Evaluation of Anti-Infection and Anti-Diabetic Activities in Methanolic and n-Hexane Plant Extracts of Indigenously Cultivated Chenopodium album

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Abstract: The Asian region of earth has a rich agriculture system that provides extensive opportunities to boost pharmaceutical and nutritional research to address the use of food crops for health benefits and potential clinical applications. The traditionally cropped green leaf vegetable indigenously known as Bathu and botanically known as Chenopodium album (C. album) is traditionally used as a sedative, blood purifier, hepatoprotectant, diuretic, and antiscorbutic laxative. In this study, we investigated the anti-infection potential, anti-diabetic potential, and mineral composition of indigenously cultivated C. album plant extracts. Methanol and n-hexane solvents were used to extract phytochemicals at different extraction conditions. The maximum yield of 12.72 ± 0.36 g/100 g extract was obtained in methanol with 200 rpm shaking, 200 mL solvent, and an 8 h extraction period. Under the same conditions, n-hexane gave 2.09 ± 0.29 g/100 g extract. Good alpha-amylase inhibition efficiency was shown by the n-hexane extracts, while the methanol extracts showed good urease inhibition potential. The H6 extract had the lowest IC50 (8.16 ± 0.2 ug/mL) as compared to the standard acarbose (9.27 ± 0.6 ug/mL). Similarly, the M6 extract revealed a significant urease inhibitory potential, i.e., IC50 of 18.77 ± 0.6 ug/mL, which was close to the standard thiourea (IC50: 19.09 ± 0.7 ug/mL).

Regarding the antibacterial study, the M6 extract showed 16.55 ± 0.57 mm ZOI against E. coli and 15.54 ± 0.55 mm in the case of S. aureus, as compared to the standard ciprofloxacin, which showed 26.08 ± 0.73 mm, and penicillin, which showed 21.12 ± 0.81 mm ZOI. Mineral profiling was investigated by ICP-OES, which showed significant amounts of Mg and Fe in all extracts. Our findings tend to show that systematic harvesting and utilization of this vegetable crop could be recommended as an alternative nutritional therapy in the management of internal infections and diabetes.

Keywords: Chenopodium album; alpha-amylase inhibition; urease inhibition; antibacterial potential; E. coli; S. aureus; mineral contents

1. Introduction

The health issues of 80% of the developing world’s population are still managed by using folk medicines obtained from medicinal plants [1]. Approximately 28,187 medicinal plant species are being used by humans to treat a variety of ailments. A quantitative description of medicinal plants listed about 20,000 medicinal plant species, issued by the World Health Organization (WHO), and showed the dominant use of medicinal plants to treat disease and the development of regulations for their use in more than 100 countries; over 1340 medicinal plants showed satisfactory antimicrobial potential, and more than
30,000 antimicrobial and antioxidant compounds were isolated [2]. The reported data reflect the inherited importance of natural flora to combat the current challenges of bacterial resistance and diseases due to oxidative stress and malfunctioning of hormonal glands.

In the present era, bacterial resistance poses a serious threat to human beings. The prevalence of bacterial resistance is largely attributed to the careless use of antibiotics. Without adopting preventive measures, bacterial infection could be the leading cause of mortality worldwide by 2050 [3]. In 2019, over 1.2 million fatalities were linked to antimicrobial resistance, and this is estimated to increase to approximately 10 million deaths annually by 2050 if antimicrobial resistance is not curbed [4]. In addition to microbial ailments, oxidation chemical reactions produce free radicals that retard cellular activity and cause damage to the cells [5,6]. When these free radicals, which mostly include superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide, are produced beyond the limit of their natural stabilizers, it results in serious disorders [7–9]. It has been widely reported that 66% of the world’s plant species have a promising amount of antioxidant chemicals. Among these, green leafy vegetables pack a big antioxidant punch. They are bearers of medicinally important compounds, such as folic acid, ascorbic acid, β-carotene, and riboflavin, as well as minerals [10]. In addition to improving many organ and biological functions, green leafy vegetables also strongly resist pathogen attack and play a crucial role in the management of diabetes mellitus disorder (DM) [11,12]. However, the maximum benefit from medicinal plants is obtained by optimizing the best possible phytochemical extraction conditions [2,13].

