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Urease Inhibitory Potential of Crude Extracts and Their Isolated Fractions of *Conyza canadensis*

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Abstract

Conyza canadensis (Canadian horseweed) has long been recognized for its therapeutic applications and could provide naturally occurring compounds with urease inhibitory effects. This paper discusses the urease inhibition properties of various solvent fractions of *C. canadensis* (n-hexane, chloroform, ethyl acetate, methanol and butanol) and compares its actions with thiourea, which is a standard urease inhibitor. The results indicated that the butanol fraction exhibited the most potent urease (85.98%), followed by methanol (78.02% and ethyl acetate (67.09%). The IC_{50} values indicated that butanol and methanol fractions had a stronger inhibitory effect on the urease activity compared to ethyl acetate fraction. The findings are indicative of the fact that *C. canadensis* can serve as a source of natural urease inhibitors, which can potentially be used as an alternative treatment of urease-based gastrointestinal disorders such as Helicobacter pylori infections. Further studies are necessary to determine which specific bioactive compounds can have the desired effect and determine their therapeutic effectiveness.

KEYWORDS

Conyza canadensis, natural products, bioactive compounds, urease inhibition, enzyme inhibition, gastrointestinal disorders, traditional medicine, Helicobacter pylori.

1.0 INTRODUCTION

Conyza canadensis, commonly known as Canadian horseweed, is a plant which has a long tradition of use in traditional medicine in most cultures. *C. canadensis* is extensively employed in the management of different diseases such as gastrointestinal diseases, inflammatory diseases, and infections because of its diverse medicinal characteristics [1, 2]. Besides, *C. canadensis* has demonstrated its possibilities in the control of various diseases, including diabetes, as demonstrated by Aslam et al., who reported *in vitro* and *in vivo* evidence of its antidiabetic properties [3]. In addition, it has also been observed that *C. canadensis* has immense antioxidant, cytotoxic, and anti-inflammatory effects, thereby enhancing its therapeutic value [4]. The diverse bioactive compounds present in *C. canadensis* have been identified to have promising bioactivity in modern pharmacological research, and this also indicates its potential in being the source of natural products used as therapy. Shakirullah et al.

discovered compounds like Conyzolide and Conyzoflavone to exhibit substantial antimicrobial effects [5].

Urease is an enzyme that breaks down urea to carbon dioxide and ammonia and is a major contributor to various GIT disorders, including peptic ulcers and Helicobacter pylori infections. Urease can cause release of ammonia, which can damage the gastric mucosa, and has a contribution to the development of such diseases [6]. As a result, the inhibition of the urease activity has become a therapeutic objective of utmost importance in the treatment of *H. pylori* infections, especially in children, with the limited spectrum of treatment options available [6]. The current study, pointing to the development of efficient urease inhibitors, has displayed encouraging findings that the targeting of urease can contribute to a substantial increase in eliminating *H. pylori* and curbing the risks posed by the emergence of antibiotic-resistance [7, 8]. Moreover, the necessity of non-antibiotic interventions, such as urease inhibitors as the method of maintaining gut microflora and achieving effective treatment of *H. pylori* infection, has been also noted in several studies [8, 9]. Other drugs such as palmatine, also have been found to have the potential of suppressing the activity of urease, as well as the growth of the *H. pylori*, which may offer a new approach to treatment [10].

Despite such developments, a significant gap in research exists in defining and characterizing new plant-based urease inhibitors that are effective and safe as clinical agents. The further study of natural products, specifically plants such as *C. canadensis*, may yield important information on the evolution of alternative treatments to *H. pylori* infections especially following the increasing concern about antibiotic resistance.

2.0 MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The plant was collected, shad dried, pulverized and 3 kg of dried powder plant materials were obtained. After plant powder maceration, crude methanolic extract was obtained according to well-established reported protocols [11-15]. Following the filtration and vacuum concentration process at 40 ^{o}C, 300 g crude methanolic extract was obtained. The extract was further fractioned with various solvents on the basis of polarity (n-hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions). The crude methanolic as well as the subsequent solvent fractions were screened for phytochemical investigation and antioxidant activities.

2.2 Urease activity

The urease inhibitory activity of n-hexane, chloroform, ethyl acetate, methanol, and butanol fractions of *Conyza canadensis* extract was assessed using the jack bean urease (EC 3.5.1.5) inhibition assay. The reaction mixture was prepared in a 96-well microplate and contained 25 µL of enzyme solution, 55 µL of phosphate buffer (100 mM, pH 8.2) with 100 mM urea as the substrate, and 5 µL of the test sample. The mixture was incubated at 30°C for 15 minutes.

Enzymatic activity was determined spectrophotometrically by measuring the amount of ammonia produced using the indophenol method. After incubation, 45 µL of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% NaOCl containing active chlorine) were transferred to each well respectively. The plates were incubated for an additional 50 minutes at room temperature, and the absorbance was recorded at 630 nm using a microplate reader.

All the experiments were conducted in triplicates and a final volume of the reaction was kept at 200 µL per well. Thiourea was employed as the reference standard inhibitor [16, 17]. The percentage of urease inhibition was calculated using the following equation:

$$\text{Percent effect} = 100 - \frac{OD\ testwell}{OD\ control} \times 100$$

2.3 Statistical Analysis

All the experimental data were expressed as mean ± standard deviation (SD) and were computed with reference to three independent replicates (n = 3). Analysis of the results was conducted through one-way analysis of variance (ANOVA), with a post hoc test of Tukey being used to assess the occurrence of significant differences between treatment means. A p-value of less than 0.05 was taken as significant.

