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Antiulcer Effect of Extract/Fractions of *Eruca sativa*: Attenuation of Urease Activity

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Abstract

Eruca sativa (Rocket salad) is known for its antiulcer properties in the traditional system of treatment. The present study was, therefore, designed to scrutinize its effect on urease activity in vitro. The results demonstrated marked attenuation of urease by the crude extract of various test concentrations with IC_{50} value of 7.77 mg/mL. On fractionation, marked change in inhibitory profile was observed. The ethyl acetate fraction was the most potent urease inhibitor with IC_{50} value of 4.17 mg/mL followed by the aqueous fraction with an IC_{50} value of 5.83 mg/mL. However, hexane did not show significant urease inhibition. In conclusion, the present study illustrated strong antagonism of urease activity and thus validated scientifically the traditional use of the plant in the treatment of ulcers.

Keywords

Eruca sativa, extract/fractions, urease inhibition

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Introduction

Eruca sativa generally known as "Taramira" or "Rocket salad" is diploid herbaceous plant that grows annually up to 80 cm and is native to Turkey, Eastern Central Europe, Iberian Peninsula, France, Switzerland, the Apennine Peninsula, Hungary, the Balkan Peninsula, the Caucasus, the Eastern Mediterranean, Afghanistan, Northwest Africa, and Libya.[1] This plant has been reported for various biological activities such as antiscorbutic, aphrodisiac, increase urination (diuretic), relief in stomach pain (stomachic), and stimulant.[2,3] It is also used as a carminative and to alleviate abdominal discomfort and improve digestion. Rocket salad also showed potent antioxidant, renal protective, and antigastric ulcer activities.[4]

Phytochemical study on rocket leaves and seeds showed the presence of glucosinolates.[5] The preliminary phytochemical screening of rocket salad revealed the presence of flavonoids, sterols, and/or triterpenes. From the leaves of *Eruca sativa*, the literature also revealed the isolation and identification 3 new quercetins. It also contains vitamins such as vitamin C, carotenoids, and polyphenols.[6]

Being used as an antiulcer, the present study was designed to investigate the role of urease inhibition of crude extract/fractions of *Eruca sativa* in an in vitro experimental model.

Materials and Methods

Plant Materials

Fresh plants of *Eruca sativa* were purchased from the local market in 2011. After collection, a plant taxonomist at the Department of Plant Sciences, KUST, Pakistan, determined the taxonomic identities of the desired plants.

Extract Preparation

Air-dried and coarsely powdered plants were extracted 3 times with methanol. The methanol extracts were evaporated under reduced pressure to give a dark-greenish residue (extract), which was further suspended in water and partitioned successively with *n*-hexane, chloroform, and ethyl acetate to obtain *n*-hexane soluble, chloroform-soluble, ethyl acetate-soluble, and aqueous fractions, respectively. The crude plant extracts and subsequent solvent-soluble fractions were then dissolved in dimethyl sulfoxide individually and stored in refrigerator at 4°C for future use.

Chemicals Used

Urea (Sigma-Aldrich), sodium nitroprusside, phenol red (BDH Chemicals Ltd, England), thiourea, sodium dihydrogen phosphate (Merck, Germany), urease Jack Beans (Avonchem Ltd, UK), sodium hypochlorite (HC Haq Chemicals, Pakistan), dimethyl sulfoxide (UNI-Chem).