Bathua (C. album), a staple green leafy vegetable crop in Asia, Europe, and the United States of America, has seen an increase in value due to its high nutrient and energy content [14]. There are several medical uses of this weedy plant. Traditionally, it is used as a sedative, blood purifier, hepatoprotectant, diuretic, and antiscorbutic laxative. Additionally, it is the main source of antioxidants, important nutritional components, and nutrients. Complete plants and early branches of C. album are also the source of β-carotene as well as vitamin C. C. album has 8-α-acetoxy crypomeridiol, cryptomeridiol, antinociceptive and antipruritic effects, and a sperm-immobilizing mediator as the growth-stimulating action [15]. Furthermore, a lot of functional food components such as alkaloid chinoalbicin, phenols, phenolic amide, apocortinoid, saponins, lignans, cinnamic acid amide, apocortinoid, and xyloside, as well as hypotensive activity, are present in this plant [16]. On the basis of the reported data, it can be estimated that the C. album green leafy vegetable possesses excellent potential to manage bacterial infections and oxidative stress-related, metabolic, and hormonal disorders. Therefore, the aim of this study is to optimize the extraction condition to obtain the maximum yield of phytochemicals from C. album leaves using methanol and n-hexane solvents; to investigate their antibacterial and anti-diabetic activities; and to explore their mineral composition.

2. Materials and Methods

2.1. Materials

All the chemicals were of analytical grade. Disodium hydrogen phosphate, sodium dihydrogen phosphate, dimethyl sulphoxide, alpha-amylase enzyme, urease enzymes, potato starch, sodium chloride, potassium sodium tartrate tetrahydrate, 3,5-dinitrosalicylic acid, sodium nitroprusside, phenol, sodium hypochloride, sodium hydroxide, and hydrochloric acid were purchased from Sigma (St. Louis, MO, USA).

2.2. Microbial Strains

Two different species of bacteria were cultured: gram-positive S. aureus (ATCC25923) and gram-negative E. coli (ATCC25922). The antibacterial activity tests were carried out at the diagnostic laboratory of GC University, Faisalabad. An agar well diffusion protocol was used in the antimicrobial activity tests.
2.3. Collection and Identification of Plant

The complete plant of *C. album* was assembled from the local area of Punjab, Pakistan. The plant was identified by the plant identification section of the Department of Botany, Government College University of Faisalabad.

2.4. Preparation of Plant Extracts

The plant was washed with distilled water, dried under shade, pulverized to fine powder (150 mesh size), and then stored at room temperature in airtight glass jars until further use. Two solvents, methanol and n-hexane, were used to prepare extracts of the plant. We mixed the plant powder and solvent in a 1/10 (weight-to-volume) ratio; the mixture was shaken at various solvent concentrations, with mechanical shaking speed and time, at room temperature. After extraction, Whatman No. 1 filter was used to filter the solvent containing plant material. The extracts were evaporated until they were completely dry. For future research, the materials were sealed in airtight bottles and kept at 4 °C. The fraction’s percentage of yield was determined using the following formula:

$$\text{Yield (\%) = \frac{\text{Weight of dry plant extract}}{\text{Weight of dry plant}} \times 100}$$  \hspace{1cm} (1)

Flasks were used in triplicate for each sample throughout the extraction process, and the findings are reported as mean ± standard deviation (SD) (n = 3).

2.5. Mineral Analysis of *C. album*

The powder of the plant, *C. album*, was examined by mineral analysis using ICP-OES. A wet digestion process was used after minor modification. One gram of precisely measured plant powder of *C. album* was put into a digestion flask along with twelve milliliters of HNO$_3$. After a few moments, we then added 4 mL of 33% H$_2$O$_2$. The resultant mixture was then set on a hot plate, followed by the addition of 10 mL of ultra-pure water, and filtered upon cooling. The filtered solution was subjected to an ICP-OES (Teledyne Leeman Labs, Hudson, NH, USA, Prodigy-7) for analysis after 5-times dilution [17].

