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Analysis of urease inhibitory activity of different extracts of *Rhododendron arboreum* Sm. and *Rhododendron campanulatum* D. Don

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Abstract
Urease responsible for the rapid hydrolysis of urea to ammonia & CO_2 plays crucial role in the persistent habitation of *Helicobacter pylori* which causes various gastrointestinal diseases such as gastritis, duodenal, peptic ulcer, gastric cancer, pyelonephritis etc. Plant-based natural products are therefore being utilised to cure disorders induced by urease enzyme. In the present investigation, inhibitory effects of different extracts prepared in different solvents (methanol, acetone & aqueous) of *Rhododendron arboreum* and *R. campanulatum* were examined against jack bean urease at a concentration range of 0.2-1.0 mg/mL. *R. arboreum* showed 61.10±0.33, 44.20±0.50 and 45.70±0.50% inhibition at a concentration of 1 mg/mL for methanol, acetone and aqueous leaf extracts respectively. On the other hand, *R. campanulatum* exhibited 51.45±2.30, 38.05±1.50 and 32.80±0.45% α-amylase inhibition for methanol, acetone and aqueous extracts respectively at 1 mg/mL. The inhibitory potential/activity increased with increasing concentration of each plant extract. The results further revealed that the methanol extracts of both the plants exhibited maximum inhibitory effects than other solvent extracts. This tends to show that the active metabolites or phyto-constituents of the different plant parts are better extracted with methanol than in other solvents. Therefore, the present study approves the medicinal value of these plants and scientifically validates them for use as a component of medicinal preparations for curing diseases associated with urease enzyme.

Keywords: *Rhododendron arboreum*, *R. campanulatum*, Jack bean urease, leaf extract, inhibition

Introduction
Urease (urea amidohydrolase: EC 3.5.1.5) is a ubiquitous enzyme which is wide spread in nature, being present in multiple forms of life ranging from bacteria to plants and animals (Karplus *et al*., 1997) [1]. Urease, the first enzyme crystallized or extracted from Jack bean (*Canavalia ensiformis*) and known to contain nickel ions which rapidly catalyse the hydrolysis of urea to ammonia and carbon dioxide, has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions which are detrimental for human and animal health and have adverse effects on agriculture (Michetti, 1998) [2]. The reaction catalysed by urease is as follows:

$$(NH)_2CO + H_2O \rightarrow CO_2 + 2\ NH_3$$

The product, ammonia, of such decomposing reactions diffuses across the cytoplasmic membrane, buffering the periplasmic space and thereby allows growth in the presence of extracellular gastric acid (Sachs *et al*., 2002) [3] and is responsible for negative effects of this enzyme activity on human health (Mobley *et al*., 1995) [4] such as causing peptic ulcers, stomach cancer etc. Formation of urinary struvite stones in urinary tract is mainly associated with urea-splitting bacterium i.e. *Ureaplasme urealyticum. Helicobacter pylori* whole cell stimulates an oxidative burst in human neutrophils (Suzuki *et al*., 1992) [5]. Hydrogen peroxide (H_2O_2) formed from the oxidative burst oxidizes chloride ions which react with ammonia liberated by *H. pylori* urease to give the highly toxic product monochloramine (Mai *et al*., 1991) [6]. It is also found that by increasing ammonia level in the body some neurological disorders are also arisen leading to Parkinson´s disease (Amtul *et al*., 2002) [7]. Moreover, *H. pylori* infection is also suspected to be associated with cardiac disorders such as coronary artery and ischemic heart diseases (Mendall *et al*., 1994; Tougas *et al*., 1999) [8, 9].

The medicinal plants are being widely used for their therapeutic potential in controlling various disorders or ailments caused by urease enzyme. Scientists all over the world are unifying traditional knowledge with experimental methodology for evaluating the efficacy and safety of herbal preparations (Ahmad *et al*., 2014) [10]. The rural population of India, like most developing countries, mostly relies on valuable heritage of medicinal plants. It is therefore of high interest to find out the possible reasons for efficacy of indigenous medicinal plant products which are commonly used by local population or traditional practitioners (Irfan *et al*., 2019) [11]. Therefore, the present study on urease inhibition of *Rhododendron arboreum* and *R. campanulatum* collected from Himachal Pradesh was undertaken. *Rhododendron* plants are traditionally used to treat numerous human ailments like blood dysentery, headache, asthma, cough, stomach ache, fever, inflammation, fungal infections etc (Prakash, 2021; Liu *et al*., 2022; Sharma *et al*., 2022) [12, 13, 14]. Jack bean urease enzyme was used in the study because it shares more than 50% similarity with the bacterial urease and also was found that the mechanism of action and the kinetics of inhibition for bacterial urease and jack bean urease are almost similar (Ciurli *et al*., 1999) [15].

