In vitro anthelmintic activity of Chenopodium album and in-silico prediction of mechanistic role on Eisenia foetida

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ABSTRACT

Helminths born diseases are related to pitiable management practices and improper control strategies. The medicinal plants contain various phytoconstituents that are liable for their anthelmintic activity. The aerial parts of the Chenopodium album were successively extracted with microwaves assisted extraction using petroleum ether, ethyl acetate, methanol, hydroalcoholic and aqueous solvents to get respective extracts (CAPE, CAEE, CAME, CAHE, and CAAE). All the extracts were analyzed for preliminary phytochemical screening for the identification of phytoconstituents. The anthelmintic activity was analyzed on Indian adult earthworms Eisenia foetida using piperazine citrate (PCT) as a standard drug. All the extracts (apart from CAAE) lead to paralysis and fatality of the earthworms. CAEE extract exhibits highly significant anthelmintic activity at a 10 mg/ml concentration by causing paralysis and fatality of the earthworms and was more potent than PCT suspension. At a concentration of 10 mg/ml, paralysis and death time for CAEE was recorded as (10.08 ± 1.11) and (65.28 ± 2.09) respectively, while for standard piperazine citrate, it was recorded as (22.96 ± 1.12) and (65.09 ± 1.23). The CAEE exhibits two major compounds by LC-MS, i.e., NG and DG, that are mainly accountable for the Chenopodium album anthelmintic activity. The plant possesses GABA-mimetic action and thereby leads to flaccid, reversible paralysis of the body wall muscle.

1. Introduction

Helminths contaminations or infections influence a vast number of global populations, especially in rising countries [1]. Approximately 819 million people globally affected with Ascaris, 438 million by means of hookworm, and 464 million by Trichuris [2, 3]. Helminths infection occurs due to the warm, humid equatorial regions and inadequate hygiene sanitation facilities [4]. Helminths are classified into various categories, i.e., Tapeworms, roundworms, and flukes, and predominantly affect humans and animals, thereby causing physiological damage. Helminths born diseases are related to pitiable management practices and deprived and improper control strategies [5, 6, 7]. Medicinal plants are vital in managing parasitic infections and possess several advantages over available synthetic medicine. The medicinal plants contain various secondary metabolites that exhibit anthelmintic activity [8, 9, 10].

The Chenopodium album Linn. is commonly recognized as bathua classified into the Chenopodiaceae family, widely spread across the globe, and contains various bioactive constituents that help treat various anticancer diseases hepatoprotective, antioxidant, antiinociceptive activity, etc. [11, 12, 13]. The plant was reported for its anthelmintic activity [14, 15, 16]. Nevertheless, the responsible secondary metabolites and their mechanism for anthelmintic activity have never been explored. Therefore, the current research attempts to estimate the in vitro anthelmintic effect of various extracts obtained from the aerial parts of Chenopodium album in earthworm Eisenia foetida to estimate and authenticate the ethnobotanical use in a scientific manner.

2. Materials and methods

2.1. Collection and authentication of plant material

Air-dried aerial parts of Chenopodium album were collected from Ludhiana's local areas (Punjab). They were authenticated by Dr. Sunita Garg, Emeritus Scientist, Department of Raw Material Herbarium &
2.2. Extraction of plant material

The aerial parts of the *Chenopodium album* were shade dried at room temperature and crushed by a pulverizer. The powder was sequentially extracted by microwaves assistant technique [17, 18] with slight modifications using petroleum ether, ethyl acetate, methanol, hydroalcoholic, and aqueous solvents to get respective extracts. The solvents were recovered by distillation under reduced pressure by a rotary evaporator. The petroleum ether extract (CAPE), ethyl acetate extract (CAEE), methanol extract (CAME), hydroalcoholic extract (CAHE), and aqueous extract (CAAЕ) were placed in desiccators till further use. All these extracts were used to evaluate anthelmintic activity.

2.3. Phytochemical screening

All the extracts CAPE, CAEE, CAME, CAHE and CAAE were analyzed for preliminary phytochemical screening for the identification of various phytoconstituents such as flavonoids, tannins, and phenolic compounds, terpenoids, alkaloids, steroid, glycosides, saponins, proteins, amino acids, and carbohydrates [19, 20, 21, 22].

2.3.1. Test for alkaloids

To the dried extracts, add dilute HCL dropwise and filter it. The resulting filtrate was screened for the preliminary tests of alkaloids.

**Test with Dragendorff’s reagent:** The reagent was mixed with the filtrate on a watch glass to observe orange, brown precipitates that reveal the alkaloids’ occurrence.