2.6. Alpha-Amylase Inhibitory Assay

The assay for α-amylase inhibition was carried out using a slightly modified version of the standard method [18]. In a 96-well plate, 50 mL of each plant extract was combined with 150 µL of starch solution and 10 mL of enzyme. The plates were incubated at 37 °C for 30 min. Then, 20 µL of sodium hydroxide and 20 µL of coloring reagent were added. Further, plates were placed into a 100 °C water bath for 20 min, and the reaction mixture was removed from the water bath and cooled down. Moreover, the activity of alpha-amylase was measured by measuring the mixture’s absorbance at 540 nm in an Elisa plate reader. To adjust the absorption of the mixture, blank samples were employed in which the enzyme was replaced with buffer solution. The maximum enzyme activity was evaluated using a negative control reaction in which the plant extract was replaced with 50 µL of DMSO. As a positive control, acarbose solution at concentrations of 0.312, 0.625, 1.25, 2.5, 5, and 10 µg/mL was used. The following formula was used to calculate the alpha-amylase inhibition percentage:

$$(\%) \text{ Inhibition of } \alpha\text{-Amylase} = 100 \times \frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}}$$  \hspace{1cm} (2)

The values of $\Delta A_{\text{control}}$ and $\Delta A_{\text{sample}}$ were calculated by using these formulas:

$$\Delta A_{\text{control}} = A_{\text{test}} - A_{\text{blank}}$$  \hspace{1cm} (3)

$$\Delta A_{\text{sample}} = A_{\text{test}} - A_{\text{blank}}$$  \hspace{1cm} (4)
2.7. Urease Inhibitory Assay

The urease assay was performed on a 96-well plate by using an Elisa plate reader. First of all, 40 µL of buffer was added; 10 µL of sample was loaded one by one in each well; and 10 µL of urease enzyme was also added to the wells. The plate was maintained in an incubator at 37 °C for 30 min after the enzyme was added. After that, 40 µL of each phenol reagent, containing a mixture of 1% phenol, 0.005% of sodium nitroprusside, and an appropriate amount of alkaline (NaOH) reagents, was added to each of the wells. After adding these reagents, the plate was returned to the incubator again for 30 min at 37 °C. The absorbance at 625 nm was measured using a micro-plate reader after the incubation process. To adjust the absorption of the mixture, blank samples were employed in which the enzyme was replaced with 10 µL of buffer solution. Inhibition (%) was computed after the measurement of optical density (OD) by using the following formula: 100 − (OD) test well/(OD) control. Thiourea was taken as the standard control, and the IC50 values were calculated by establishing a regression curve [19–21].

2.8. Antibacterial Activity

*C. album*’s antibacterial activity against *S. aureus* (ATCC25923) and *E. coli* (ATCC25922) was tested using agar well diffusion [22]. These bacterial strains were incubated at 37 °C for 24 h. After dissolving 1.38 g of Mueller–Hinton agar in 60 mL of water, the mixture was heated to boiling. The following items were autoclaved for 90 min at 121 °C: inoculation loop, tweezer, Eppendorf, distilled water, cotton swabs, micropipette (200 µL) tips, micropipette (1000 µL) tips, and boiled agar. Each sterile petri dish was filled with 15 mL of M-H agar and left in the laminar flow cabinet to solidify. The petri plates, with respective strains of bacteria, were marked for streaking after solidification of agar. After streaking, 8 mm wells were created on each plate using tips with a diameter of 1 mm. The samples and controls were then labeled. After that, every plate’s well was filled with samples (20 mg/mL) and positive (20 mg/mL) and negative controls. As a positive control, penicillin was used for *S. aureus* and ciprofloxacin for *E. coli*. Petri plates were loaded with samples and controls and incubated for 24 h at 37 °C. The inhibition zones were measured after 24 h.

