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In vitro urease inhibition screening of some edible and medicinal herbs to combat *Helicobacter pylori* related gastric diseases

Hina Zahid[1], Bushra Hina[2] and Khizar Abbas[3]*

[1]Department of Pharmacognosy, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[2]Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
[3]Department of Pharmacognosy, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan

Abstract: Prevalence of gastric diseases caused by *Helicobacter Pylori* bacteria is very common especially in developing countries. *H. pylori* is not only responsible for initiating gastric complaints like gastritis and peptic ulcer but may also lead to gastric cancer. The aim of this research study is to explore natural flora that exhibit anti urease potential. For this purpose, fifteen edible and medicinal herbs (*Silybium marianum*, *Fagonia arabica, Nigella sativa, Curcuma longa, Ocimum sanctum, Salvia rosmainus, Hyssopus officinalis, Antrirrhinum majus, Salvia splendens, Tropaeolum majus, Dalbergia sisso, Aloe barbadensis, Abelmoschus esculentus*, *Cuscuta reflexa, Hibiscus schizopetalus)* were screened for anti-urease activity at three different concentration i.e, 25µg/ml, 50µg/ml and 75µg/ml. The results indicated significant outcomes for urease inhibitory activity for all tested medicinal plants. However, *F. arabica* (87.2±1.47)*, N. sativa* (90.4±0.09)*, O. sanctum* (75.6±0.95)*, H. officinalis* (78.9±0.69)*, T. maju* (87.3±0.14)*, A. esculentus* (90.3±0.86), *C. reflexa* (94.1±0.92) showed significant results at 75µg/ml when compared to Thiourea. Moreover IC_{50} values were also calculated for urease inhibitory activity. It can be concluded that utilization of these valuable medicinal plants can not only decrease the prevalence of gastric diseases caused by *H. pylori* bacteria but a good candidate for therapeutic purposes.

Keywords: Herbs, GIT diseases, peptic ulcer, stomach cancer, urease inhibitory activity.

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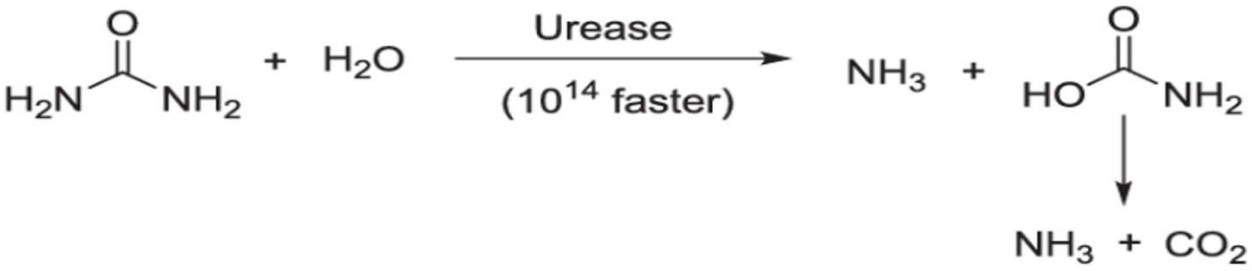
INTRODUCTION

Prevalence of gastric diseases caused by *H. pylori* bacteria is very common worldwide. Its manifestations start from gastritis, peptic ulcer and may lead to stomach cancers. According to World Gastroenterology Association (WGA) Helicobacter pylori has been a major disease-causing human pathogen for humanity since last four decades. *H. pylori* infection is a key reason of morbidity and mortality infecting about half of the world's population via spread of peptic ulcer diseases and gastric cancer. It is very challenging situation for health care professionals and researchers to prevent, treat and control the spread of diseases caused by *H. pylori* due to difference in spectrum of diseases as well as wide worldwide variation of suffering population (WGO, 2021; Chan and Lau, 2021). *H. pylori* infection is related to socioeconomic status and lifestyle pattern. Well-resourced population of developed countries is at lower risk, while prevalence of infection remains high in the regions of world that belong to developing countries where resources are limited (Cover and Blaser, 2020).