**Test with Mayer’s reagent:** The reagent was mixed with the filtrate on a watch glass to observe cream-colored precipitates that reveal the alkaloids’ occurrence.

**Test with Wagner’s reagent:** The reagent was mixed with the filtrate on a watch glass to observe reddish-brown precipitates that reveal the alkaloids’ occurrence.

**Test with Hager’s reagent:** The reagent was mixed with the filtrate on a watch glass to observe yellow precipitates that reveal the alkaloids’ occurrence.

2.3.2. Test for tannins & phenolic compounds

All the dried extracts were mixed with ethanol and filtered for performing the various preliminary tests for the tannins.

**5% FeCl₃ solution:** To the filtrate, FeCl₃ solution was mixed, monitored for the appearance of a dark blue-black color that reveals the tannins’ occurrence.

**Lead acetate solution:** The lead acetate was mixed with the filtrate, monitored for white precipitates’ appearance that reveals the tannins’ occurrence.

**Gelatin solution:** The gelatin solution was mixed with the filtrate, monitored for white precipitates’ appearance revealing the tannins’ occurrence.

**Iodine solution test:** The dilute iodine solution was mixed with the filtrate, monitored for red color appearance reveals the tannins’ occurrence.

2.3.3. Test for flavonoids

All the dried extracts were mixed with ethanol, filtered for performing the various preliminary identifications for the flavonoids.

**Shinoda test:** The 95% ethanol (5 ml) and HCL was added to the filtrate and after that, magnesium turning was added, monitored for the presence of pink color reveals the occurrence of flavonoids.

**Lead acetate solution:** The solution was mixed with the filtrate, monitored for the appearance of yellow-colored precipitates reveal the occurrence of flavonoids.

2.3.4. Test for steroids

All the dried extracts were mixed with chloroform and filtered for performing the various preliminary tests for the steroids.

**Salkowski test:** The chloroform (2 ml) and conc. H₂SO₄ were mixed with filtrate. Afterward, it was monitored for red and greenish-yellow fluorescence for the CHCl₃ and acid layer, respectively, which reveals the occurrence of steroids.

### Table 1. Color, consistency and % yield of *Chenopodium album* extracts.

| S. No | Chemical Test           | CAPE     | CAEE | CAME     | CAHE | CAAE |
|-------|-------------------------|----------|------|----------|------|------|
| 1     | Alkaloids               | —        | +    | +++      | +    | —    |
| 2     | Flavonoids              | —        | +++  | ++++     | +    | —    |
| 3     | Tannins and phenolic compounds | — | +++ | +++    | +    | —    |
| 4     | Saponins                | —        | —    | ++       | +    | —    |
| 5     | Triterpenoid            | +++      | —    | —        | —    | —    |
| 6     | Steroids                | ++       | +    | —        | —    | —    |
| 7     | Carbohydrates           | —        | —    | ++       | ++   | +    |
| 8     | Protein and amino acids | —        | —    | ++       | ++   | ++   |

:+ Weak positive test; +:+ Low positive test; +++: Strong positive test; -: Negative test. CAPE: *Chenopodium album* petroleum ether extract; CAEE: *Chenopodium album* ethyl acetate extract; CAME: *Chenopodium album* methanolic extract; CAHE: *Chenopodium album* hydro alcoholic extract; CAAE: *Chenopodium album* aqueous extract.

### Table 2. Phytochemical screening of the various extracts from *Chenopodium album*.

| S. No | Chemical Test       | CAPE | CAEE | CAME | CAHE | CAAE |
|-------|---------------------|------|------|------|------|------|
| 1     | Alkaloids           | —    | +    | +++  | +    | —    |
| 2     | Flavonoids          | —    | +++  | ++++ | +    | —    |
| 3     | Tannins and phenolic compounds | — | +++ | +++    | +    | —    |
| 4     | Saponins            | —    | —    | ++    | +    | —    |
| 5     | Triterpenoid        | +++  | —    | —     | —    | —    |
| 6     | Steroids            | ++   | +    | —     | —    | —    |
| 7     | Carbohydrates       | —    | —    | ++    | ++   | +    |
| 8     | Protein and amino acids | — | —    | ++    | ++   | ++   |

:+: Weak positive test; +:+: Low positive test; +++: Strong positive test; -: Negative test. CAPE: *Chenopodium album* petroleum ether extract; CAEE: *Chenopodium album* ethyl acetate extract; CAME: *Chenopodium album* methanolic extract; CAHE: *Chenopodium album* hydro alcoholic extract; CAAE: *Chenopodium album* aqueous extract.
Represents the anthelmintic activity of the different extracts of *Chenopodium album*.