2.9. Statistical Analysis

The results are presented as mean ± SEM and were analyzed by two-way ANOVA, followed by Dennett’s multiple comparisons test using GraphPad Prism 5 software [23]. The % inhibition of α-amylase and urease enzyme activity was compared with all concentrations of extracts (Run M1–M12 and H1–H12), while for antibacterial activity the results were compared with the standard drug. A p value > 0.05 is considered as non-significant (ns), <0.05 as significant (*), <0.01 as more significant (**), and <0.001 as highly significant (***)

3. Results and Discussion

3.1. Yield of Extract

In the present investigation, methanol and n-hexane were used as solvents for extraction. The extraction yield (g/100 g plant extract) for each set of extractions is tabulated in Table 1. For this analysis, it was noted that methanolic extracts showed high yields as compared to n-hexane extracts, which is in good agreement with the literature [24]. M6 showed the highest yield (12.72 ± 0.36 g/100 g), while M7 gave the lowest (10.11 ± 0.39 g/100 g). It was found that the methanolic extracts had the following yield order: M6 > M11 > M5 > M3 > M9 > M10 > M2 > M12 > M8 > M4 > M1 > M7. On the other hand, H6 gave the highest yield (2.09 ± 0.29 g/100 g), while H1 showed the lowest yield (1.14 ± 0.12 g/100 g). The order of yield for the n-hexane extracts was H6 > H5 > H11 > H9 > H3 > H8 > H10 > H12 = H2 > H4 > H7 > H1. It was noted that the extraction parameters had a great influence on the extraction yields. Methanol gave a high yield, which might be due to the good interaction with plenty of phytochemicals, such as phenolic compounds, flavonoids, terpenoids, and alkaloids, present in the *C. album* plant extract. The n-hexane extracts showed a low yield
due to their non-polar nature. On the other hand, it was noted that the extraction yields increased after increasing the solvent concentration and mechanical shaking time. However, the literature regarding the length of the extraction is more contradictory. Some researchers used short extraction periods and some used a long duration of extraction [25]. There was no significant effect of varying rpm values on the extraction yield.

Table 1. Yield of C. album plant extracts of methanol (M1–M12) and n-hexane (H1–H12) under various conditions of extraction.

<table>
<thead>
<tr>
<th>Run</th>
<th>Factors</th>
<th>Yield (g/100 g of Dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanolic Extracts</td>
<td>n-Hexane Extracts</td>
</tr>
<tr>
<td></td>
<td>Solvent Concentration (mL)</td>
<td>Speed (rpm)</td>
</tr>
<tr>
<td>M1</td>
<td>H1</td>
<td>100</td>
</tr>
<tr>
<td>M2</td>
<td>H2</td>
<td>100</td>
</tr>
<tr>
<td>M3</td>
<td>H3</td>
<td>100</td>
</tr>
<tr>
<td>M4</td>
<td>H4</td>
<td>200</td>
</tr>
<tr>
<td>M5</td>
<td>H5</td>
<td>200</td>
</tr>
<tr>
<td>M6</td>
<td>H6</td>
<td>200</td>
</tr>
<tr>
<td>M7</td>
<td>H7</td>
<td>100</td>
</tr>
<tr>
<td>M8</td>
<td>H8</td>
<td>100</td>
</tr>
<tr>
<td>M9</td>
<td>H9</td>
<td>100</td>
</tr>
<tr>
<td>M10</td>
<td>H10</td>
<td>150</td>
</tr>
<tr>
<td>M11</td>
<td>H11</td>
<td>200</td>
</tr>
<tr>
<td>M12</td>
<td>H12</td>
<td>150</td>
</tr>
</tbody>
</table>

3.2. Mineral Profile

The findings of this study revealed that significant amounts of Mg and Fe are present in C. album plant leaves, while Cd and Si were found in moderate amounts. The results are given in Table 2. Similarly, traces of Pb, Ni, Zn, Cr, and Mn were also found in this plant. The zinc contents in C. album are comparable to the quantity reported for many green leafy vegetables [26]. Moreover, our mineral composition results are similar to those of earlier studies [12]. It is also reported, as the plant grew older, its nutrient content decreased. However, high mineral contents were reported by researchers compared to other green leafy vegetables [14].