Survival of *H. pylori* in the highly acidic environment of stomach is mainly dependent on a nickel and enzyme Urease present in bacterial cytoplasm. Urease is responsible for metabolizing urea into ammonia and carbon dioxide adjusting the pH necessary for bacterial survival (Amin *et al.,* 2013). The schematic representation of urea hydrolysis by urease is depicted in scheme 1 (Yuri *et al*., 2018). The current regime of treatment of digestive diseases caused by *H. pylori* infection is based on triple therapy including two broadband antibiotics and proton pump inhibitor. However conventional therapy regimens of *H. pylori* are becoming complicated and ineffective due to antibiotic resistance as is counted in one of 16 antibiotic resistant dangerous bacterial strains (Suzuki *et al.,* 2010; Cunha *et al.,* 2021). Apart from bacterial resistance, high therapeutic cost and complex regimen of multiple daily doses makes the treatment unapproachable. As gastric infections caused by *H. Pylori* are highly prevalent around the globe as well as communicable through oral to oral and oral to fecal transmission, there is an urgent need to find a highly specific and targeted remedy (Sharaf *et al.,* 2022).

In current scenario a remarkable trend is noticed to explore nature as search engine for the discovery of phytochemicals having great therapeutical potentials. A lot of medicinal plants have been proved to have antibacterial properties. Keeping this fact in mind it can be believed that *H. pylori* gram negative bacteria responsible for digestive problems can also be eradicated with natural herbs. Main objective of this research study is to explore natural flora to identify and authenticate medicinal plants having anti-Urease activity. Blocking the Urease enzyme will result in eradication of bacteria hence protect from all complications like peptic ulcer and

**Corresponding author:* e-mail: Khizarabbas@bzu.edu.pk



Scheme 1: Urea hydrolysis catalyzed by ureases.

cancers. In this regard we selected 15 medicinal plants *Silybium marianum*, *Fagonia arabica, Nigella sativa, Curcuma longa, Ocimum sanctum, Salvia rosmainus, Hyssopus officinalis, Antrirrhinum majus, Salvia splendens, Tropaeolum majus, Dalbergia sisso* (flowers)*, Dalbergia sisso (*leaves)*, Aloe barbadensis, Abelmoschus esculentus* (aerial parts), *Abelmoschus esculentus* (seeds), *Cuscuta reflexa, Hibiscus schizopetalus)* for anti-urease screening.

MATERIALS AND METHODS

Material collection and identification

The selected 15 medicinal plants 100 gm each were purchased from the local herbal market of Karachi city and identified and authenticated by Prof. Dr. Ghazala H Rizwani, Department of Pharmacognosy, Faculty of Pharmacy Hamdard University. Botanical descriptions and Pharmacognostic profiles of all tested plants are mentioned in table 1.

Extract preparation

All plant materials were garbled, washed and shade dried individually. Extraction of each plant was performed separately by soaking in ethanol for 15 days at room temperature. It was then filtered through whatman filter paper No 1, followed by solvent evaporation under reduced pressure using rotary evaporator (Buchi, Switzerland), at a temperature of 46°C to give a residue (extract). After complete solvent evaporation, each of 15 extracts were weighed and stored in airtight bottles for further use. The % yield of extracts obtained were *Silybium marianum* (7.2%), *Fagonia arabica* (5.9%)*, Nigella sativa* (7.8%)*, Curcuma longa* (6.6%)*, Ocimum sanctum* (6.3%)*, Salvia rosmainus* (4.3%)*, Hyssopus officinalis* (9.8%)*, Antrirrhinum majus* (5.6%)*, Salvia splendens* (4.5%)*, Tropaeolum majus 3.9%, Dalbergia sisso* (flowers) (6.0%)*, Dalbergia sisso* (leaves) (10.0%)*, Aloe barbadensis* (5.0%)*, Abelmoschus esculentus* (aerial parts) (7.5%), *Abelmoschus esculentus* (seeds) (6.1%), *Cuscuta reflexa* (5.1%)*, Hibiscus schizopetalus* (8.02%)

Chemicals and reagents

All chemicals and reagents used were Analytical grade. Canavalia ensiformis (Avonchem Ltd, UK), Sodium Hypochlorite (HC Haq Chemicals, Pakistan), Sodium Nitroprusside (BDH Chemicals Ltd, England), Thiourea (Merck, Germany), Urea (Sigma-Aldrich).

