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Antibacterial, antioxidant and cytotoxic activities of *Conyza Canadensis* ethanolic extract

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ABSTRACT

Background: Medicinal herbs are widely valued for their therapeutic potential Conyza canadensis, with a long history of treating various ailments was the subject of this recent research. *Aim*: The study aimed to evaluate the total phenols and flavonoids, antioxidant, antibacterial and cytotoxicity activity of *Conyza canadensis* ethanolic extract *in vitro*. *Methodology*: The aerial parts of the plant were extracted with 70% ethanol. The extract was analyzed for total phenolic and flavonoid content, antioxidant capacity using DPPH assay, antimicrobial effects against selected pathogens, and cytotoxicity on HepG2 cells using the MTT assay. *Results*: The extract showed high phenolic (226.28 ± 12.67 mg/g GAE) and flavonoid content (123.1 ± 16.25 µg/mL). The DPPH assay indicated dose-dependent scavenging activity, surpassing that of vitamin C. Antibacterial activity was moderate to strong, with inhibition zones ranging from 13 mm to 31 mm depending on concentration and organism. The MTT assay revealed significant cytotoxicity against HepG2 cells at higher doses. *Conclusion*: *Conyza canadensis* exhibits promising antioxidant, antibacterial, and cytotoxic properties attributed to its phytochemical content.

KEYWORDS

extract, herb, health, antioxidant, cell line

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1. INTRODUCTION

Conyza canadensis, commonly known as Canadian fleabane or horseweed, is a resilient and widely distributed plant species with a rich history of traditional medicinal use [1]. Throughout the years this plant has caught the eye of researchers for its healing properties. Communities have long prized the *Conyza canadensis* plant for its ability to reduce inflammation, relieve pain, and promote healing of wounds. Such study of this historic application serves to uncover how natural biological processes actually work [2]. However, there is a mass of raw material nearby waiting to be exploited which can be refined into a variety of useful substances like alkaloids, flavonoids, terpenes and phenolics all have life activities [3]. The purpose of the present study is to investigate various *in vitro* capabilities of *Conyza canadensis* extract, focusing on its cytotoxic, anti-inflammatory, antiox-

idative, and antibacterial effects. Antioxidants are life preservers. No stress-related ailment can be staved off without them. It has been said that *Conyza canadensis* flower bioflavonoids also derive their antioxidant properties from scavenging free radicals and thus help to keep cells safe from oxidative injury. This would change the way we eat. Also, *Conyza canadensis* ethanolic extract has been used by people seeking relief from pain but immune system responses to the extracts suggested potential inflammation-alleviating effects, *in vivo*. This study explores the *in vitro* biological activities of *Conyza canadensis*, focusing on its antioxidant, cytotoxic, anti-inflammatory, and antibacterial properties. *Conyza canadensis* is traditionally known for its wound-healing and anti-inflammatory effects, which may be attributed to its rich phytochemical content, such as flavonoids and phenolic compounds [4]. These bioactive constituents help neutralize oxidative stress by scavenging free radicals. Investigating the extract's mechanisms of action *in vitro* may provide insights into its potential therapeutic applications.

2. METHODOLOGY

2.1. Ethanolic extraction of *Conyza canadensis*

The aerial portions of the plant were collected between September 2022 and March 2023 from Erbil, Iraq, and subsequently categorized by the National Herbarium of Iraq. 50 grams of the powdered plant were extracted with 70% ethanol at 65°C for 3 h using a Soxhlet device, filtered and evaporated until it was dry [5].

2.2. Total phenolic and flavonoid contents assay

In brief, the T.F. assay involved dissolving 3.2 mg of plant ethanolic extract in 5 mL of 50% methanol, adding 1 mL of 5% (w/v) sodium nitrite solution, adding 1 mL of 10% (w/v) aluminum chloride solution after 6 min, and waiting an additional 5 min before adding 10 mL of 10% (w/v) NaOH solution. The volume was brought to 50 mL [5] with distilled water. Following that, the absorbance at 450 nm was measured for 15 min using a spectrophotometer. Rutin (as standard) at the same concentrations was used to draw a standard curve.