Materials and Methods
Collection and processing of plant material
Leaves of *Rhododendron arboreum* and *R. campanulatum* were plucked and collected from Churdhar Wildlife

Sanctuary area of District Sirmaur, Himachal Pradesh, India. For further analysis, the collected plant material was brought to the laboratory. Leaves of both the plants were washed thoroughly under tap water and then with 2% Mercuric chloride. After this, the leaves were cut into smaller pieces for quick drying. The dried plant material was crushed into fine powder with the help of pestle mortar & finally the fine powder was stored in an air tight container at room temperature.

Urease inhibition assay

The enzyme inhibition was determined through catalytic effects of urease on urea by measuring change in absorbance in the absence as well as presence of inhibitor at 640 nm by using UV-VIS spectrophotometer. All the plant extracts (methanol, acetone and aqueous) were tested for their urease inhibitory activity at a concentration of 1.0 mg/mL. The herbal extracts that exerted significant inhibition were tested in a concentration range of 0.2 to 1.0 mg/mL (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL). For urease inhibition assay, after addition of 10 mL of phosphate buffer to accurate weight of enzyme, sonication was performed for about 60 seconds followed by centrifugation and absorbance of upper solution was measured at 280 nm. By applying equation A= εbc, where c is concentration of solution (mol/L), b is length of the UV cell and ε represents molar absorptivity, the concentration of initial urease solution was calculated. After proper dilution, the concentration of enzyme solution was adjusted to 2 mg/mL. Reaction mixture containing 1.2 mL of phosphate buffer solution (10 Mm potassium phosphate, 10 Mm lithium chloride and 1 Mm EDTA, pH 8.2 at 37ºC), 0.2 mL of urease enzyme solution and 0.1 mL of the test compound was subjected to incubation for 5 minutes. After pre-incubation, 0.5 mL (66 Mm) of urea was added to the reaction mixture and incubated for around 20 minutes. Urease activity was determined by measuring the ammonia released during the reaction by modified spectrophotometric method as described by by the method of Weatherburn, 1967 [16]. Briefly, 1 mL each of phenol reagent (1% w/v sodium nitroprusside) and an alkaline reagent (1% w/v NaOH and 0.075% active chloride NaOCl) were added to all the test tubes. The control contained all the reagents except the sample. The increase in absorbance at 640 nm was measured after 30 minutes and the percent inhibition was determined using the formula:

$$\% \text{ Urease Inhibition} = \left(\frac{Ac - As}{Ac}\right) \times 100$$

Here A_s is the absorbance of the sample under study whereas A_c is the absorbance of the control. Each experiment was repeated thrice and average was thus calculated. Thiourea was used as a positive control. Data were expressed as mean ± standard deviation (SD). IC_{50} values were also determined from the dose response curves.

Results and Discussions

In the present study, leaf extracts (methanol, acetone and aqueous) of *Rhododendron arboreum* and *R. campanulatum* were tested for their enzyme inhibitory activity against jack bean urease and it was observed that the plant extracts showed concentration dependent inhibition of urease enzyme as shown in Table 1 & 2 and in Figure 1. In case of *Rhododendron arboreum*, the inhibitory activity of urease at a concentration of 1 mg/mL was 61.10±0.33, 44.20±0.50 and 45.70±0.50% for methanol, acetone and aqueous extract respectively. On the other hand, *R. campanulatum* showed 51.45±2.30, 38.05±1.50 and 32.80±0.45% inhibition against urease enzyme at a concentration of 1 mg/mL. The inhibitory activity increased with increasing the concentration of each plant extract in the range of 0.2-1.0 mg/mL. The results further indicated that methanol extracts exerted maximum inhibitory effects than other solvent extracts. This tends to show that the active metabolites of the different plant part/s are better extracted with methanol than other solvents. As per literature survey, there is no previous clear report found on urease inhibitory activity of *Rhododendron arboreum* and *R. campanulatum*.

Table 1: Urease Inhibitory activity (%) of *Rhododendron arboreum* extracts at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Thiourea
0.2	20.22±1.25	15.20±0.35	13.33±0.25	28.38±0.78
0.4	29.95±0.55	23.00±0.00	20.50±1.45	41.58±0.55
0.6	36.05±0.75	30.00±0.00	28.30±0.00	56.30±1.20
0.8	48.60±2.15	36.65±0.25	36.00±2.00	69.20±0.50
1.0	61.10±0.33	44.20±0.50	45.70±0.50	81.26±1.25
IC_{50} (µg/mL)	0.81	1.16	1.12	0.51

Values are given as mean ± SD

Table 2: Urease Inhibitory activity (%) of *Rhododendron campanulatum* extracts at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Thiourea
0.2	16.42±0.26	15.90±1.40	11.70±0.60	28.38±0.78
0.4	24.47±1.20	21.45±1.25	14.05±3.00	41.58±0.55
0.6	33.60±2.00	28.10±0.05	20.50±0.30	56.30±1.20
0.8	42.00±0.10	34.42±1.10	26.30±0.22	69.20±0.50
1.0	51.45±2.30	38.05±1.50	32.80±0.45	81.26±1.25
IC_{50} (µg/mL)	0.97	1.38	1.66	0.51

