IN- VITRO ANTIUREASE ACTIVITY OF AQUEOUS-ETHANOL EXTRACT OF SOME MEDICINAL PLANTS

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ABSTRACT: Study was planned to evaluate urease inhibitory efficacy of aqueous-ethanol extract of medicinal plants including *Mentha piperita*, *Violo odorata*, *Trachyspermum copticum*, *Metricarea chamomile* and *Foeniculum vulgare*, traditionally used for management of gastrointestinal disorders. Phenol hypochlorite method was adopted to evaluate the urease inhibitory efficacy. The extracts were also phytochemically evaluated. The results showed that aqueous-ethanol extract of *Mentha piperita*, *Violo odorata*, *Trachyspermum copticum*, *Metricarea chamomile* and *Foeniculum vulgare* exhibited significant antiurease efficacy. The IC_{50} values of *Mentha piperita, Metricarea hamomile, Foeniculum vulgare* and *Violo odorata* extracts were 124±0.71, 119± 0.65, 119± 0.65 495±0.80 and 333±0.98 µg/ml respectively. Extract of *Mentha piperita* showed more antiurease potential in comparison to other plants extracts. Phytochemical screening of the extracts revealed the presence of alkaloids, tannins, saponins, flavonoids, flavones and sterol. The present study therefore, showed that the medicinal plants exhibit antiurease activity.

Key words: Antiurease activity, Medicinal plants, Inhibition, Phytochemical screening.

(Received 29-07-2016 Accepted 10-03-2017)

INTRODUCTION

Urease is a substrate specific nickel dependent enzyme that speeds up the hydrolysis of urea to yield ammonia and carbon dioxide. Urease is urea amidohydrolase, instituted by different organisms that catalyze the synthesis of ammonia and carbon dioxide from urea (Khan *et al*., 2012). Carbamate hurriedly and instinctively decomposes, producing a second molecule of ammonia. Major increase in pH caused by these reactions causing the environment alkaline and is also responsible for deleterious property of urease which affects the human health (Lateef *et al*., 2012). The most essential role of urease in the stomach is to maintain the survival of urease producing bacteria for example *Helicobater pylori* (*H. pylori*) (Stingl *et al*., 2002). Ureases are a broad group of naturally occurring enzymes, essential in providing nitrogen to organisms for the formation of the structure of ammonia (Koper *et al*., 2004).

Mentha piperita (*M. piperita*) belongs to family *Lamiaceae*. It is commonly known as mint in English and pudina in Urdu. Leaves are used for medicinal purpose. *M. piperita* is an aromatic plant and is traditionally known for medicinal properties (Neha *et al.,* 2014). It has antioxidant and antimicrobial properties. Menthol is a biochemical cyclic terpene alcohol that is a major constituent of *M. piperita. M. piperita* has antibacterial, antiviral, anti-oedema, analgesic, digestive, decongestant, antiflatulent, antitussive, carminative, antimutagenic, antispasmodic and mild stimulant properties **(**Galib and Al-Kassie, 2010). It is used in digestive disorders, irritable bowel syndrome, tropical treatment of headaches, metabolic diseases i.e. diabetes mellitus and hypercholesterolemia, gall bladder and biliary tract disorders, Chron's disease and ulcerative colitis. Methanolic leaf extract has showed activity against methicillin resistant *S. aureus* (Arzani *et al*., 2009). Methanolic extract of mint leaves contains tannins and flavanoids *(*Kaur *et al.,* 2010).

Foeniculum vulgare (*F. vulgare*) belongs to family *Umbelliferae.* It is commonly known as fennel in English and saunf in Urdu. *F. vulgare* is an annual, biennial and perennial aromatic herb and it has antioxidant activity. It has antidiabetic, antioxidant, hepatoprotective, antifungal, antibacterial, antimicrobial, antithrombotic, antispasmodic, antiosteoporotic, carminative, diuretic, expectorant, anti-inflammatory, laxative and analgesic properties. It possesses antibacterial effect against *Escherichia coli* and *S. aureus* (Zadeh, 2007). Anethole, a chemical component has structural resemblance with dopamine thus, exhibit dopamine like effects such as intestinal relaxation and infentile colic (Gurinder and Daljit, 2010).