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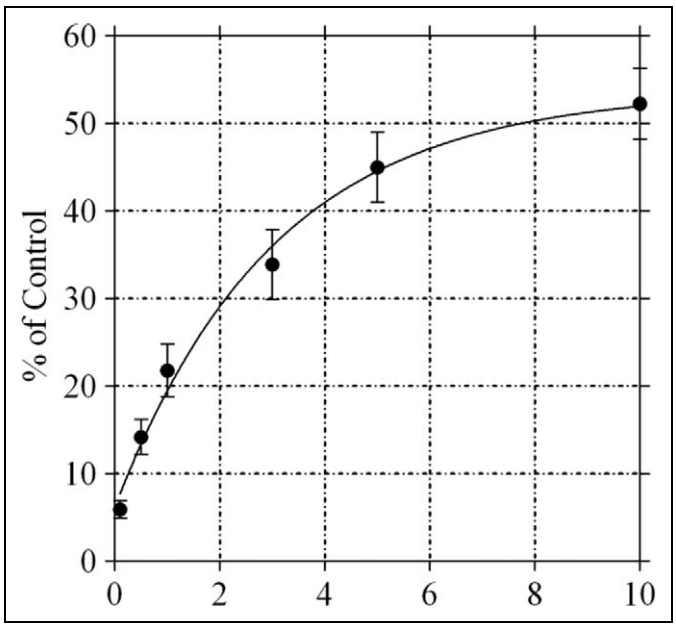

Figure 1. The percent effect of the crude extract of *Eruca sativa* against urease.
The data shown are mean ± SEM of 3 independent assays. One-way analysis of variance was carried out for the determination of difference between groups. $P < 0.05$ was considered as significant.

In Vitro Urease Assay

Twenty-five microliters of enzyme (Jack Bean Urease) solution and 5 µL of test articles (0.5 mM concentration) were incubated for 15 minutes at 30°C.[7] The aliquot was taken after 15 minutes and again incubated with 55 µL of buffer containing 100 mM urea for 15 minutes at 30°C. Ammonia production was measured as a urease activity by the indophenol method as described earlier.[8] Final volumes were maintained as 200 µL by adding 45 µL phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprussside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl). The increase in absorbance was measured at 630 nm after 50 minutes at pH 8.2. The results (change in absorbance per minute) were calculated spectrometrically on different concentrations of drugs. Thiourea was used as the standard inhibitor, and percentage inhibitions were calculated as follows:

$$\% \text{ Inhibition} = 100 - (OD_{testwell}/OD_{control}) \times 100$$

The IC_{50} values were calculated using the statistical software GraphPad PRISM 6.

Statistical Analysis

The resulting data were expressed as the mean ± SEM (n = 3) in each group. To determine the differences between groups, one-way analysis of variance was performed (GraphPad PRISM 6, San Diego, CA) using the least significant difference test at $P < .5$ or $P < .01$.

Results

Urease Inhibitory Effect of Crude Extract of Eruca sativa

The urease inhibitory effect of the crude extract of various concentrations of *Eruca sativa* is illustrated in Figure 1. It had marked attenuation of urease in a concentration-dependent manner. Maximum inhibitory effect (52.70%) was observed at a dose of 10 mg/mL. The estimated IC_{50} value was 7.77 mg/mL (Table 1).

Table 1. The Estimated IC_{50} Values of Extract/Fractions of *Eruca sativa* Against Urease[a].

Extract/Fractions	IC_{50} (mg/mL)
Crude methanolic extract	7.77 ± 0.11
Hexane fraction	N/A
Chloroform fraction	6.37 ± 0.12
Ethyl acetate fraction	4.17 ± 0.67
Aqueous fraction	5.83 ± 0.19

[a] The data are mean ± SEM of 3 independent assays.