Table 2. Mineral composition of C. album.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Mineral Profile with Peak Intensity</th>
<th>Values in ppm (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zinc (Zn213.8)</td>
<td>0.12 ± 0.014</td>
</tr>
<tr>
<td>2</td>
<td>Cadmium (Cd226.5)</td>
<td>20.29 ± 1.9</td>
</tr>
<tr>
<td>3</td>
<td>Lead (Pb283.3)</td>
<td>0.75 ± 0.057</td>
</tr>
<tr>
<td>4</td>
<td>Chromium (Cr267.7)</td>
<td>0.20 ± 0.023</td>
</tr>
<tr>
<td>5</td>
<td>Magnesium (Mg279.5)</td>
<td>3546.23 ± 14.01</td>
</tr>
<tr>
<td>6</td>
<td>Nickel (Ni361.9)</td>
<td>0.62 ± 0.047</td>
</tr>
<tr>
<td>7</td>
<td>Manganese (Mn257.6)</td>
<td>0.13 ± 0.015</td>
</tr>
<tr>
<td>8</td>
<td>Silicon (Si251.6)</td>
<td>3.59 ± 0.43</td>
</tr>
<tr>
<td>9</td>
<td>Iron (Fe259.9)</td>
<td>3413.36 ± 12.49</td>
</tr>
</tbody>
</table>
3.3. Alpha-Amylase Inhibitory Activity

The current study assessed the inhibitory potential of methanolic and n-hexane extracts of C. album on α-amylase by an in vitro method. The results are shown in Figures 1 and 2. It was noted that the methanolic extracts at various concentrations possessed a lower α-amylase percentage of inhibition activity as compared to the extracts of n-hexane, while the standard reference drug, acarbose, showed an α-amylase inhibitory activity of 17%–52% at lower to higher concentrations, with an IC₅₀ value of 9.27 ± 0.6 µg/mL. The IC₅₀ (µg/mL) values of the methanolic and n-hexane samples are shown in Figure 3. The IC₅₀ values of the methanolic fractions were lower than those of the extracts of n-hexane. Amongst the n-hexane extracts, H6 had the lowest IC₅₀ value (8.16 ± 0.2 µg/mL), while H7 showed the highest (11.05 ± 0.7 µg/mL). Similarly, M1 had the highest IC₅₀ value (41.76 ± 0.9 µg/mL) while M6 had the lowest IC₅₀ value (33.40 ± 1.3 µg/mL). The extracts of n-hexane had the following IC₅₀ (µg/mL) order: H6 < H11 < H5 < H3 < H9 < H10 < H2 < H12 < H8 < H4 < H1 < H7. The order of IC₅₀ (µg/mL) values for the methanol fractions were M1 > M4 > M7 > M2 > M12 > M10 > M8 > M3 > M9 > M11 > M5 > M6. The n-hexane fraction of C. album showed good inhibitory activities as compared to the IC₅₀ value of the standard drug. Despite the higher phenolic contents in the methanol fractions, they showed lower α-amylase enzyme inhibitory activity as compared to the n-hexane extracts [27]. Presumably, non-phenolic components or any potential synergistic interactions between phenolic and non-phenolic molecules could be the cause of this lower inhibitory activity of the methanolic extracts. However, enzyme inhibitory activities showed a significant effect, with p value < 0.05, as shown by various studies [28,29].

![Figure 1. α-amylase enzyme inhibition with methanolic (M1–M12) fractions of C. album.](image)

### 3.4. Urease Inhibitory Activity

The ability of the fractions, methanol and n-hexane, to inhibit urease enzyme was examined in vitro. All the fractions of methanol revealed a higher percentage of urease inhibition than the fractions of n-hexane, as demonstrated in Figures 4 and 5. Thiourea, the standard control, showed urease inhibitory activity in the range of 22% to 36% at 0.312 to 10 µg/mL concentrations, with an IC₅₀ value of 19.09 ± 0.7 µg/mL. The IC₅₀ values of the methanol extracts were recorded as lower than those of the n-hexane extracts, as shown in Figure 6. Our findings are also in agreement with those in [30]. Urease was significantly (p < 0.05) less inhibited by the n-hexane fractions, which might be due to the presence of more non-polar/volatile compounds in the extracts. Among the methanolic extracts, M6 had the lowest IC₅₀ value (18.77 ± 0.6 µg/mL), while M7 had the highest...
value (24.47 ± 0.3 µg/mL). Similarly, H1 had the highest IC50 value (55.10 ± 1.7 µg/mL) and H6 had the lowest (47.84 ± 1.1 µg/mL). The methanolic fractions had the following IC50 value order: M6 < M11 < M5 < M3 < M10 < M9 < M12 < M2 < M8 < M4 < M1 < M7. The order of IC50 values for the n-hexane extracts was H6 < H5 < H11 < H9 < H3 < H8 < H10 < H12 < H2 < H7 < H4 < H1. It is reported that this medicinal herb crop could be considered in situations where it is necessary to slow down the urease activity, such as in the management of urease-induced stomach ulcers [30].