The Folin-Ciocalteau reagent was utilized. In short, 1 mL of ethanolic extract was combined separately with 4.0 mL of sodium carbonate solution (75 g/l) and 5.0 mL of Folin Ciocalteu reagent. Following one h, the absorbance at 765 nm was measured [6].

$$C = C_1 \times \frac{V}{m}$$

where C = total phenolic content in mg/g, in GAE (Gallic acid equivalent), C_1 = concentration of Gallic acid established from the calibration curve in mg/mL, V = volume of extract in mL, and m = the weight of the plant extract in g. A similar procedure was applied for Gallic acid to draw a standard curve.

2.3. Antioxidant activity using Radical Scavenging Activity of 2,2- diphenyl-1-picrylhdrazyl (DPPH) assay

About 3.9 mL of DPPH solution was mixed with 0.1 mL of each concentration (0.625, 0.125, 0.250, and 0.500 mg/mL). The mixture was then incubated for 30 min at 37°C. The absorbance of each solution was measured using a spectrophotometer at 517 nm, and the result was computed using the following equation:

$$\text{DPPH scavenging} = 1 - \left(\frac{\text{ABS. of sample}}{\text{ABS. of standard}}\right) \times 100$$

2.4. Antimicrobial assay for *Conyza canadensis* extract

The assay of antimicrobial activity includes estimation of both antibacterial and antifungal of *Conyza canadensis* against one gram negative and one positive bacteria in-addition to one type of Candida albicans. Anti-bacterial method estimated against different pathogenic bacteria and fungi (*Staph aureus, Escherichia coli. Candida albicans*) [7]. Different concentrations of the plant ethanolic extract (25, 50, 75, and 100%) were tested. Muller Hinton agar plates were previously prepared, and after solidification, they were stored at 4°C to give wells a firm surface. Subsequently, 50 µL of various plant percentages were added to them, and the plates were cultured for 18–24 h at 37ºC. Following incubation, the widths of the inhibitory zones were measured in millimetres using a ruler, and the results were then recorded [6].

2.5. Cytotoxicity of *Conyza canadensis* extract on hepg2 cells via MTT assay (3-(4, 5 – dimethylthiazol – 2 - yl) – 2, 5 diphenyltetrazolium bromide)

Using an MTT ready kit, the cytotoxic effects of five different doses of *Conyza canadensis* (25, 50, 100, 200, and 400 µg/mL) were assessed *in vitro* on HepG2. Following cell line maintenance,

HepG2 cells ((1 × 10^{-4} – 1 × 10^{6}) cells mL^{-1} were seeded at final volume (200 µL) and cultivated at 37 °C for 24 h using micro-titer plates (96-well). Following the addition of the produced concentrations, each well was incubated for 24 h.

Next, 10 millilitres of MTT solution was added, and the mixture was incubated for 4 h at 37 °C with 5% CO_2. After draining the medium, 100 mL of the solubilization solution was added, and it was further incubated for five min. An ELISA microplate reader (Bio-Rad, USA) was utilized to measure formazan formation at 570 nm. [7]

2.6. Statistical analysis

The computer program SPSS version 13.1 was utilized to perform an analysis of variance (ANOVA).

3. RESULTS

The phytochemical analysis of *Conyza canadensis* ethanolic extract revealed a substantial presence of both flavonoids and phenolic compounds. The total flavonoid content was quantified as 123.1 ± 16.25 µg/mL, while the total phenolic content, expressed in terms of gallic acid equivalents, was 226.28 ± 12.67 mg/g. These values suggest a high concentration of bioactive secondary metabolites in the extract.