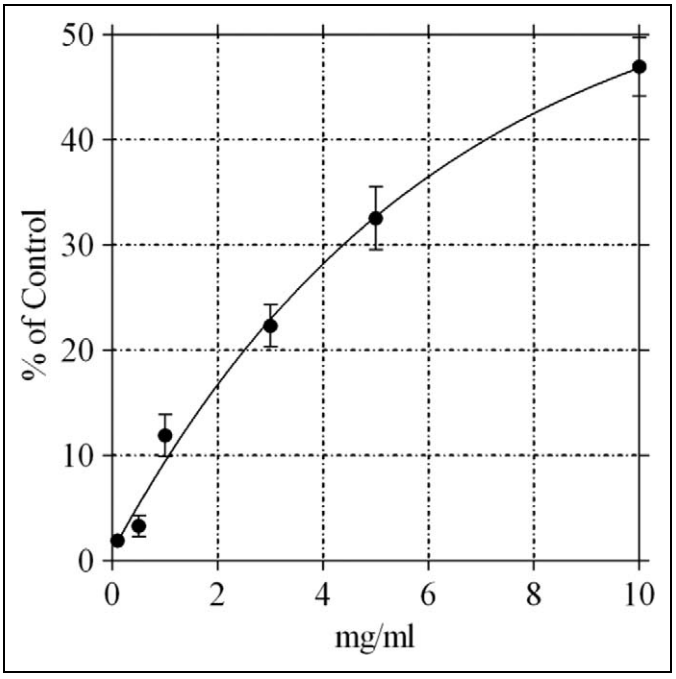

Figure 2. The percent effect of the hexane fraction of *Eruca sativa* against urease.
The data shown are mean ± SEM of 3 independent assays. One-way analysis of variance was carried out for the determination of difference between groups. $P < 0.05$ was considered as significant.

Urease Inhibitory Effect of Hexane Fraction of Eruca sativa

The result of various concentrations of the hexane fraction of *Eruca sativa* against urease is shown in Figure 2. The fraction caused dose-dependent inhibition of the enzyme with maximum activity of 47.10% at 10 mg/mL.

Urease Inhibitory Effect of Chloroform Fraction of Eruca sativa

The effect of various concentrations of the chloroform fraction of *Eruca sativa* against urease is presented in Figure 3. The

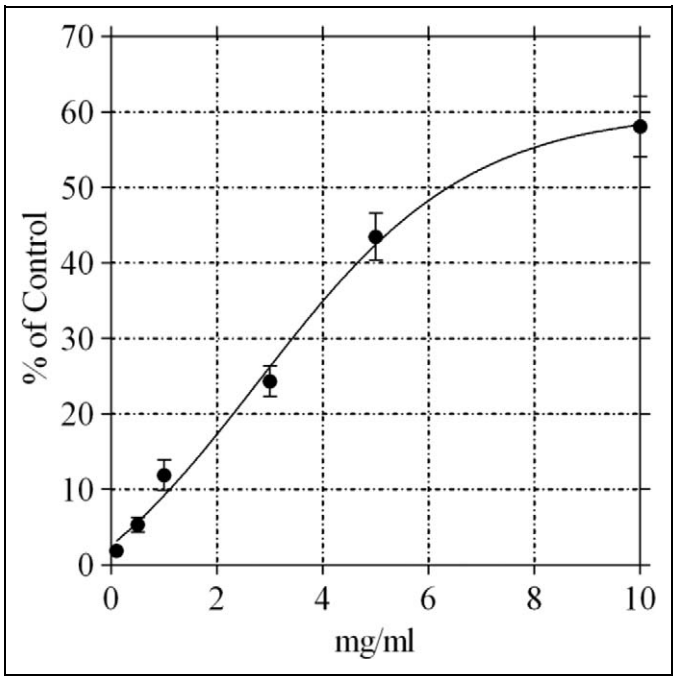

Figure 3. The percent effect of the chloroform fraction of *Eruca sativa* against urease.
The data shown are mean ± SEM of 3 independent assays. One-way analysis of variance was carried out for the determination of difference between groups. $P < 0.05$ was considered as significant.

fraction provoked significant blockade of urease activity at various test concentrations. The maximum effect (58.07%) was produced at a dose of 10 mg/mL. The half-maximum concentration (IC_{50}) was calculated at 6.33 mg/mL (Table 1).

Urease Inhibitory Effect of Ethyl Acetate Fraction of Eruca sativa

Figure 4 shows the results of urease inhibition of various concentrations of the ethyl acetate fraction of *Eruca sativa*. It demonstrated marked attenuation of urease activity at test concentrations with maximum action of 69.56% at 10 mg/mL. The half-maximum concentration (IC_{50}) for the ethyl acetate fraction was 4.17 mg/mL (Table 1).

