

SHORT COMMUNICATION

Urease inhibitory activity of *Hippophae rhamnoides* and *Cassia fistula*

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Abstract: The rational use of plants as medicine is traced back over five epochs to ancient documents of early civilizations and is certainly as old as mankind. These medicines originally developed from crude drugs like tinctures and tinctures. Minimum 119 chemical substances are derived from 90 plant species and used all over the world as medicines, several of them containing compounds derived from or modelled after naturally occurring lead molecules and 74% of these derived from orthodox medicinal plants. 252 drugs (11%) are believed to be basic and essential by the WHO and are exclusively of plant origin. We have examined anti-urease activity of ethyl alcohol (Et-OH) and methyl alcohol (Me-OH) extracts of *H. rhamnoides* and *Cassia fistula*. Berthelot assay was used for the determination of anti-urease activity. The enzyme activity and inhibition was measured through catalytic effects of urease on urea by measuring change in absorbance in the absence and in the presence of inhibitor at 625nm using UV spectrophotometer. In the study, both Et-OH and Me-OH extracts of *H. rhamnoides* (91.69%±1.21) and *C. fistula* (79.44%±0.55) showed stronger action against urease activity. An overview on the medicinal uses of *H. rhamnoides* and *C. fistula* showing anti-urease activity may predict their possible alternative use for stomach problems. This study may help to explain the beneficial effects of these plants against stomach infection associated with pathogenic strains of *H. pylori* as Urease is the most prominent protein component of *H. pylori*.

Keywords: *H. pylori*, Ulcer, Urease

INTRODUCTION

Enzyme inhibition studies continue a significant area of pharmaceutical research since these studies resulted in the findings of drugs useful in a diversity of physiological conditions. Urease inhibitors have attracted much devotion as prospective new anti-ulcer drugs. Atypically, urease was the first enzyme crystallized but its mechanism of action is still largely mis-understood (Amtul *et al.*, 2002).

Urease is a nickel enzyme produced by plants, fungi, algae, and bacteria. It is involved in nitrogen turnover and in crop fertilisation as well as in human and animal pathologies. It catalyses the hydrolysis of urea giving rise to ammonia and carbon dioxide.

Gastritis and gastro-duodenal ulcers can be caused by infection with *H. pylori*, known to be the main risk factor for the development of stomach cancer and lymphomas. The bacterium was isolated and for the 1st time grown on agar plates. Based on its constant presence in a high percentage of biopsies from patients affected by ulcers they proposed it to be the cause of the disease. To prove this Marshall exposed himself to contamination with a

bacterial culture and got infected. Urease production is the main factor allowing the bacterium to survive in the very acidic environment of the stomach lumen before reaching the mucus layer, a natural protection against the low pH. The main role of urease in *H. pylori* infection is the creation of a “cloud” of ammonia used for bacterial cell protection. *Hippophae rhamnoides* is a source of seed oil, which is much unsaturated and valuable, because of its light absorption and emollient properties, as an ingredient in cosmetics, phyto-pharmaceuticals. *Hippophae rhamnoides* is used in cancer therapy, cardiovascular therapy, liver diseases, skin diseases, gastrointestinal disorders (Sumner, 1996). *Cassia fistula* contains tannins, fatty acids isoflavonoids, flavonoids, glycosides, anthraquinones, and phenolic compounds (Dixon *et al.*, 1975). *Cassia fistula* possesses medicinal properties useful in the treatment of skin diseases, inflammatory diseases, rheumatism, anorexia and jaundice (Alper, 1993). *Cassia fistula* and *Hippophae rhamnoides* are used extensively in folk medicines; however, they are not explored for anti-urease activities scientifically.

MATERIALS AND METHODS

Jack bean urease (Shanghai Ruji Biology Technology Co., Ltd.), Thiourea (Dalian Meilun Biotech Co., Ltd.),

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Indophenol, Sodium hydroxide, NaOCl 0.1%, Phenol, Sodium, nitroprusside. (Crescent urea diagnostic kit was purchased from Dia Sys).

Plants collection

Hippophae rhamnoides berries were purchased from Pak Sea Buckthorn International Skardu, Pakistan. *Cassia fistulas* pods were collected from the old campus. The identification of plants was performed at Chulistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan.

Preparation of plant extracts

Cassia fistula leaves: 150Gms shade-dried ground plant material for each sample was extracted with 70% methanol for 72 hours at room temperature in a 5liter beaker. The residues were extracted twice with the same fresh solvent and extracts combined.