3.0 RESULTS AND DISCUSSION

3.1 Urease Inhibitory Activity of *Conyza canadensis* Extract Fractions

Urease inhibitory activity of different solvent fractions of *Conyza canadensis* was measured at a concentration of 0.2 µg/mL, and the results were presented in Table 1. The butanol extract had the best inhibitory activity (85.98%), which was then followed by methanol (78.02%) and ethyl acetate (67.09%). The chloroform and the *n*-hexane extracts had a relatively lower inhibitory effect with a percentage of 42.09% and 39.87%, respectively. Thiourea, which was used as reference standard, had highest urease inhibition (98.40%), and this emphasizes its strong inhibitory effect. The different inhibitory potential of the fractions of the different fractions indicates that solubility and polarity of compounds in *Conyza canadensis* might be a major contributor to its activity against urease.

The most potent extracts were also determined as to their IC_{50} values, indicating the concentration at which 50% the inhibition is achieved. The lowest IC_{50} was that of the butanol fraction (36.10 ± 0.80 µg/mL) then methanol (42.09 ± 2.01 µg/mL) and ethyl acetate (102.66 ± 1.23 µg/mL). These results point to the fact that the butanol extract is highly effective, and the least concentration is needed to obtain 50% inhibition of the urease activity. Conversely, the ethyl acetate fraction had a relatively higher value of IC_{50}, which means that more concentration of the fraction is needed to attain the same level of urease inhibition.

Table 1: Urease inhibitory activity of hexane, chloroform, ethyl acetate, methanol, and butanol fractions of *Conyza canadensis*

S.NO	Extract/standard	Concentration (µg/mL)	% inhibition	IC_{50}
01	n-Hexane	0.2	39.87	-
02	Chloroform	0.2	42.09	-
03	Ethyl Acetate	0.2	67.09	102.66±1.23
04	Methanol	0.2	78.02	42.09±2.01
05	Butanol	0.2	85.98	36.10±0.80
06	Thiourea	0.2	98.40	22.01±0.09

The identified urease inhibitory effect of *C. canadensis* is consistent with its conventional application in the treatment of gastrointestinal conditions, which are commonly associated with urease activity and infection with Helicobacter pylori. *H. pylori* produces an enzyme, urease, that facilitates the breakdown of urea into ammonia that can cause damage to the gastric mucosa and cause peptic ulcers to develop [6]. As a result, urease inhibition has emerged as an important treatment alternative to *H. pylori* and other gastrointestinal illnesses associated with excessive urease production [7].

In earlier researches it has been shown that a number of plant species such as *Conyza canadensis* have a huge potential in the form of natural sources of urease inhibitors. Indicatively, Polat et al. (2022) have demonstrated that some medicinal plants like *C. canadensis* possess antioxidant, cytotoxic, and anti-inflammatory effects, which can be translated to their therapeutic effects [4]. Similarly, Shakirullah et al. (2011) reported that *C. canadensis* compounds have antimicrobial properties, thereby emphasizing its therapeutic value [5]. The current study is relevant to this literature since the study reveals the urease inhibitory effect of the plant and thus, as a result, it is possible to consider the use of the plant in management of urease-related conditions.

Recently, the use of urease inhibitors, including those of *C. canadensis*, has also become important in the treatment of *H. pylori*. Hassan and Šudomova (2017) highlighted the

therapeutic importance of urease inhibitors in the treatment of *H. pylori* infections, and particularly in children, where the use of antibiotics to treat the condition is usually limited due to the side effects [6]. Urease inhibition has been also considered potentially effective in minimizing occurrence of antibiotic resistance since urease inhibitors may be employed on the preservation of gut microbiota and treatment of H. pylori infection [7]. Furthermore, it has been proven that such compounds as palmatine are capable of inhibiting the activity of urease and the growth of *H. pylori*, thus offering the alternative treatment options [10].

The results of this research are promising, and further research is required in order to isolate and identify the specific bioactive compounds that lead to the observed urease inhibition in *C. canadensis*. Previous studies had suggested that flavonoids, alkaloids and other polyphenolic compounds may be involved in urease inhibition process [4]. Further studies involving the application of modern chromatography techniques will be required to identify these compounds and their associated functions in urease inhibition. In addition to this, *in vivo* studies will be crucial in determining the therapeutic potential of these extracts, as well as their safety information, particularly in the treatment of the *H. pylori* infections and also in the treatment of other gastrointestinal diseases.

Conclusively, the urease inhibitory properties of *Conyza canadensis* fractions especially butanol and methanol are considerable, and this fact underscores the prospect of using this plant as a natural source of urease inhibitors. These findings provide a foundation of future research on *C. canadensis* as a potential therapeutic agent in the treatment of *H. pylori* infections and other urease-related gastrointestinal diseases.

4.0 CONCLUSION

In conclusion, *Conyza canadensis* showed a high urease inhibitory activity, particularly in butanol and methanol fractions. Such fractions can be potential sources of natural urease inhibitors and can potentially be used in the treatment of Helicobacter pylori infection and other gastrointestinal diseases. Further research must be done to isolate the active compounds and identify their therapeutic potential and safety to be applied to clinical practice

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CONFLICT OF INTEREST

"The authors declare no conflict of interest

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