Urease Inhibitory Effect of Aqueous Fraction of Eruca sativa

When the aqueous fraction of *Eruca sativa* was studied at various concentrations, it displayed profound inhibition on urease as shown in Figure 5. The effect was in a concentration-dependent manner with maximum effect of 59.44% at 10 mg/mL. The calculated half-maximum concentration (IC_{50}) for the aqueous fraction was 5.83 mg/mL (Table 1). The standard test drug thiourea showed most dominant effect, that is, 91.05% inhibition at 100 µg/mL (Figure 6).

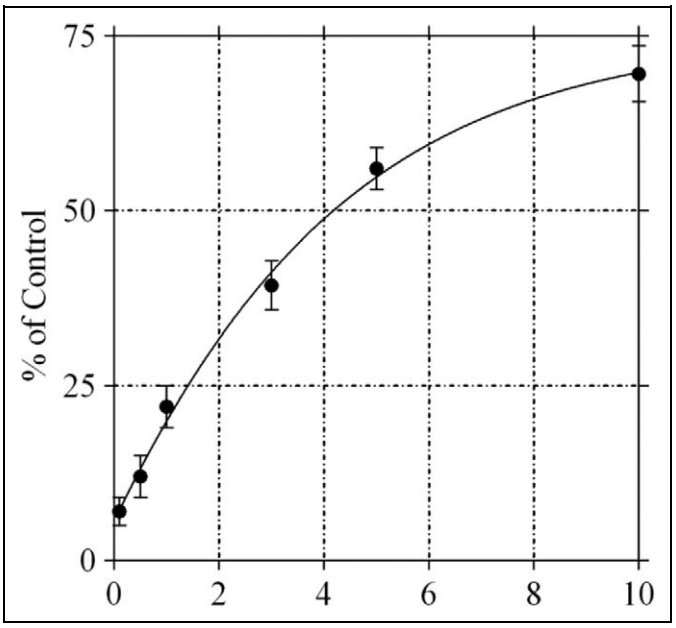

Figure 4. The percent effect of the ethyl acetate fraction of *Eruca sativa* against urease.
The data shown are mean ± SEM of 3 independent assays. One-way analysis of variance was carried out for the determination of difference between groups. $P < 0.05$ was considered as significant.

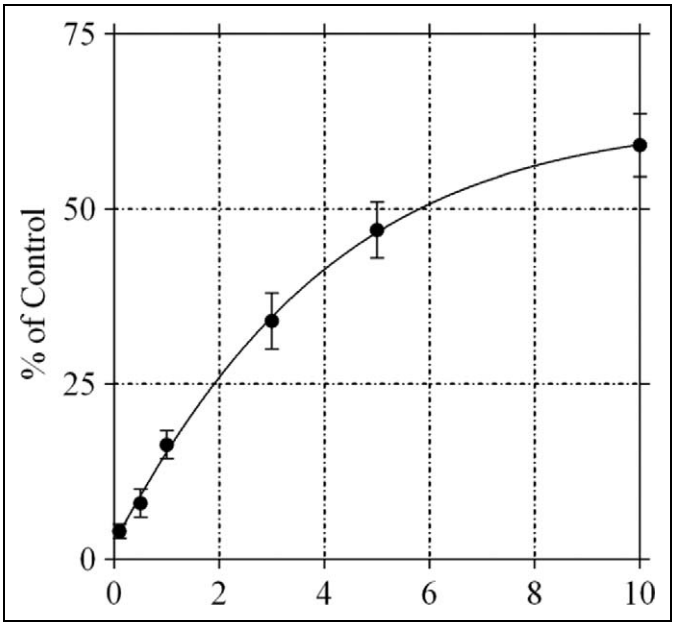

Figure 5. The percent effect of the aqueous fraction of *Eruca sativa* against urease.
The data shown are mean ± SEM of 3 independent assays. One-way analysis of variance was carried out for the determination of difference between groups. $P < 0.05$ was considered as significant.

