methanol root extracts; ERE= Ethyl acetate root extracts

Table: 5 Alpha amylase activity of methanol and ethyl acetate extract of C. album

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (µg/mL)</th>
<th>Alpha amylase inhibition activity</th>
<th>Acarbose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MLE</td>
<td>ELE</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>63.74±0.06</td>
<td>63.00±0.76</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>52.01±0.18</td>
<td>54.20±0.81</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>47.00±0.41</td>
<td>50.10±0.06</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>28.72±0.61</td>
<td>30.48±0.13</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>14.18±0.07</td>
<td>14.70±0.00</td>
</tr>
</tbody>
</table>

(N=3), Data represents: mean ± SEM; MLE= methanol leaves; ELE= Ethyl acetate leaves extract; MRE= methanol root extracts; ERE= Ethyl acetate root extracts

Graph 1 showing results for standard curve of Gallic acid

Table: 6 Showing results for standard Gallic acid

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration(µg/mL)</th>
<th>Absorbance(STD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>0.463</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.590</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>0.711</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0.822</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>0.901</td>
</tr>
</tbody>
</table>
Table 7 showing absorbance of standard Quercetin

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration(µg/mL)</th>
<th>Absorbance(STD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>0.380</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.471</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>0.590</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0.683</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>0.811</td>
</tr>
</tbody>
</table>

Table 8 showing results of Total phenolic content (%w/w) and flavonoid content (%w/w) of *C. album* extracts

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/mL)</th>
<th>MLE</th>
<th>ELE</th>
<th>MRE</th>
<th>ERE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500</td>
<td>12.60</td>
<td>8.2</td>
<td>10.17</td>
<td>6.9</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>11.62</td>
<td>7.2</td>
<td>11.73</td>
<td>5.58</td>
</tr>
</tbody>
</table>

*a=Phenolic content, b=flavonoid content*

Graph 2. showing results for standard curve of quercetin

\[ y = 0.0043x + 0.2647 \]

\[ R^2 = 0.9969 \]

Figure: 1 showing percentage inhibition of DPPH of methanol and ethyl acetate leaves extract

```
% DPPH Methanoliic and Ethyl Acetate leaves extract

- Methanol
- Ethyl acetate
- Ascorbic acid
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Figure: 1 showing percentage inhibition of DPPH of methanol and ethyl acetate leaves extract
Figure: 2 showing percentage inhibition of DPPH of methanol and ethyl acetate root extract.

Figure: 3 showing percentage inhibition of reducing power of methanol and ethyl acetate leaves extract.
Figure: 4 showing percentage inhibition of reducing power of methanol and ethyl acetate root extracts.

Figure: 5 showing $\text{H}_2\text{O}_2$ Radical Scavenging of methanol and ethyl acetate leaves extract.

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Sunil Kumar et al  
*Der Pharmacia Sinica, 2015, 6(12):48-61*
Figure: 6 showing $\text{H}_2\text{O}_2$ Radical Scavenging of methanol and ethyl acetate roots extract

Figure: 7 showing phosphomolybdate Scavenging of methanol and ethyl acetate leaves extract
Figure: 8 showing Phosphomolybdate scavenging activity of methanol and ethyl acetate roots extract

Figure: 9 showing results for alpha amylase activity of leaves extract

% Inhibition of leaves extract of alpha amylase activity

Phosphomolybdate Scavenging Activity of roots

- Methanol
- Ethyl Acetate
- Ascorbic acid
CONCLUSION

Thus, our study shows that *Chenopodium album* extracts have antidiabetic and antioxidant effects. Furthermore, phytochemical and pharmacological investigations are needed to isolate and identify the active constituents responsible for the activity.

REFERENCES