Anti-Urease activity

Sample preparation and incubation

Each of the extract weigh accurately (1 gm) and dissolve in solvent to prepare litre of solution. Then take 1 ml of it and dilute again to one litre to get µg/ml concentration. To evaluate the urease inhibitory activity of different medicinal plants the exact 20µl of enzyme (Canavalia ensiformis- Jack Bean) solution mixed with 55µl of phosphate buffer (0.2M) in each extract the mixture having pH of 7.4 (Weatherfourn, 1967). Then, the mixture was kept on incubation for 10 minutes at 30℃, later add 15µl urea and kept it again for incubation using the same time and temperature. After incubation, the known volume of each extract of medicinal plant (25µg/ml, 50µg/ml and 75µg/ml) respectively were added in 96 wells. Now add 25µg/ml of Thiourea (standard solution). Properly mixed it and kept it for 10 minutes at 37℃. Then add 40µl Alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) and 60µl phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) in all wells and leave it at room temperature for 10 minutes (Khan *et al.,* 2015). Experiments were performed in a triplicate fashion and thiourea was used as standard inhibitor.

Absorbance using UV spectrophotometer

Measure the absorbance spectrophotometric at 625 nm. Urease inhibition percentage was calculated by using the mentioned formula:

% inhibition = [1- (A_{625} of sample/A_{625} of control) × 100]

IC 50 value calculations

IC_{50} values of all medicinal plants and standard Thiourea were calculated by plotting graph between % urease inhibitory activity verses doses/concentration of extracts, then fitting the data with a straight trendline. Linear regression Equation thus obtained was used to IC_{50} determination. Y = M* X + C

STATISTICAL ANALYSIS

Anti-urease activity of all 15 medicinal plants along standard thiourea is represented as mean ± S.E.M. In order to find the significant differences in activity among them One way ANOVA was employed followed by Tukey's posthoc test at $p<0.05$. SPSS version 20 was used for statistical calculations.

RESULTS

Anti-urease activity of ethanolic extracts of all 15 medicinal plants (three different doses 25, 50 and 75µg/ml) has been analyzed using UV spectrophotometer keeping thiourea as standard. Fig. 1a, 2a, 3a showed the graphical representation of % urease inhibition of extracts of medicinal plants and standard thiourea at the concentration of 25, 50 and 75µg/ml respectively. However, *F. arabica* (87.2±1.47), *N. sativa* (90.4±0.09), *O. sanctum* (75.6±0.95), *H. officinalis* (78.9±0.69), *T. maju* (87.3±0.14), *A. esculentus* (90.3±0.86), *C. reflexa* (94.1±0.92) showed significant results at 75µg/ml when compared to Thiourea.

After estimation of % urease inhibitory activity of all plant extracts, IC_{50} was calculated that is shown in table 2. IC_{50} value of standard thiourea was found 35.6µl. Extracts having excellent IC_{50} values were *Cuscuta reflexa* (35.4µg/ml), *Aloe barbadensis* (36.5µg/ml), *Nigella sativa* (37.1µg/ml), *Fagonia arabica* (39µg/ml), *Tropaeolum maju* (40.17µg/ml), *Abelmoschus esculentus* (seed 40.4µg/ml), *Ocimum sanctum* (41µg/ml), *Abelmoschus esculentus* (Aerial parts 41.2µg/ml) and *Hyssopus officinalis* (42µg/ml). IC_{50} values of medicinal plant extracts and standard Thiourea for Anti-urease activity were showed in table 2. Comparison of three different concentrations (25ug/ml, 50ug/ml and 75ug/ml) was depicted in fig. 2.

DISCUSSION

H. pylori is a causative bacteria responsible for widespread spectrum of gastric diseases including multifocal atopic gastritis, stomach cancers and peptic ulcers. Stomach cancer is listed as the 3rd main cause of morbidity among population due to cancer and behind 90% of such cases the causative organism was detected as *H. pylori* (Cunha *et al.,* 2021). It is the need of time to explore natural flora to find medicinal and edible plants that are capable of destroying the colonies of this gram-negative bacterial pathogen in the stomach. Our current research study is designed to investigate some common edible as well as medicinal herbs for their urease inhibitory effect so that increasing morbidity and mortality due to *H. pylori* can be minimized and controlled.