Regarding the antioxidant potential, the DPPH radical scavenging assay demonstrated a concentration-dependent effect. At 250 mg/mL, the extract exhibited a scavenging activity of 58.20 ± 2.23%, which increased to 74.51 ± 3.69% at 0.500 mg/mL. These results indicate that the antioxidant efficacy of the extract is enhanced at higher concentrations and outperforms vitamin C in free radical neutralization across all tested levels.

The antimicrobial activity of the extract was also assessed against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* at concentrations of 25%, 50%, 75%, and 100%. The inhibition zones observed increased proportionally with concentration. *S. aureus* showed the highest sensitivity, with an inhibition zone of 31 mm at 75% and 30 mm at 100%, whereas *Candida albicans* exhibited a smaller inhibition zone of 13 mm at 25% and 18 mm at 100%. *E. coli* demonstrated moderate susceptibility with inhibition zones ranging from 15 mm to 21 mm.

The cytotoxicity of the ethanolic extract was evaluated on HepG2 liver cancer cells and compared to normal human dermal fibroblast (HdFn) cells using the MTT assay. A dose-dependent cytotoxic effect was observed. HepG2 cell viability decreased progressively with increasing extract concentrations: 75.22 ± 1.66% at 25 µg/mL, down to 52.33 ± 2.37% at 400 µg/mL. In contrast, HdFn cells maintained relatively higher viability, starting from 96.21 ± 2.14% at 25 µg/mL and decreasing to 68.57 ± 2.41% at 400 µg/mL. This indicates selective cytotoxicity of the extract toward cancerous cells over normal cells.

These findings collectively support the biological activity of *Conyza canadensis* ethanolic extract, suggesting its potential use as an antioxidant, antibacterial, and cytotoxic agent.

Table 1. Antimicrobial activities and Cytotoxic potentials of *Conyza Canadensis* extract against liver cancer cell line.

Types of microorganism	100%	75%	50%	25%
Candida albicans	18	16	15	13
Escherichia coli	20	21	18	15
Staphylococcus aureus	30	31	26	20
Concentration µg/mL^{-1} (Cytotoxic potential)	**Mean viability (%) HdFn**		**HepG2**	
400	68.57±2.41		52.33± 2.37	
200	85.90±1.92		56.98±2.80	
100	90.25±1.51		63.92±1.28	
50	93.28±1.33		70.04±2.70	
25	96.21±2.14		75.22±1.66	

The cytotoxicity assay revealed a concentration-dependent decrease in HepG2 cell viability. At the lowest concentration (25 µg/mL), cell viability was 75.22 ± 1.66%, while at the highest tested concentration (400 µg/mL), viability dropped significantly to 52.33 ± 2.37%. In contrast, the normal HdFn cells showed higher viability, ranging from 96.21 ± 2.14% at 25 µg/mL to 68.57 ± 2.41% at 400 µg/mL

4. DISCUSSION

Some possible reasons for the extract's anti-oxidant effects are the presence of phytochemicals such as phenolic compounds and flavonoids. Its antioxidant features of flavonoid and phenol suggested that it could be used as a source of antioxidant-rich food and supplements.
The extract's potential as herbal agent is indicated by its antibacterial assessment, which is significant given the current rise in antibiotic resistance [8]. The extract showed activity against a variety of bacteria and other pathogens. Although further investigation is necessary to comprehend the mechanisms underlying these findings fully, *Conyza canadensis* ethanolic extract may provide a source for the development of novel antimicrobial medicines or serve as an additional therapeutic option.[9]. Additionally, cytotoxicity studies demonstrated that the extract should be used at limited concentration and doses potential medicinal applications [10].

5. CONCLUSION

The conducted study detailed the ability of *Conyza canadensis* plant to scavenge free radicals and antibacterial potential in addition to inhibit cancer cell growth in comparison to normal cell. All these activities may attribute to secondary metabolite present in the plant.

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CONFLICT OF INTEREST STATEMENT:
The authors declare no conflicts of interest

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