| Conc. (mg/ml) | Paralysis Time (Minutes) | Death Time (Minutes) |
|--------------|--------------------------|----------------------|
|              | Mean ± SD                | VC PCT CAPE CAEE CAME CAHE CAAE | Mean ± SD                | VC PCT CAPE CAEE CAME CAHE CAAE |
| 2            | 153.43 /C6 1.49*         | 161.10 /C6 1.34*#     | 135.45 /C6 1.61*#       | 216.25 /C6 1.99*#              |
| 3            | 352.49 /C6 2.46*         | 380.23 /C6 2.01*#     | 320.66 /C6 2.15*        | 390.89 /C6 2.33*#              |
| 4            | 101.52 /C6 1.43*         | 109.10 /C6 1.50*#     | 85.95 /C6 1.36*#        | 128.40 /C6 1.67*#              |
| 6            | 68.19 /C6 1.53*          | 77.74 /C6 1.20*#      | 54.86 /C6 1.47*         | 86.45 /C6 1.76*#               |
| 8            | 34.14 /C6 1.23*          | 35.52 /C6 1.35*#      | 29.02 /C6 1.23*         | 41.28 /C6 1.47*#               |
| 10           | 22.86 /C6 1.12*          | 23.55 /C6 1.15*#      | 19.08 /C6 1.13*         | 25.56 /C6 1.47*#               |

**Liebermann Burchard test:** The chloroform (2 ml), acetic anhydride (1–2 ml), and conc. H2SO4 were mixed with filtrate, monitored for red, blue, and green color, revealing the steroids’ occurrence.

**2.3.5. Test for saponins**

All the dried extracts were mixed with water and filtered for performing the various preliminary tests for the saponins.

**Foam test:** The alcoholic and aqueous filtrate was vigorously shaken with water, monitored for the appearance of tenacious foam, revealing the saponins’ occurrence.

**2.3.6. Test for amino acids and proteins**

**Biuret test:** Reagent was added to the test solution, monitored for the appearance of violet or pink color, revealing the proteins’ occurrence.

**Million’s reagent test:** The reagent was added to the test solution, observed for white precipitates’ appearance. After that, white precipitates were warmed and kept aside to form a dark red color solution that reveals the proteins’ occurrence.

**Ninhydrin reagent test:** The 5% ninhydrin solution was mixed with the test sample and kept for 10 min on a water bath, monitored for the presence of purple or bluish color reveals the occurrence of the proteins.

**2.3.7. Test for carbohydrates**

All the extracts were mixed with water and filtered to perform the various preliminary tests for the carbohydrates.

**Test with Molisch’s reagent:** The α-naphthol solution prepared in alcohol and concentrated sulfuric acid was mixed with the filtrate, monitored for the development of the violet ring between the two layers test tube reveals the occurrence of the carbohydrates and glycosides.

**Test with Fehling solution:** Both A and B Fehling solution was mixed, boiled for a minute, and then added to the filtrate and further kept for 5–10 min on a water bath, monitored for yellow, dark red precipitates reveal the occurrence of carbohydrates and glycosides.

**Benedict’s reagent:** To the filtrate, add reagent and kept for 5 min on a water bath, monitored for a green, yellow and red color solution. The color of the solution depends on the availability of the reducing sugar in the sample.

**Barfoed test:** The reagent was mixed with the filtrate and kept for 1–2 min on a water bath, monitored for the presence of red-colored precipitates, which exhibits the occurrence of the monosaccharides.

**2.3.8. Test for triterpenoid**

All the extracts were mixed with ethanol and filtered for performing the various preliminary tests for the triterpenoid.

**Thionyl chloride test:** Tin metal bead and thionyl chloride solution were mixed with the filtrate, monitored for the appearance of pink color with effervescence exhibiting the triterpinoid occurrence.

**2.4. Anthelmintic activity**

The anthelmintic activity was analyzed on Indian adult earthworms (*Eisenia fetida*) that possess similarity with human intestinal parasites. The earthworms were purchased and authenticated by Mahavir Organic Manure, Phillaur, Punjab, India, having reference No 82 and were kept under standard conditions of living.

**2.5. Chemicals**

Piperazine citrate (PCT) was purchased from (GlaxoSmithKline Pharmaceuticals), Sodium carboxymethyl cellulose (CMC), and Sodium chloride from (Loba Chemicals, Mumbai).