It is very important to understand the mechanism of *H. pylori* survival to eradicate the gastric diseases caused by this bacterium. The bacterial mechanism behind survival in highly acidic environment of stomach has been explained by various researchers. It can be concluded from literature that Urease is the key bacterial enzyme composed of two genes *Ure* A and *Ure* B, responsible for increasing the pH of stomach by liberating ammonia. Moreover, gene transcription and reduced mucin viscoelasticity also help of *H. pylori* in adherence to the surface epithilum cells of stomach promoting vacuolating cytotoxic activity and growth- inhibitory factor for stomach cell proliferation (Smoot, 1997; Cellia *et al.*, 2009; Graham and Miftahussurur, 2018; Khan *et al.,* 2017). This available scientific data on mechanism of *H. pylori* survival suggests inhibiting the urease enzyme in the stomach hence maintaining the lower acidic pH resulting in bacterial death. In our research study we have explored fifteen medicinal and edible plants from Pakistan for their anti-urease potential to combat gastric diseases caused by *H. pylori*. Utilization of these plants will inhibit the bacterial urease enzyme resulting in bactericidal effect. Analytical results indicate that all plants showed positive anti-urease activity *in vitro*. Among the tested samples some plants such as *Nigella sativa* (Black cumin), *Ocimum sanctum* (basil), *Abelmoschus esculentus* (lady finger), *Salvia rosmainus* (rosemary) and *Curcuma longa* (turmeric) are edible.

These plants are easily available and their incorporation in daily cuisine of general population may not only help in spread of gastric diseases but also fighting against *H. pylori* via inhibition of bacterial urease. On the other hand, medicinal plants like *Silybum marianum, Fagonia arabica, Hyssopus officinalis, Antrirrhinum majus, Salvia splendens, Tropaeolum majus, Dalbergia sisso, Aloe barbadensis, and Cuscuta reflexa* has been proved as good candidates to be employed for therapeutic use among patients of gastric diseases.

Upon comparison of extracts with standard thiourea, it was concluded that most of the tested medicinal plants (*Fagonia arabica, Nigella sativa, Ocimum sanctum, Hyssopus officinalis, Tropaeolum maju, Abelmoschus esculentus* (Aerial parts), *Abelmoschus esculentus* (seed), *Cuscuta reflexa*) showed significant urease inhibitory activity at all doses of 25µg/ml, 50µg/ml and 75µg/ml. On the other hand, *Silybum marianum*, *Aloe barbadensis*, *Hibiscus schizopetalus* showed moderate activity and found statistically significant at doses of 50µg/ml and 75µg/ml, while flowers of *Dalbergia sisso* was found significant at dose of 75µg/ml when compared to thiourea.

However, *Curcuma longa, Salvia rosmainus, Antrirrhinum majus, Salvia splendens* although showed anti-urease activity at all doses but not as significant as Thiourea. Regarding mechanism it is evident from results that urease enzyme inhibitory activity of all medicinal plants is dependent on concentration, and it is increasing in dose dependent manner (Figs. 1a, 1b and 1c). Our research findings suggest that the use of edible herbs having anti urease potential in daily routine can minimize the spread of *H. pylori* infections. Moreover, natural flora can serve as search engine for the discovery of new anti *H. pylori* drugs due to the presence of active phytochemicals having therapeutic potential. Extraction, isolation and structure elucidation of such active constituents from these herbs can serve as model for developing their synthetic analogue.

Table 1: Detail Description of Medicinal plants selected for anti- Urease screening.