Metricarea chamomile (*M. chamomile*) belongs to family *Asteraceae.* It is commonly known as

chamomile in English and its Urdu name is babuna. *M. chamomile* has anti-inflammatory, anti hyperglycemic, anti-cancer, anti pruritic, anti microbial, poliovirus inhibitory, antiulcer, anti allergic, antispasmodic, virucidal, immunomodulatory, sedative and wound healing properties (Enas, 2013). It has ovicidal and repellent property. Chamomile is used to treat gastrointestinal disorders, oral mucositis, osteoporosis, insomnia, haemorrhoids, infant botulism, stress and depression (Singla *et al.*, 2010).

Trachyspermum copticum (*T. ammi*) belongs to family *Apiaceae or Umbelliferae*. Its English name is Carom seed and Urdu name is Ajwain. Its seeds are used as medicine. Ajwain has diuretic, sputum expelling, antispasmodic, antiseptic, anthelmintic, carminative, laxative, stomachic, antihyperlipidaemic, kidney stone inhibitory, bactericidal, lipolysing, digestive, hypotensive, hepatoprotective, bronchodilatory, abortificient, antiinflammatory, intestinal dysbiosis properties. It is given for flatulence, dyspepsia and diarrhea frequently suggested for cholera, influenza, colic, indigestion, edema, rheumatism and earache (Asif *et al*., 2013). Urease is sorted out from different microbes and plants. Essential oil of *Trachyspermum copticum* is effective against fungal species *Epidermophyton floccsum, Microsporum canis* and *Trichophyton mentagrophyts*, thymol (Gurinder and Daljit, 2010). In another study, *Vibrio cholera, S. typhi, E. coli* and *S. aureus* are inhibited by essential oil extracted from *Trachyspermum copticum* (Patel *et al*., 2008).

Viola odorata (*V. odorata*) belongs to family *Violaceae*. Its English name is sweet violet and Urdu name is banafsha. Its chemical constituents include glycosides, steroids, saponins, flavonoids, alkaloids, terpenes, phytosterols, triterpenoids, anthocyanins, phenols, tannins and asteroids. Whole plant is used for medical purpose. It has expectorant, diuretic, anti-inflammatory, antihypertensive, antidyslipidemic and antioxidant activity. *Viola odorata* is used in various skin diseases, cystitis, bronchitis, cold, whooping cough, upper respiratory tract infections, cancer, migraine, headache, insomnia and chronic bronchial asthma (Ines *et al*., 2014).

In the present research work, anti urease activity of traditional medicinal plants including *Mentha piperita* (pudina), *Violo odorata* (banafsha), *Trachyspermum copiticum* (ajwain), *Metricarea chamomile* (babuna) and *Foeniculum vulgare* (saunf) found in the vicinity of Rawalakot, Azad Kashmir were evaluated.

MATERIAL AND METHODS

Five medicinal plants including *Mentha piperita* (pudina), *Violo odorata* (panafsha), *Trachyspermum copiticum* (ajwain), *Metricarea chamomile* (babuna) and *Foeniculum vulgare* (saunf) were purchased from the local market of the Rawalakot Azad Jammu & Kashmir, Pakistan. The plants were confirmed from Department of Botany, The University of the Poonch, Rawalakot.

Plants extract preparation: Plants were washed with distilled water to remove all the external dirt and unwanted material and shade dried for seventy two hours. The dried material was crushed and grinded to powder. The powder (50 g, each) was soaked in one liter of 70 percent ethanol for 48 hours with occasional stirring and shaking. After 48 hours, the soaked material of each plant was filtered separately by whattman no. 1 filter paper. The residual mass was evaporated at room temperature for achieving desiccation to get final volume of 100 ml. The residues were measured and dissolved in phosphate buffer saline (25 ml) and stored at 4 ^{o}C prior to use.