Figure 2. α-amylase enzyme inhibition with n-hexane (H1–H12) fractions of C. album.

Figure 3. IC50 values of methanolic (M1–M12) and n-hexane (H1–H12) fractions against α-amylase.

3.5. Antibacterial Activity

The antibacterial potential of the C. album plant extracts was tested against E. coli and S. aureus bacteria.
µg/mL). Similarly, H1 had the highest IC50 value (55.10 ± 1.7 µg/mL) and H6 had the lowest (47.84 ± 1.1 µg/mL). The methanolic fractions had the following IC50 value order: M6 < M11 < M5 < M3 < M10 < M9 < M12 < M2 < M8 < M4 < M1 < M7. The order of IC50 values for the n-hexane extracts was H6 < H5 < H11 < H9 < H3 < H8 < H10 < H12 < H2 < H7 < H4 < H1. It is reported that this medicinal herb crop could be considered in situations where it is necessary to slow down the urease activity, such as in the management of urease-induced stomach ulcers [30].

Figure 4. Urease enzyme inhibition with methanolic (M1–M12) fractions of C. album.

Figure 5. Urease enzyme inhibition with n-hexane (H1–H12) fractions of C. album.

3.5.1. E. coli Growth Inhibition Activity

E. coli bacterial strain ATCC25922 was used to record the bacterial growth inhibition potential of the n-hexane extracts along with the methanolic fractions in terms of ZOI (mm). The results are shown in Table 3. Run H6 had the highest 15.70 ± 0.65 mm ZOI in comparison to run M6, which exhibited 16.55 ± 0.57 mm ZOI. Previously reported data on the methanolic extract of C. album showed E. coli growth ZOI of ~18 mm. Our study showed a maximum of 16.55 ± 0.57 mm ZOI against E. coli, which is slightly lower than that reported in the literature, which might be due to geographical and cultivation method differences. Moreover, the influence of the genetic variability of the plant, environmental factors, and microbial resistance differences from one region to another may also be reasons for the lower methanolic extract activity against E. coli [31,32]. Our results showed that the n-hexane extracts are slightly less potent than the methanolic extracts. The inhibition zone formed by the control, ciprofloxacin, against E. coli was 26.08 ± 0.73 mm, and the negative control (blank, containing no sample) showed no significant inhibition zone. The
methanolic fractions had the following ZOI (mm) order: M6 > M11 > M5 > M3 > M9 > M2 > M10 > M1 > M12 > M8 = M4 > M7, while the n-hexane extracts showed the following order: H6 > H5 > H11 > H9 > H3 > H12 > H8 > H10 > H1 > H2 > H4 > H7. It was noted that a significant inhibition zone was shown by all the extracts of both solvents.

### Figure 6. IC_{50} values of methanolic (M1–M12) and n-hexane (H1–H12) extracts of C. album for urease enzyme inhibition.

Table 3. Antibacterial studies of methanol (M1–M12) and n-hexane (H1–H12) C. album plant extracts under various conditions of extraction.