S. No.	Scientific names	Local names	Family	Part used	Phytochemicals	Therapeutic uses
1.	*Silybium marianum*	Milk thistle	Asteraceae	Flower	Silybin, silidianin, Silicristin	Liver disorder, hepatitis, cirrhosis, jaundice. diabetes. Indigestion (Wang *et al.,* 2020)
2.	*Fagonia arabica*	Dhamasa/ sachi booti	Zygophyllaceae	Aerial part	Glycoside, flavonoids, terpenoids, Saponin, Alkaloids	Antidiabetic, anticancer, antipyretic, laxative antioxidant, anti inflammatory (Iftikhar *et al.,* 2022)
3.	*Nigella sativa*	Kalonji	Ranunculaceae	Seed	Fatty acid, terpene, alcohol, volatile oil	Antioxidant, inflammation, Asthma, hypertension, Cancer (Sharma *et al.,* 2009)
4.	*Curcuma longa*	Turmeric	Zinigiberaceae	Root and Rhizome	Flavonoids, curcumin, volatile oil, sugar, protein, Resin	Inflammation, Digestion, Antioxidant, Depression, Cancer (Omosa *et al.,* 2017)
5.	*Ocimum sanctum*	Holy Basil	Labiateae	Leaf	Flavonoids, tannin, saponin, phenolic, essential oil	Bronchitis, bronchial asthma, diarrhea, skin disease, Malaria (Pattanayak *et al.,* 2010)
6.	*Salvia rosmainus*	Rosemary	Lamiaceae	Aerial part	Phenolic acid, flavonoids, carnosic acid	Headache, dysmenhorrea, epilepsy, rheumatic pain, spasm (Choukairi *et al.*, 2019)
7.	*Hyssopus officinalis*	Hyssop/ Zofa	Lamiaceae	Aerial part	Beta pinene, limonene, caryphyllene	Asthma, sore throat, antioxidant, anorexia, Brochitis (Said *et al.,* 2015)
8.	*Antrirrhinum majus*	Snap dragon/ gul e meymoon	Scrophulariaceae	Flower	Amino acid, pigments, flavonoids, oil	Diuretic, sucrvy, liver disorder, tumor (Al sanafi, 2015)
9.	*Salvia splendens*	Scarlet sage	Lamiaceae	Leaves	Flavonoids, triterpenes, saponin, tannin, alkaloids, phenolic content	Wound dressing, dysentery, colic hemorrhides (Moharram *et al.,* 2012)
10	*Tropaeolum majus*	Nasturtium	Tropaeolaceae	Flower	Ascorbic acid, flaconoid, phenoiliv content	Antimicrobial, hypotensive, expectorant, anti cancer (Garzón and Wrolstad, 2009)
11	*Dalbergia sisso*	Sheesham	Fabaceae	Leaves	Carbohydrate, protein, amino acid, flavonoids, phenolic content	Skin disorder, peptic ulcer, Bleeding, Leprosy (Bhattacharya *et al.,* 2014)
12	*Dalbergia sisso*	Sheesham	Fabaceae	Flower	Carbohydrate, protein, amino acid, flavonoids, phenolic content	Skin disorder, peptic ulcer, Bleeding, Leprosy (Bhattacharya *et al.,* 2014)
13	*Alo barbadensis*	Alo vera	Liliaceae	Aerial parts	Anthraquinone, carbohydrate, saponin, steroids, tannin	Wound healing, Diabetes, constipation (Manvitha and Bidya, 2014)
14	*Abelmoschus esculentus*	Lady finger	Malvaceae	Aerial parts	Cellulose, lignin, Hemicellulose	Diabetes, weight loss, constipation (Jain *et al.,* 2012)
15	*Abelmoschus esculentus*	Lady finger	Malvaceae	Seed	Cellulose, lignin, Hemicellulose	Diabetes, weight loss, constipation (Jain *et al.,* 2012)
16	*Cuscuta reflexa*	Giant dodder	Cuscutaceae	Sap	Alkaloid, flavonoid, terpenoids, saponin, tannin, steroids	Purgative, cough, jaundice, muscle pain (Saini *et al.*, 2015)
17	*Hibiscus schizopetalus*	Shoe- flower	Malvaceae	Leaves	Terpenoids, steroids, Phenolics	Arthritis, diabetes, cough, malaria (Wong *et al.,* 2016)