Activity of urease and its inhibition: Enzyme activity and inhibition was measured through seeded up property of enzyme on urea by quantifying variation in absorbance using spectrophotometer at 625 nm. Absorbance of blank test mixture was prepared by taking buffer (500µl), reagent 1 (2.5ml) and reagent 2 (2.5ml) and was adjusted at 'zero' by turning the spectrophotometer at auto zero. For the enzyme assay reaction mixture cocktail containing urea (15 µl) and reagent 1 (2.5 ml) was incubated at 37 ^{o}C for 5 minutes and subsequent addition of reagent 2 (2.5 ml) and incubated for 10 minutes. The study was performed by the incorporation of substrate at different amounts subsequently rest of the enzyme assay procedure.

Phytochemical analysis: Phytochemicals screening of plant extracts was carried out by standard methods to identify the presence of active constituents such as, tannins, alkaloids, flavonoids and saponins.

Statistical analysis: One way ANOVA was carried out to find out the variations between various groups. IC_{50} values were calculated by using graph pad prism. $P<0.05$ was considered as significance level.

RESULTS AND DISCUSSION

Phytochemical analysis of the aqueous ethanolic extracts of *M. piperita, T. copticum, F. vulgare, M. chamomile and V. odorata* showed the presence of compounds like glycosides, tannins, alkaloids, flavonoids and saponins.

In the present study, Thiourea was considered as a standard antiurease drug. The IC_{50} values were determined by investigating the *M. piperita, M. chamomile, V. odorata, T. copticum* and *F. vulgare* extracts showing inhibitory activity to positive control employing spectrophotometeric measurements. The percentage urease inhibition of standard thiourea was 99.26 ± 1.18^{f} %, IC_{50} was 19±0.08 µg/ml. The maximum

urease inhibitory activity was shown by the leaf extract of *M. piperita* (71±0.45[c] %)and IC_{50} was 124±0.71 µg/ml. Inhibition shown by the seed extract of *M. chamomile* was 68± 0.97[a] and IC_{50} was 119± 0.65 µg/ml. Anti-urease activity of the flowers extract of *V. odorata* was 65±0.53[d] and IC_{50} was 104±0.94 µg/ml. The inhibition of urease shown by the seed extract of *F. vulgare* was 51±0.76[e] and IC_{50} was 495±0.80 µg/ml. Enzyme inhibitory effect was shown by the seed extract of *T. copticum* was 51±0.1[b] and IC_{50} was 333±0.98 µg/ml.

Table 1: Phytochemical constituents of five medicinal plants.

Class of compounds	*M. piperita*	*T. copticum*	*F. vulgare*	*V. odorta*	*M. chamomile*
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Flavonols	+	+	+	+	+
Flavones	+	+	+	+	+
Flavonoids	+	+	+	+	+
Sterols	+	+	+	+	+

(+); indicates the presence of active constituents

Table 2: Antiurease activity of medicinal plants.

Plant	% inhibition	IC_{50} (µg/ml)
Mentha piperita	71±0.45[c]	124±0.71
Matricarea chamomile	68±0.97[a]	119± 0.65
Trachyspermum copticum	51±0.1[b]	333±0.98
Foeniculum vulgare	51±0.76[e]	495±0.80
Violo odorata	65±0.53[d]	104±0.94
*Standard drugs	99.26±1.18[f]	19±0.08 µmol

Superscript a, b, c, d, e and f showed that mean± standard deviation are significantly different (p<0.05)
(*Standard drugs: Thiourea)

Urease is a specific nickel dependent enzyme which catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide. Synthesis of ammonia and CO_2 is catalyzed by urease from urea mostly found in stomach of vertebrates (Khan *et al*., 2012). The most essential role of urease in the stomach is to maintain the survival of urease producing bacteria most importantly *H. pylori* in the acidic environment (Stingl *et al*., 2002).

