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Original Article

Screening of 20 Commonly Used Iranian Traditional Medicinal Plants Against Urease

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Abstract

Infection with *Helicobacter pyloriis* the most common cause of stomach and duodenal ulcers. About more than 80 % of people are infected with *H. pylori* in developing countries. *H. pylori* uses urease enzyme product "ammonia" in order to neutralize and protect itself from the stomach acidic condition and urease enzyme activity has been shown to be essential to the colonization of *H. pylori.* Inhibitory activity of 20 traditional medicinal plants were examined and evaluated against Jack bean urease activity by Berthelot reaction to obtains natural sources of urease inhibitors. Each herb was extracted using 80% aqueous methanol, then tested its IC_{50} value was determined. Eight of the whole 20 studied plants crude extracts were found the most effective with IC_{50} values of less than 100 µg/mL including *Laurus nobilis, Zingiber officinale, Nigella sativa, Angelica archangelica, Acorus calamus, Allium sativum,Curcuma longa, and Citrus aurantium* extracts, from which most potent urease inhibitory was observed for *Zingiber officinale, Laurus nobilis*, and *Nigella sativa* with IC_{50} values of 48.54, 48.69 and 59.10 µg/mL, respectively.

Keywords: Urease; *H. pylori;* Medical plants; Extracts; Urease inhibitor.

Introduction

Urease (E.C 3.5.1.5), considered as the most proficient protagonist in biochemistry, is a nickel containing enzyme carrying out the rapid catalysis and hydrolysis of urea to produce ammonia and carbon dioxide (1) It diffuses along the cytoplasmic membrane, increases the preplasmic space pH and as a result allows the bacteria growth in the present of extra cellular gastric acid(2). Additionally, urease activity will lead to kidney stones formation and also conduct the development of urolithiasis, pyelonephritis and hepatic encephalopathy (3, 4).

It has been shown that *H. pylori,*a pathogen which is colonized in the digestion system of human beings and considered as one of the important factors leading to gastric disease,is incapable of causing infection in the absence of urease (5). Natural medicines especially medicinal plants have been considered as one of the options to cure the diseases in some cases for many decades and their basic ingredients are used in medicine industry at present time. Unfortunately along with improvements in discovery of new chemicals, drugs and different antibiotics, not only the harmful side effects of these medicines have emerged, but also the bacteria developed resistance to them. Therefore, discovering of new active chemicals from medicinal plants with possible urease inhibitory activity could help to cure ulcer and gastritis caused by *H. pylori* infection (6, 7). In

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this study, the urease inhibitory activities of 20 herbal medicine extract against Jack bean urease were evaluated (8,9).

Experimental

Sodium nitroprussid and urease (EC 3.5.1.5) from Jack beans were purchase from sigma (St. Louis, MO, USA)and deionized water was used in all experiments. Potassium phosphate buffer(100 mM), pH=7.4, was prepared in distilled water. The studied plants were randomly obtained from local medicinal herb shops, Tehran, Iran (June 2012) based on their traditional uses for gastritis and were identified by one of our authors of the presented article (F. Mojab). The authenticated samples were deposited in the Herbarium of Shahid Beheshti University of Medical Science.

Extract preparation

0.5 g of air dried and powdered plant material was extracted in 10 mL, 80:20 methanol: water at room temperature (25±1ºC) for 24 hours. The resulting liquid extract was then filtered and concentrated to dryness under reduced pressure. The dry extracts were stored at -20 ºC till used (9).

Determination of urease activity

All 20 extracts were tested for their urease inhibitory activity at concentration of 125µg/mL by the modified spectrophometric method developed by Berthelot reaction. Inhibition assays were first performed for herbal extracts that were proven to exert significant inhibition and also for positive controls. The plant extract were tested in a concentration range of 1 to 125 µg/mL. Hydroxyurea was used as standard inhibitor. The solution assay mixture consisted of urea (850 µL) and (135 µL) crud extract with a total value of 985µL. The reactions were initiated by the addition of 15µL of urease enzyme solution in phosphate buffer (100 mM, pH 7.4, 1 µg/mL). Urease activity was determined by measuring ammonia concentration after 60 minutes of enzymatic reaction. The ammonia was determined using 500 µL of solution A (contained 0.5 g phenol and 2.5 mg of sodium nitroprusside in 50 mL of distilled water) and 500µL of solution B (contained of 250 mg sodium hydroxide and 820 µL of sodium hypochlorite 5% in 50 mL of distilled water) at the temperature of 37 °C for 30 minutes. The absorbance was read at 625 nm. Activity of uninhibited urease was designated as the control activity of 100%.

Determination of IC50 values and Data processing

The extent of the enzymatic reaction was calculated based on the following equation:

$$I\ (\%) = 100 - 100 * (T / C)$$

Where *I* (%) is the inhibition of the enzyme, T (test) is the absorbance of the tested sample (plant extract or positive control in the solvent) in the presence of enzyme,C(control) is the absorbance of the solvent in the presence of enzyme. Data are expressed as mean ± standard error (SD) and the results were taken from at least three times.