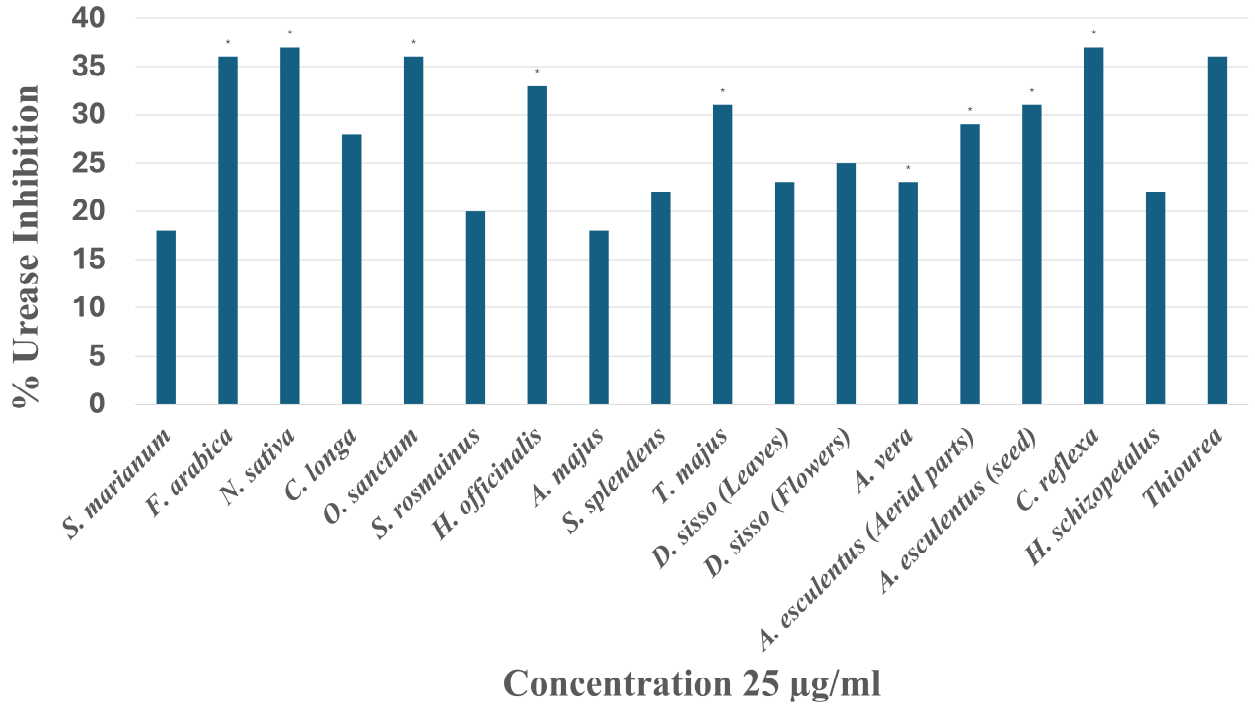


Fig. 1: Effect of extracts at 25ug/ml on urease inhibition activity. Data are shown as the mean ± SD (n = 3). Asterisk represents statistically significant.