Most abundant source of urease in human being is bacteria. Urease plays central role in *H. pylori* metabolism, virulence and is essential for colonization of pathogen in the gastric mucosa. It is also effective immunogen that elicits a strong immune response by host (Ha *et al*., 2001). As a result, it plays the main role in gastric diseases by providing colonization facility to bacterium. Consequently, elimination of the organism can be achieved by urease inhibition. Inhibitors of urease are successful in favor of rehabilitation of a lot of infectious conditions caused by the emission of urease from *H. pylori* (Amtul *et al*., 2006). *H. pylori* is acid susceptible and simply multiplies at 7-8 pH (Stingl *et al*., 2002). It is expected that greater than 80 percent of people in the developing nations suffer from *H. pylori* infection (Logan and Walker, 2001).

Many plants are known to have urease blockage properties such as *H. oblongifolium*, *A. bracteata*, *A. millefolium*, *T. officinale*, *E. globules* and *M. longifolia* (Arfan *et al*., 2010).

It was observed that M. *piperita*, *M. chamomile*, *V. odorata*, *T. copticum* and *F. vulgare* extracts exhibited significant urease inhibitory activity. Furthermore, the phytochemical analysis of the plants extracts showed the presence of phytoconstituents such as glycosides, terpenoids, cardiac glycosides, glycosides, carbohydrates, tannins, alkaloids, flavonoids and saponins.

To eradicate and control bacterial infections urease production is targeted by therapeutic regimens such as urease inhibitors. Inhibitors of urease are useful against some infectious disorders including gastric pathologies and urinary tract infection, pyelonephritis, urinary catheter obstruction, peptic ulceration and cancer. Infection caused by *H. pylori* can be prevented by inbitting urease activity. Urease inhibitors are consequently measured to be potential antiulcer drugs (Arfan *et al*., 2010). Accordingly, urease inhibition property of medicinal plants has recently attracted pharmaceutical industries as they exhibit potent anti-ulcer effects. *Helicobacter pylori* has become resistant to many antibiotics, therefore there is a need to search new agents

from natural resources. Substances isolated from herbs are assumed as synthetic inhibitors of urease and can be a considerable alternatives to antibiotics because of bacteriostatic activities rather than bactericidal. Nitrogen is the weapon against hazardious microbes but urease reduces it to facilitate the infectious agents to spread and grow (Amtul *et al*., 2006).

Naturally occurring herbs are used for embarrassment of anti-urease have less side effects. Herbs are used to clear *H. pylori* from its habitat. Medicinal plants can be used as traditional remedies in treating gastrointestinal diseases and their *in-vitro* anti-*H. pylori* activity has been widely examined (Shih *et al*., 2007). Extracts from fifty Taiwanese folk medicinal plants were reported possessing higher anti-*H. pylori* activity (Wang and Huang, 2005). Medicinal plants used in the present study showing antiurease activity are also used traditionally for gastric plight. *M. piperita* grows frequently in Northern hilly areas. Whole plant is useful in gasrtointestinal diseases. *T. copticum* and *F. vulgare* are found in North and are used in gas trouble. *V. odorata* is used as hepatotonic and relieves pyrexia. *M. chamomile* is good anti-bilious agent.

Conclusion: The current findings availed scientific grounds for use of *M. piperita* (Pudina), *V. odorata* (Banafsha), *T. copticum* (Ajwain), *M. chamomile* (Babuna) and *F. vulgare* (Saunf) plants in stomach related disorders. The antiurease activity of these plants was expected to be due to inhibitory effects on *H. pylori*. However, further study is recommended to search active constituents for urease inhibitory activity.

Acknowledgement: The authors would like to express sincere thanks to Dean and chairman of Department of Eastern Medicine, University of Poonach Rawlakot Azad Jammu and Kashmir and providing support and necessary facilities for research.

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