



Original Article

In Vitro Urease Inhibitory Activity of Four Selected Medicinal Plant Extracts

Shaik liakhat Ali *, Shaik Mabunni, N Mounica, P Kuldeep, T Jeevan Kumar
Nirmala College of Pharmacy, Mangalagiri, Guntur, Andhra Pradesh, India

ARTICLE INFO

A B S T R A C T

Received: 05 Oct 2015
Accepted: 29 Dec 2015

Aim: To study the jack bean urease inhibitory property of *Abelmoschus Esculentus* {Ethanolic Extract of Seeds}, *Schleichera Oleosa*{Ethanolic Extract of Bark Powder}, *Achyranthus Aspera*{Ethanolic Extract of Leaves}, *Murraya Paniculeta*(leaves Powder). **Method:** Urease inhibition activity assay: 100microlitre(2mg/ml jackbean urease)+100 microlitres of test compound+0.2ml of 100millimolar phosphate buffer and pH 6.8 urea containing 25 millimolar, Incubation for 30min in water bath at 37°C, 600microlitres of, 4% sulphuric acid+500microlitres of solution A and solution B, Incubation for 30min in water bath at 37°C, absorbance at 625nm in UV-Visible spectrophotometer. **Results:** Ethanolic extract of fruit of *Abelmoschus esculentus* shows % inhibition of 173.15, 110.26 and Ethanolic extract of leaves of *Achyranthes aspera* shows %inhibition of 101.57. shows significant activity. **Conclusion:**In the preliminary jack bean urease inhibitory studies using “Berthelot alkaline phenol-hypochlorite method. *Abelmoschus esculentus*, *achyranthes aspera* has shown a very potential urease inhibitory property compared to other drugs and extracts. For further conformation this study should be carry out in depth to establish mechanism of action of urease inhibition by using *in vivo* and *in vitro* methods. This study helps in developing anti-H-pylori drugs which can be adjuvant to the anti-ulcer regimen.

key words: jack bean urease, medicinal plants, ulcer, H.pylori.

1. INTRODUCTION¹⁻³

Gastric and peptic ulcer are the leading diseases of hospitalization in urban and rural areas across the world, which are characterized by epigastric pain, bleeding, erosion of mucus membrane, heart burning, etc... because of imbalance of protective mechanisms and aggressive mechanisms of stomach, uncontrolled acid release and which will be exacerbated by

Corresponding author *

Shaik Liakhat Ali
Nirmala college of pharmacy, Atmakur
Guntur, Andhra pradesh
Email: liakhat761@gmail.com

Helicobacter pylori- a bacteria which erodes mucous layer of stomach, decreases protective mechanism and causes damage to the underlying layers of stomach. now a days *H.pylori* infected ulcers are more due to poor hygiene in developing countries. *H.pylori*, to survive in acidic environment, it produces and releases an enzyme called Urease. Urease enzyme helps in maintaining a basic environment around the bacterial outer membrane to get rid of acid present in stomach by converting urea, an excreta of bacteria itself, into ammonia. Many antibacterial agents in use to kill the *H.pylori* but they affects microbial flora and may develop resistance and may cause side effects. To eliminate these disadvantages, we conducted a research to inhibit the enzyme Urease.

Most of the plants available to mankind are capable of curing many diseases and disorders, here we worked on four plants which are having several other pharmacological activities for their urease inhibitory property. *Abelmoschus Esculentus* {Ethanollic Extract of Seeds}, *Schleichera Oleosa*{Ethanollic Extract of Bark Powder}, *Achyranthus Aspera*{Ethanollic Extract of Leaves}, *Murraya Paniculeta*(leaves Powder).

urease enzyme inhibition will decrease the survival of *H.pylori* due to absence of ammonia, so that these plants will help in the treatment of ulcer as adjunctive drugs along with proton pump inhibitors and other anti ulcer agents.

2. MATERIALS AND METHODS^{4,5}

Four Medicinal plants which are listed above are taken from the college premises. Each plant sample was individually powdered and 1gm was extracted by maceration method using aqueous ethanol as solvent for 24hrs. . Each extract was filtered, concentrated under reduced pressure to dryness and stored at 0°C until time of analysis. The percentage of inhibition at different concentration of extracts, dissolved in same solvent was accurately defined.

For urease inhibition assays after addition of 10 mL of phosphate buffer to accurately weight of enzyme, sonication was performed for 60s, followed by centrifugation and evaluating absorbance of upper solution in $\lambda = 280$ nm which is attributed to enzyme. By using the following equation $A = bc$ where c is the concentration of solution (mol/L), b is the length of the UV cell and ϵ represents molar absorptivity in the specific wavelength, we can calculate the concentration of initially urease solution. After proper dilution, the concentration of enzyme solution adjusts at 2 mg/mL.

The assay mixture, containing 100 μ L (2 mg/mL) of Jack-bean urease and 100 μ L of the test compound with 0.2 mL of 100 mM phosphate buffer pH 6.8 containing 25 mM urea was pre-incubated for 30 min in water bath at 37°C. The urease reaction was stopped after 30 min incubation with 600 μ L of 4% H₂SO₄ acid. This product then reacts with phenol to form blue-colored indophenols whose absorbance is measured at 625 nm.