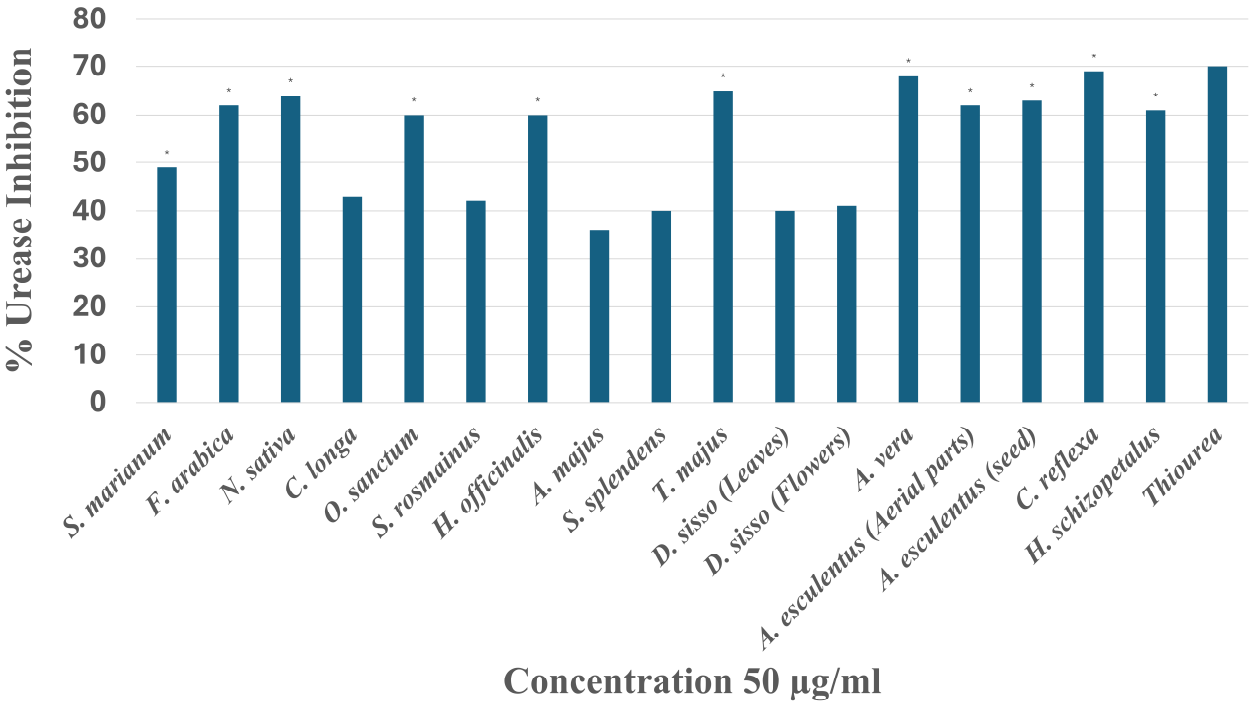


Fig. 2: Effect of extracts at 50ug/ml on urease inhibition activity. Data are shown as the mean ± SD (n = 3). Asterisk represents statistically significant.

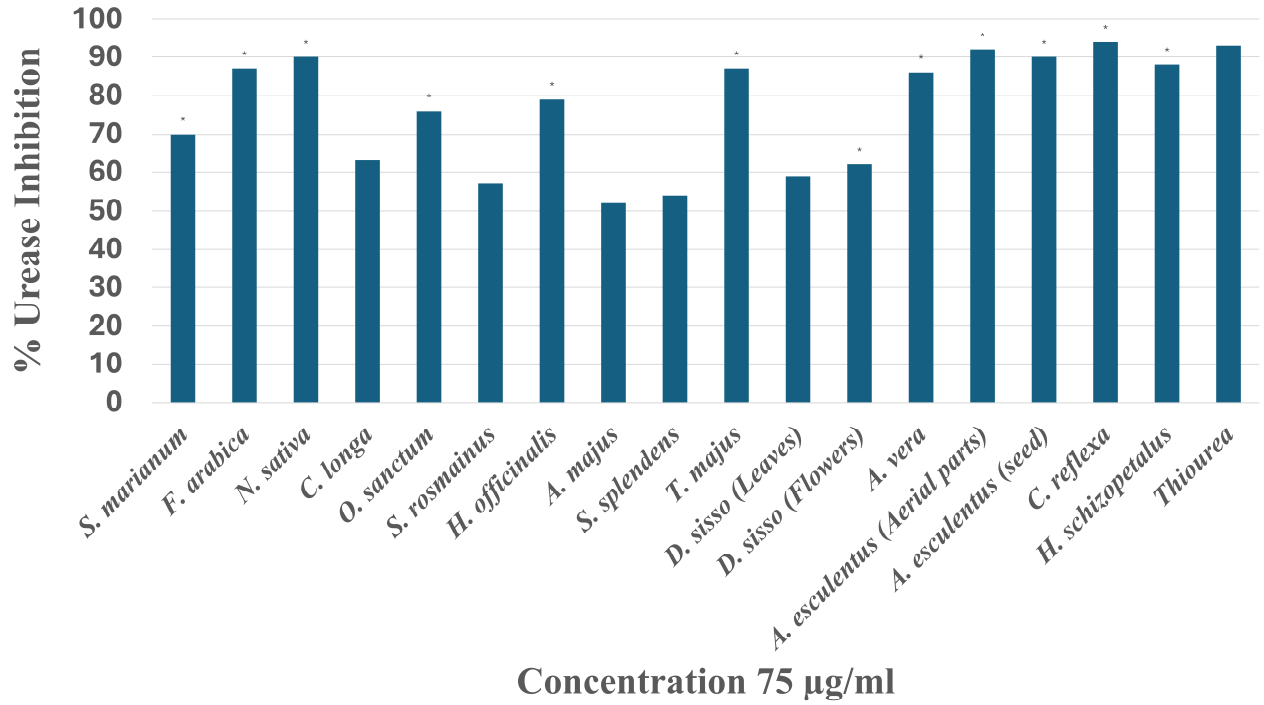


Fig. 3: Effect of extracts at 75ug/ml on urease inhibition activity. Data are shown as the mean ± SD (n = 3). Asterisk represents statistically significant.