**2.6. Experimental design**

The anthelmintic activity was assessed on *Eisenia fetida* with slight modifications [23, 24, 25]. The adult earthworms (E. fetida) with 6–12
cm length and 0.2–0.4 mm diameter were separated into 35 groups consisting of five earthworms in each group. The vehicle control (Group I–V) was administrated with normal saline and 1% CMC at different concentrations, i.e., 2, 4, 6, 8, and 10 mg/ml, respectively. The standard groups (Group VI–X) received the PCT suspension at concentrations of 2, 4, 6, 8, and 10 mg/ml, respectively. All the test samples CAPE (Group XI–XV), CAEE (Group XVI–XX), CAME (Group XXI–XXV), CAHE (XXVI–XXX), and CAAE (Group XXXI–XXXV) were also administrated with different concentrations, i.e., 2, 4, 6, 8 and 10 mg/ml respectively. The time taken by each group for causing paralysis and fatality to *Eisenia fetida* was observed separately for the individual worm. The paralysis was confirmed when there is the loss of movement and contraction in earthworms when it is pressed by a finger, whereas the fatality of the earthworm is represented by the loss of their motility with the fading of their body color. The experiment was carried out in triplicate.

### 2.7. Preparation of standard

The oral suspension of piperazine citrate (PCT) was dissolved in normal saline to prepare the stock solution (20 mg/ml). The various dilutions (2–10 mg/ml) were prepared with normal saline from the stock solution. These dilutions were administrated as a standard drug in the earthworm.
2.8. Preparation of test sample

The stock solution (200 mg/ml) was prepared from all the extracts (CAPE, CAEE, CAME, CAHE) using carboxymethylcellulose (1% CMC), and normal saline was used for preparing the CAAE extract. Further various dilutions (2–10 mg/ml) were prepared from the stock solutions for all the extracts.

2.9. LC-MS of the bioactive extract

The bioactive extract (CAEE) obtained from the aerial parts of the plant was characterized by LC-MS using Micromass Q-Tof Micro (waters) instrument for the phytoconstituents’ presence responsible the activity. It is equipped with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APcI) sources having a mass Range of 4000 amu in quadruple and 20000 amu in Time of Flight. A hexapole collision cell between the two mass analyzers is used to induce fragmentation to study the structural investigations while using an MS/MS mode instrument. The Mass Spectrometer is coupled with Waters 2795 HPLC having qua-

terinary pumping configured for flow rates from 0.05- 5.0 ml/Min, and water + 0.1 formic acid/ Acetonitrile was used as a mobile phase. The autosampler is configured with a 100 micro-liter syringe. The identified components were correlated with the compounds’ mass fragments reported in available literature [26, 27].

2.10. Molecular docking studies

For this molecular modeling software, Autodock-vina [28, 29, 30] was used by the pdb or protein selected 4MS3 as a GABA (gamma-aminobutyric acid) receptor of (human) due to the unavailability of nematode GABA protein crystal structure and were downloaded from the protein data bank. The GABA receptor is a well-known target for piperazine exhibits weak GABA-mimetic and causes reversible paralysis of body wall muscle [31, 32, 33]. The structures of these molecules were drawn by ChemDraw and changed to 3D. Further minimizations of energy were carried out using the MM2 Interface program on ChemBio3D Ultra 12.0, and molecules were saved in pdb format (Cambridge Soft). To identify the most active molecule, removing the internal ligand was initially done, and docking was carried out in a similar pattern to an actual ligand.

3. Results

3.1. Extraction of the plant material

The aerials parts of the plant Chenopodium album was successfully extracted with different solvents. The % yield of the various extracts CAPE, CAEE, CAME, CAHE, and CAAE were found to be 6.83, 8.58, 12.15, 10.12, and 14.56 (w/w), respectively (Table 1).
3.2. Phytochemical screening

All the extracts of the plant were analyzed for the occurrence of various secondary metabolites. The phytochemical screening exhibits triterpenoid and steroids in CAPE extract, flavonoids, alkaloids, steroids, and tannins in CAEE extract. Furthermore, the flavonoids, alkaloids, tannins, saponins, carbohydrates, and proteins were present in CAME and CAHE, whereas the CAAE showed carbohydrates and proteins (Table 2).

3.3. Anthelmintic activity

The aerial parts of the Chenopodium album showed potent anthelmintic activity when evaluated on Indian earthworm Eisenia fetida. The vehicle control does not produce any effect on the earthworm. However, all the extracts (apart from CAAE extract) lead to the earthworm’s paralysis and fatality. The CAEE extract posses highly significant potent anthelmintic activity compared to the standard PCT at a concentration of 10 mg/ml. The paralysis and death time for CAEE at 10 mg/ml concentration was recorded as (10.08 ± 1.11) and (65.28 ± 2.09) respectively, while for standard PCT, it was recorded as (22.96 ± 1.12) and (65.09 ± 1.23). The CAPE and CAME also showed significant anthelmintic activity but less than that of standard PCT. However, the CAHE showed very mild anthelmintic activity at 10 mg/ml concentration only, while the CAAE did not possess anthelmintic activity (Table 3, Figures 1 and 2). The results represent that CAEE extract showed a highly significant anthelmintic effect by causing paralysis and death to the earthworm compared to the standard drug. Therefore, CAEE extract was further explored for the identification of the responsible phytoconstituents.