<table>
<thead>
<tr>
<th>Methanolic Extract</th>
<th>Zone of Inhibition (mm)</th>
<th>n-Hexane Extract</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>E. coli</td>
<td>S. aureus</td>
</tr>
<tr>
<td>M1</td>
<td>12.33 ± 0.04 **</td>
<td>14.12 ± 0.38 *</td>
<td>H1</td>
</tr>
<tr>
<td>M2</td>
<td>14.13 ± 0.55 ns</td>
<td>14.34 ± 0.64 ns</td>
<td>H2</td>
</tr>
<tr>
<td>M3</td>
<td>15.37 ± 0.27 *</td>
<td>15.19 ± 0.01 **</td>
<td>H3</td>
</tr>
<tr>
<td>M4</td>
<td>12.34 ± 0.57 ns</td>
<td>13.21 ± 0.55 ns</td>
<td>H4</td>
</tr>
<tr>
<td>M5</td>
<td>15.36 ± 0.42 *</td>
<td>16.10 ± 0.56 ns</td>
<td>H5</td>
</tr>
<tr>
<td>M6</td>
<td>15.55 ± 0.55 ns</td>
<td>16.55 ± 0.57 ns</td>
<td>H6</td>
</tr>
<tr>
<td>M7</td>
<td>10.33 ± 0.60 ns</td>
<td>11.98 ± 0.60 ns</td>
<td>H7</td>
</tr>
<tr>
<td>M8</td>
<td>13.33 ± 0.61 ns</td>
<td>13.21 ± 0.57 ns</td>
<td>H8</td>
</tr>
<tr>
<td>M9</td>
<td>15.32 ± 0.45 *</td>
<td>14.54 ± 0.02 **</td>
<td>H9</td>
</tr>
<tr>
<td>M10</td>
<td>14.22 ± 0.52 ns</td>
<td>13.33 ± 0.52 ns</td>
<td>H10</td>
</tr>
<tr>
<td>M11</td>
<td>15.47 ± 0.55 ns</td>
<td>16.25 ± 0.25 *</td>
<td>H11</td>
</tr>
<tr>
<td>M12</td>
<td>13.29 ± 0.58 ns</td>
<td>13.85 ± 0.60 ns</td>
<td>H12</td>
</tr>
</tbody>
</table>

### 3.5.2. S. aureus Growth Inhibition Activity

*S. aureus* bacterial strain ATCC25923 was used to record the bacterial growth inhibition potential of the n-hexane extracts compared with the methanolic extracts in terms of ZOI (mm) and with penicillin (standard drug). The results are summarized in Table 3. The positive control, penicillin, showed 21.12 ± 0.81 mm ZOI, while the negative control showed no ZOI. The n-hexane extract (H6) showed 15.14 ± 0.57 mm ZOI, while the methanolic extract (M6) showed slightly more (15.55 ± 0.55 mm ZOI). It has been reported that the
methanol extract of *C. album* has significant effectiveness against *S. aureus*, with ZOI of 20.5 mm, as compared to extracts in petroleum ether, dichloromethane, and ethyl acetate. Our results recorded a maximum of 15.55 ± 0.55 mm ZOI of *S. aureus* bacteria using the methanolic extract and 15.14 ± 0.57 mm ZOI using the n-hexane extract. In addition to geographical and cultivation method differences, the genetic variability of the crop, and environmental factors, bacterial mutation might be the main cause of the comparatively lower activity of the methanol extract as compared to that previously reported [32]. The following *S. aureus* bacterial growth inhibitory order of the n-hexane fractions was recorded: H6 > H8 > H5 > H11 > H9 > H3 > H10 > H2 > H12 > H4 > H7 > H1, while that of the methanol extracts was M6 > M11 > M3 > M5 > M9 > M10 > M2 > M8 > M12 > M4 > M1 > M7. In summary, the extracts of n-hexane solvent at its particular concentration showed notable ZOI against *S. aureus* and *E. coli*, which could be considered for further herbal antibacterial medicine in addition to methanolic extract which has already been reported as a potent antibacterial extract [33]. The antibacterial activity of these extracts is attributed to the phenolic content present in the sample extract and influenced by locality variations of *C. album*.

4. Conclusions
Most staple crops have been used in traditional ways since the beginning of human civilization, with no significant value-addition processes. And many green leafy vegetables were also used as food for livestock, including *C. album*. Due to its traditional medicinal importance, we determined its antibacterial activity against two infection-causing bacteria, i.e., *S. aureus* and *E. coli*, and also determined its enzyme inhibition activity to assess its potential to manage diabetes mellitus and ulcer diseases. We recorded excellent results in both the antibacterial and enzyme inhibition studies. This suggests that the extraction of phytochemicals in methanol from *C. album* could be formulated into herbal medicine for treating bacterial infections, diabetes mellitus, and stomach ulcers. Moreover, the presence of important minerals, e.g., Zn, Mg, and Fe, make *C. album* an attractive option for addressing mineral deficiency issues.


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