Discussion

Urease (urea amidohydrolase) is usually found in different bacteria, fungi, algae, and plants. It is responsible for the hydrolysis of urea to ammonia and carbamate, which is the final

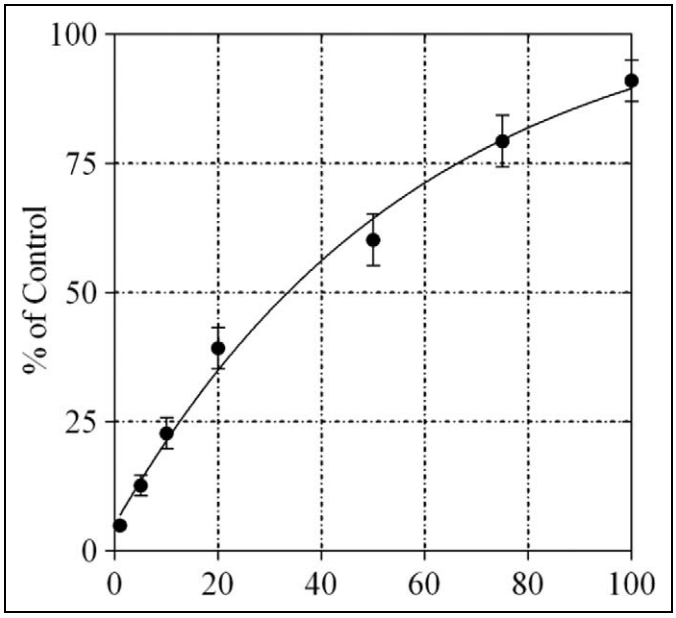

Figure 6. The percent effect of thiourea against urease.
The data shown are mean ± SEM of 3 independent assays. One-way analysis of variance was carried out for the determination of difference between groups. $P < 0.05$ was considered as significant.

step of nitrogen metabolism in living organisms.[9] The carbamate in turn quickly and spontaneously decomposes, yielding a second molecule of ammonia. These reactions may cause significant increase in pH and are therefore responsible for negative effects of urease activity in human health and agriculture.[10,11]

The experimental findings suggested that infections produced by bacteria such as *Helicobacter pylori* and *Proteus mirabilis* usually have a high urease activity. Urease is central to *Helicobacter pylori* metabolism and virulence, necessary for its colonization in gastric mucosa.[12] It is a potent immunogen that elicits a strong immune response. Urease represents an interesting model for metalloenzyme studies. Before the discovery of *Helicobacter pylori*, urease production was limited to human physiology. But now the contribution of this bacterium in urease production is well established. It contributes in urinary tract and gastrointestinal infections, probably augmenting the severity of several pathological conditions like peptic ulcers and stomach cancer. Ureases are also involved in the development of different human and animal pathogenicity such as urolithiasis, pyelonephritis, hepatic encephalopathy, hepatic coma, and urinary catheter encrustation.[13,14] Overproduction of urease is also contributing to environmental hazards.

As one of the key agents in the pathophysiology of multiple human and animal disorders, targeting urease for treating pathogenic disorders caused by urease-producing bacteria has already opened a new line of treatment for infections caused by such bacteria. In reality, more effective and potent compounds are mandatory with a complete new level of safety and specificity. Urease inhibitors for this purpose have gained incredible attention in recent times and have resulted in the discovery of numerous inhibitors.[7,14-16]

The results of our study showed profound inhibition of urease (Jack Bean) by the extract and various subsequent fractions of *Eruca sativa* when studied at different concentrations. The crude extract evoked marked inhibition against urease, which on fractionation produced prominent changed in activity. The most dominant fraction was ethyl acetate followed by the aqueous extract, but the hexane fraction did not exhibit significant inhibition on urease. However, further details on the isolation of the active constituents from the plant can explain the real chemical background of the current study.

In summary, the crude extract and fractions of *Eruca sativa* illustrated profound attenuation of urease in vitro and thus the study provided scientific evidence for the traditional uses of the plant in the treatment of ulcers. However, isolation and characterization of secondary metabolites from the plant is most warranted to know the chemical background of this activity.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

This study does not require ethical approval.

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