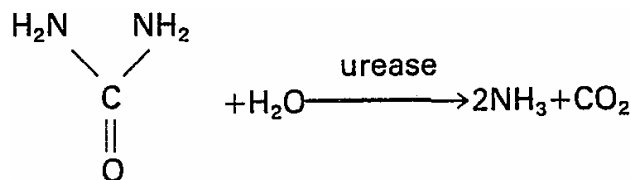


Fig. 1: Enzymatic conversion of urea to ammonia by urease

Hippophae rhamnoides: *Hippophae rhamnoides* berries were grinded and consecutively extracted. 320 Gms were macerated by a mixture of 2litres of analytical grade Methanol and Distilled water in 1:1 ratio in a Glass beaker. The macerated plant material was filtered through 08 layers of muslin cloth for coarse filtration and then through filter. The filtrate was evaporated under reduced pressure at 40°C in a Rotary vacuum evaporator.

Enzyme assay

Berthelot assay with slight modification was used for the enzyme assay. It is briefly discussed here. 85µL of assay mixture with 10µL of phosphate buffer (pH 7.0) was taken in each well of the 96-well plate. It was trailed by the totting 10 µL of sample solution and 25µL of enzyme solution. This mixture was then pre-incubated at a temperature of 37°C for 05 min. After this, 40µL of urea standard solution (20mM) was supplemented to each well and incubation was continued at 37°C for additional 10 minutes. After 10 min, 115µL of phenol hypochlorite reagents was incorporated in each well (This was prepared freshly by mixing 45µL phenol reagent with 70 µL of alkali reagent). Further incubation was done at 37°C

for another 10min for color development. After that, absorbance was taken at 625 nm using the 96-well plate reader Synergy HT [4]. The following formula was used to calculate the percentage enzyme inhibition

$$\% \text{age Inhibition} = 100 - (\text{Absorbance of test sample} / \text{Absorbance of control}) \times 100$$

Ez-Fit enzyme kinetics software was used for IC₅₀ values (concentration at which 50% enzyme catalysed reaction occurs) calculation.

RESULTS

Ethyl alcoholic (Et-OH) and methyl alcoholic (Me-OH) extracts of *H. rhamnoides* and *Cassia fistula* were assayed for the determination of anti-urease activity by Berthelot assay. Both plants showed stronger action against urease activity. The IC₅₀ value of each extract was calculated from straight-line equation given in table 1 and % age inhibition values are given in table 2.

Table 1: The slope and r² of % inhibition equation

Plant name	Equation for alcoholic extracts	
	Slope	r ²
<i>C. fistula</i>	0.5844	0.8564
<i>H. rhamnoides</i>	0.3654	0.9269

DISCUSSION

There is considerable interest in alternative approaches for the eradication of *Helicobacter pylori* using biologically active compounds including antioxidants from a wide range of natural sources. The urease inhibition activity of the hydroxy acid isomers in this study can be attributed to the complex building ability of the hydroxy acid isomers with nickel active centre of the urease. The presence of -OH and -COOH group of mono-hydroxyeicosanoic acid isomers in the plant extracts may play together a great role in the inhibition of urease activity (Weatherburn, 1976; Torres & Drumm, 2000).

An overview on the medicinal uses of the plants showing anti-urease activity is given here which may predict their potential folk use for GIT disorders. *C. fistula* is most abundant throughout Pakistan. Its pods are especially useful in spasm and constipation (Kattak *et al.*, 1986). *H. rhamnoides* is a popular plant, widely distributed throughout the Baltistan, Northern areas of Pakistan, having carminative and digestive properties.

Table 2: %age inhibition of plant extracts in comparison to standard (Avg±SEM)

S. No	Sample	Concentration (mg/ml)	Inhibition Et-OH (%)	Inhibition Me-OH (%)
1	<i>H. rhamnoides</i>	0.05	91.69±1.21	93.08±0.88
2	<i>C. fistula</i>	0.05	79.44±0.55	78.94±0.76
3	Thiourea	0.01	98.60±0.51	94.90±0.91

CONCLUSION

Helicobacter pylori are the primary causative agent of gastric ulcer and related gastro-duodenal disorders. Current triple-regimen therapy of two antibiotics and one proton-pump inhibitors has been effective; however, adverse effects, patient non-compliance and subsequent relapse of *Helicobacter pylori* infections are common. The present findings provide a scientific basis for the folk use of *C. fistula* and *H. rhomboids* in GIT disorders. The anti-urease activity of the extracts of these plants may be associated to inhibit *H. pylori*, a key cause of stomach infections. The study is a step towards developing new pharmaceuticals for *Helicobacter pylori* infections.

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