3.4. LC-MS characterisation of CAEE

The bioactive extract CAEE was analyzed by LC-MS spectra and predicted two significant compounds, i.e., notoginsenoside R2 (NG) having retention time 7.14 with a mass of m/z 770.98 (49%) and 1,6-Digalloyl glucose (DG) at 9.13 retention time with a mass of m/z 484.36 (18.53%) (Table 4). This represents that both these compounds are the primary active constituents of the CAEE extract and are primarily responsible for the plant’s anthelmintic activity.

3.5. Molecular docking

Both the phytochemical NG and DG were found to be most active with binding affinities of -6.4 kcal/mol, -7.4 kcal/mol, respectively, in comparison to internal ligand gamma-aminobutyric acid (-4.2 kcal/mol) and also from piperazine (-3.3 kcal/mol) on GABA receptor (Table 5). A detailed interaction for NG and DG was represented in Figure 3a and b respectively at the GABA receptor binding site. An overlay of NG and DG was also represented in Figure 3c at the GABA receptor binding site. NG showed hydrogen bonds (green color cylinder shown in Figure 3a) to the amino group of ARG162 at the binding site while other hydrophobic interactions were observed with LEU135, polar electrostatic interaction with THR134, SER131, THR199, and PRO105 (Figure 3a). DG also showed similar interactions and found to be better than NG. It showed hydrogen bond GLU200, while hydrophobic interaction with GLY64 and LEU195. Different amino acid residues like GLU138, THR134, ARG162, THR198, and GLU253 are involved in electrostatic polar interaction with DG (Figure 3b).

4. Discussion

Helminths infection occurs due to the warm, humid equatorial regions and inadequate hygiene sanitation facilities [34, 35]. The medicinal plants contain various secondary metabolites that exhibit anthelmintic activity [36, 37, 38]. The plant Chenopodium album plant was earlier reported for its anthelmintic activity [14, 15, 16]. Nevertheless, the responsible secondary metabolites and their mechanism for anthelmintic activity have never been explored. In this context, the Chenopodium album aerial parts were extracted using the microwave-assisted technique with different solvents, and the preliminary phytochemical screening of the extracts represents the occurrence of various secondary metabolites. The plant extracts CAPE, CAE, CAME, CAHE and CAAE were screened for their anthelmintic activity on adult Indian earthworm Eisenia fetida. All the extracts (apart from CAAE...
extract) lead to paralysis and fatality of the earthworm. CAEE extract exhibits potent anthelmintic activity at a 10 mg/ml concentration by causing paralysis and death time of earthworms and was more potent than PCT suspension. At a concentration of 10 mg/ml, paralysis and death time for CAEE was recorded as (10.08 ± 1.11) and (65.28 ± 2.09) respectively, while for standard PCT, it was recorded as (22.96 ± 1.12) and (65.09 ± 1.23).

Further, LC-MS spectra analyzed the bioactive extract CAEE and predicted two significant compounds, i.e., NG and DG. It is predicted from LC-MS that both these compounds are the plant's primary active constituents, i.e., primarily responsible for the plant's anthelmintic activity. Moreover, we explored the molecular docking studies to predict the mechanism of action of the identified compounds. Both the phytochemical NG and DG were most active with binding affinities of -6.4 kcal/mol, -7.4 kcal/mol, respectively, compared to internal ligand on GABA receptor. The PCT possess weak GABA-mimetic action that leads to reversible paralysis of body wall muscle [33, 39, 40]. The NG and DG are the two primary compounds present in the plant Chenopodium album primarily responsible for its GABA-mimetic action, causing reversible paralysis of body wall muscle in the earthworm. This finding supports the potential role of the Chenopodium album as an anthelmintic agent.

5. Conclusion
All the extracts (apart from CAEE extract) lead to paralysis and fatality of the earthworm. CAEE extract showed potent anthelmintic activity at a concentration of 10 mg/ml by causing paralysis and death time of earthworms and was more potent than PCT suspension. The CAEE exhibits two significant compounds, i.e., NG and DG, mainly responsible for the plant's anthelmintic activity. The NG act and DG possess GABA-mimetic action and thereby leads to reversible paralysis of body wall muscle.

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