Values are given as mean ± SD

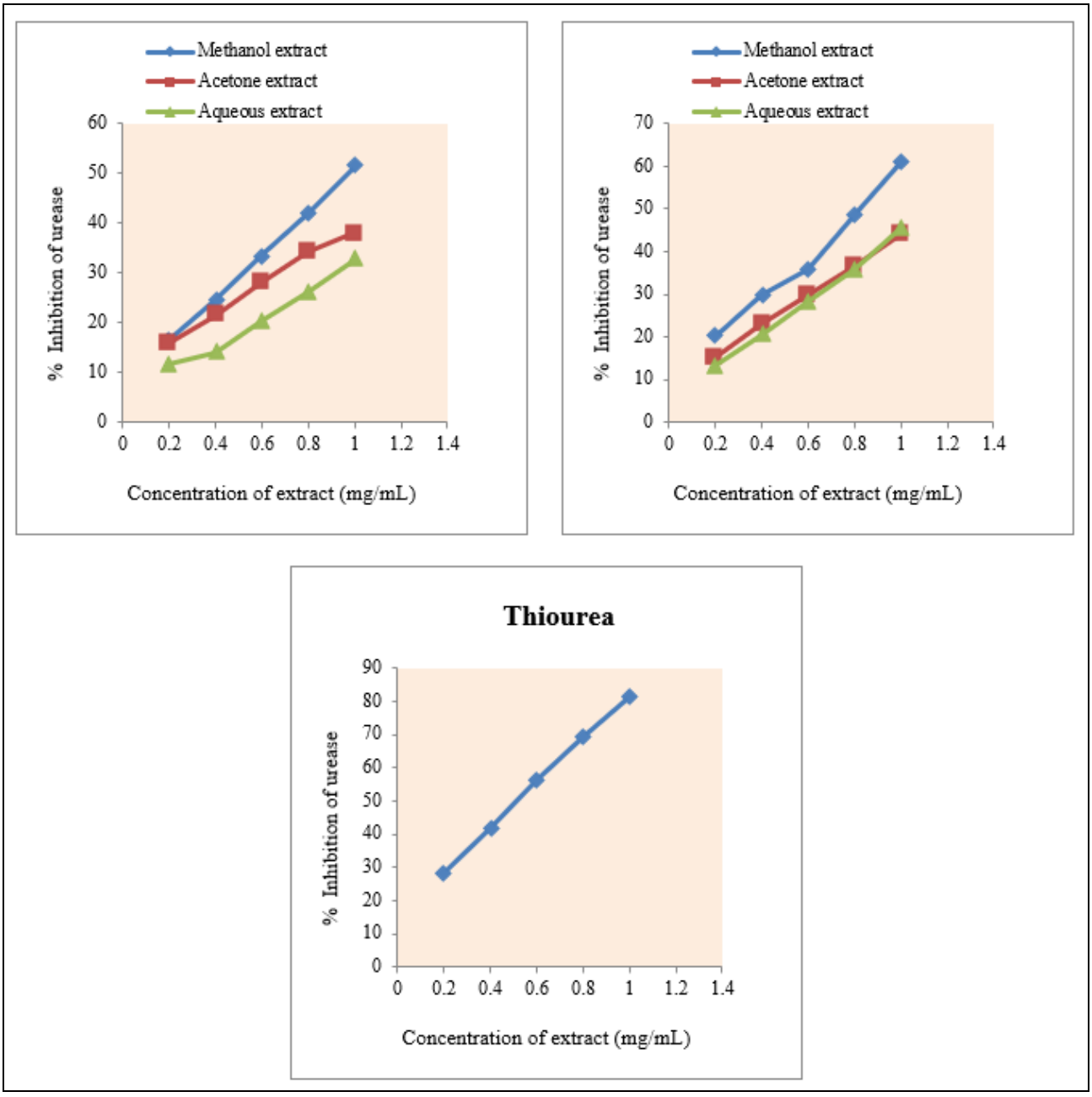

Fig 1: Inhibition profile of different extracts of medicinal plants against Jack-bean urease at concentration range of 0.2-1.0 mg/mL: (A) *Rhododendron arboreum* (B) *Rhododendron campanulatum* (C) Standard curve of Thiourea

Conclusion

As a conclusion, it could be speculated that the results of urease inhibitory studies are encouraging as all the tested leaf extracts (methanol, acetone and aqueous) of *Rhododendron arboreum* and *R. campanulatum* showed significant inhibition. Urease inhibitory activity ranged from 13.33±0.25 to 61.10±0.33% and 11.70±0.60 to 51.45±2.30% suggesting a strong urease inhibitory effects of these plants. Besides this, methanol leaf extracts were found to be more effective compared to acetone and aqueous extracts. Hence it is clear from the results that leaf extracts under study displayed variable enzyme (urease) inhibitory activities thereby confirming their roles in the treatment of various diseases/disorders caused by the malfunctioning of urease enzyme. Further research is needed to find the exact mechanism of action and the chemical constituents which are responsible for its anti-urease activity of *Rhododendron arboreum* and *R. campanulatum*.

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Conflict of Interests

Author hereby declares no conflict of interest.

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