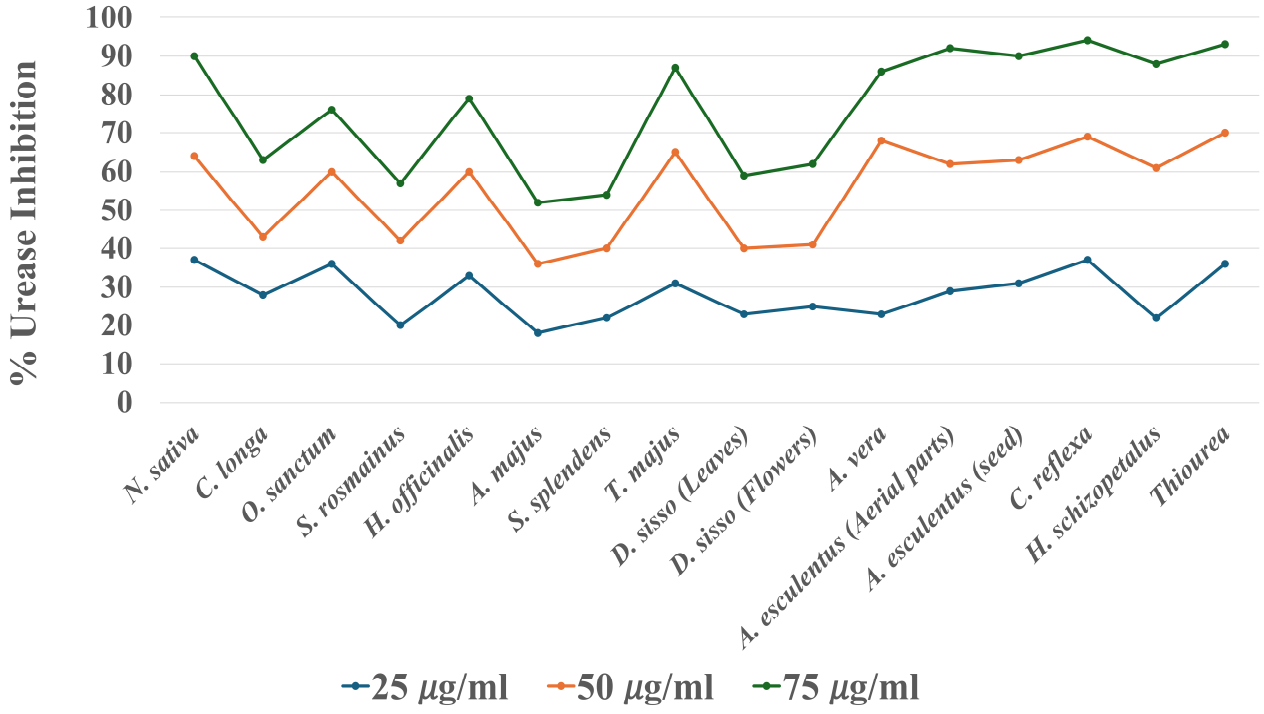


Fig. 4: Representation of comparison of three different concentrations (25ug/ml, 50ug/ml and 75ug/ml)

Table 2: IC_{50} values of medicinal plant extracts and standard Thiourea for Anti-urease activity.

S No.	Medicinal Plants (Extracts)	IC_{50} (µg/ml)
1	*Silybum marianum*	54.16
2	*Fagonia arabica*	39
3	*Nigella sativa*	37.1
4	*Curcuma longa*	58
5	*Ocimum sanctum*	41
6	*Salvia rosmainus*	64
7	*Hyssopus officinalis*	42
8	*Antrirrhinum majus*	71.6
9	*Salvia splendens*	68
10	*Tropaeolum majus*	40.17
11	*Dalbergia sisso (Leaves)*	63
12	*Dalbergia sisso (Flowers)*	60
13	*Aloe vera*	36.5
14	*Abelmoschus esculentus (Aerial parts)*	41.2
15	*Abelmoschus esculentus (seed)*	40.4
16.	*Cuscutare flexa*	35.4
17.	*Hibiscus schizopetalus*	44.7
Standard	Thiourea	35.6

CONCLUSION

Gastric problems caused by *H. pylori* are prevalent among at least 50% of the population. It is evident from this research study that utilization of medicinal plants will provide highly effective, cheap and safe treatment to people suffering from such gastric diseases. Incorporation of food, vegetables and herbs having anti-urease potential in our daily routine will prevent disastrous effects of *H. pylori* on stomach health and upgrade the quality of life. However, to achieve optimum therapeutic benefits from these herbs it is mandatory to utilize best quality genuine herbs free from adulteration and all kinds of environmental contamination. Our research stipulate prospects for developing reliable and precise anti-*H. pylori* drugs from natural sources.

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