The liberated ammonia was estimated using 500 μ L of solution A (contained 5.0 g phenol and 25 mg of sodium nitroprusside) and 500 μ L of solution B (contained of 2.5 g sodium hydroxide and 4.2 mL of sodium hypochlorite in 500 mL of distilled water) at 37°C for 30 min and the absorbance was measured at 625 nm against the control. All reactions were performed in triplicate in a final volume of 1 mL.

Percentage of inhibitions were calculated using the formula $(100 - (\text{OD sample} / \text{OD control}) \times 100)$.

SOLUTION:A:5gms of phenol,25mg of sodium nitroprusside.

SOLUTION:B:2.5gms of NaOH,4.2ml of sodium hypochlorite in 500ml water.

PHOSPHATE BUFFER:0.5 Liters of 1M K₂HPO₄ at 174.18gm.mol(for 500ml)

17.4gms of K₂HPO₄

0.5 Liters of 1M KH₂PO₄ at 136.09gm.mol

3. RESULTS

Table 1: Effect of Extracts on Urease Enzyme

Compound name	Test dose	Percentage of inhibition (%)
Abelmoschus esculentum (ethanol extract,fruit)	5 mg/ml	110.26
Abelmoschus esculentum (ethanol extract,fruit)	10 mg/ml	173.15
Schleichena oleosa(ethanol extract,)	5 mg/ml	73.7
Schleichena oleosa(ethanol extract,)	10 mg/ml	78.47
Achyranthes aspera(ethanol extract)	5 mg/ml	97.64
Achyranthes aspera(ethanol extract)	10 mg/ml	101.57
Murraya paniculata	5 mg/ml	74.5
Murraya paniculata	10 mg/ml	47.3

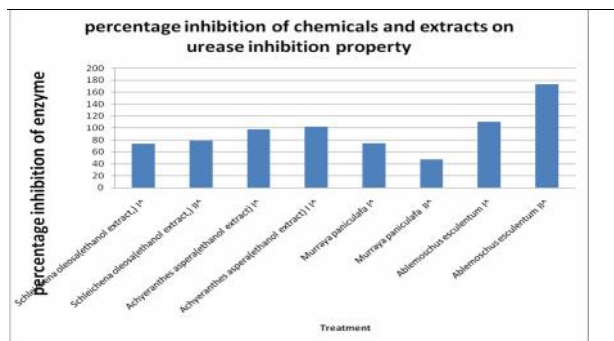


Fig 1: Effect of Extracts on Urease Enzyme As Percentage Inhibition

I[^]= 5 mg/ml

II[^]=10 mg/ml

4. DISCUSSION

As evidence in beneficial effects of medicinal plants traditionally used to manage different disorders, four extracts were examined against jack bean urease by "Berthelot alkaline phenol-hypochlorite method and results revealed inhibitory activities. Ethanolic extract of fruit of *Abelmoschus esculentus* shows % inhibition of 173.15, 110.26 and Ethanolic extract of leaves of *Achyranthes aspera* shows %inhibition of 101.57 and Duloxetine as shown the % inhibition of 185.26. Medicinal plants serve as a useful sources of novel drugs. In developing countries, since the application of antibiotics is still under a poor management as a whole, there is a growing need for finding new medicinal plants especially anti-H pylori agents that can help

eradicate the invasion and presence of survived *H. pylori* strains to avoid relapse of gastric ulcer. In this regard, the literature has reported extracts of certain plants such as cashew apple, cinnamon and Chinese tea inhibit growth of *H.pylori* and some urease inhibitory activity. We assume that the urease inhibitory property of plant extracts may be due to presence of flavanoids as previously reported in other plant extracts.

5. CONCLUSION

In the preliminary jack bean urease inhibitory studies using "Berthelot alkaline phenol- hypochlorite method. *Abelmoschus esculentus*, *achyranthes aspera*, has shown a very potential urease inhibitory property compared to other drugs and extracts. For further conformational this study should be carry out in depth to establish mechanism of action of urease inhibition by using invivo and invitro methods. This study helps in developing anti *H-pylori* drugs which can be adjuvant to the anti-ulcer regimen.

6. REFERENCES

1. U Spandana, Shaik Liakhat Ali, T Nirmala, M Santhi, SD Sipai Babu. A Review on *Tinospora cordifolia*, International Journal of Current Pharmaceutical Review and Research, 4(2): 61-68.
2. Kochlar S.I; okra (lady finger) in tropical crops, a text book of economic botany. 1986; 1: 263-264.
3. Franklin W. Martin. "Okra, Potential Multiple-Purpose Crop for the Temperate Zones and Tropics". Economic Botany, 1982; 36 (3): 340–345.
4. Mahmood Biglari, Khadijeh Soltani, Farzaneh Nabati, Roya Bazl,Faraz Mojab and Massoud Amanlou, A Preliminary Investigation of the Jack-Bean Urease Inhibition by Randomly Selected Traditionally Used Herbal Medicine, Iranian Journal of Pharmaceutical Research (2012), 11 (3): 831-837.

5. Shabnam Mahernia, Kowsar Bagherzadeh Faraz Mojab and Massoud Amanlou, Urease Inhibitory Activities of some Commonly Consumed Herbal Medicines, Iranian Journal of Pharmaceutical Research (2015), 14 (3): 943-947

Conflict of Interest: None

Source of Funding Nil