IC_{50} values (concentration of test compounds that inhibits the hydrolysis of substrates by 50%) were determined by studying the extracts urease inhibitory activity at their different concentrations in comparison to their individual positive control employing spectrophotometric measurement. IC_{50} values were obtained from dose-response curves by linear regression, using Graphpad software, prism 5.

Results and Discussion

In the presented study, urease enzyme inhibition potency of 20 herbal extracts was investigated from which 8 extracts including *Zingiber officinale, Laurus nobilis, Nigella sativa, Angelica archangelica, Acorus calamus, Allium sativum, Curcuma longa, and Citrus aurantium* extracts have shown inhibitory activity with IC_{50} values of less than 500 µg/mL(Table 1). Further examinations and IC_{50} determination revealed that the most potent urease inhibitory was observed for *Zingiber officinale* (48.54 µg/mL), *Laurus nobilis* (48.69 µg/mL), *Nigella sativa* (59.10 µg/mL), *Angelica archangelica* (64.03 µg/mL), and *Acours calamus* (88.77 µg/mL), respectively (Table 2).

Most traditional medicines are herbal ones which our information about is still insufficient.

Table 1. Urease inhibitory activity of plants extract.

No.	Scientific Name	Family	Common Name (English)	Part used	IC_{50} (µg/mL)
1	*Achillea millefolium*	Compositae	Yarrow, Milfoil	Flower	774.82
2	*Acorus calamus*	Araceae	Sweet flag	Root	88.77
3	*Allium sativum*	Lilliaceae	Garlic	Root	170.42
4	*Angelica archangelica*	Apiaceae	Garden angelica	Leaf	64.30
5	*Brassica nigra*	Brassicaceae	Black Mustard	Seed	691.48
6	*Cinchona officinalis*	Rubiaceae	Quinine bark tree	Bark	740.11
7	*Citrus aurantium*	Rutaceae	Bitter orange	Peel	465.24
8	*Curcuma longa*	Zingiberaceae	Turmeric	Rhizome	310.54
9	*Daturastramonium*	solanaceae	Thon – apple	Seed	763.23
10	*Foeniculum vulgare*	Apiaceae	Fennel seed	Seed	580.17
11	*Gentiana lutea*	Gentianaceae	Yellow gentian	Root	634.67
12	*Humulus lupulus*	Cannabinaceae	Hops	Twig	651.91
13	*Hyssopus officinalis*	Labiatae	Hyssop	Herb	703.12
14	*Laurus nobilis*	Lauraceae	Bay tree , Laurel tree	Leaf	48.69
15	*Malva sylvestris*	malvaceae	Common Mallow	Flower	786.71
16	*Nigella sativa*	RanunCulaceae	Black Cumin	Seed	59.10
17	*Piper nigrum*	Piperaceae	Black pepper	Seed	603.32
18	*Rubia tinctorum*	Rubiaceae	Madder	Root	725.36
19	*Trigonella foenum – graceum*	Leguminosae	Fenugreek	Herb	523.74
20	*Zingiber officinale*	Zingiberaceae	Ginger root	Rhizome- root	48.54

Even though medicinal plants have long been known as one of the most appropriate sources of active chemicals and their derivatives to be used as templates for designing and developing more effective compounds, preferably with less side effects, most plants having medicinal properties have not yet been thoroughly evaluated for their biological activities. With the increasing flow of medicinal plants application in the world, there is an urgent need of assessment of their complete chemical compositions (10, 11).Gastrointestinal diseases, particularly gastritis, duodenal, peptic ulcer, and gastric cancer are caused as a result of *H. pylori* infection whose habitance in the acidic medium of the stomach is highly dependence on the urease enzyme activity (11, 12). According to the literature, herbal medicines have the capability of suppressing the bacteria through inhibiting or reducing urease activity and as a result leading its ellipsis (9-12).

References

(1) Michetti K. Pathogenesis of *Helicobacter pylori* infection. *Curr. Opin. Gastroenterol.* (1998) 14: 57-63.
(2) Dixon NE, Gazzola C, Blakeley RL and Zerner B. Jack bean urease (EC 3.5.1.5), A metaloenzyme, a simple biological role for nickel? *J. Am. Chem. Soc*. (1975) 97: 4131-413.
(3) Mobley HLT and Hausinger RP. Microbial ureases: significance, regulation, and molecular

Table 2. IC_{50} (µg/mL) and medicinal uses of most active plants.

	Scientific name	Effects and medicinal uses	IC_{50} (µg/mL)
1	*Zingiber officinale*	Antiseptic, Digestive, Expectorant, Antiemetic, Antiseptic, Analgesic	48.54
2	*Laurus nobilis*	Carminative, Stomachic, Digestive	48.69
3	*Nigella sativa*	Anticancer, Analgesic, Carminative, Stomachic, Antibacterial	59.10
4	*Angelica archangelic a*	Stomachic, Antiseptic, Febrifuge, Digestive, Carminative	64.03
5	*Acours calamus*	diuretic, Antigoutal, Antiphlogistic, Stomachic, Cholagogue	88.77

characterization. *J. Microbiol. Rev.* (1989) 53: 85-108.
(4) Bauerfeind P, Garner R, Dunn BE and Mobley HL. Synthesis and activity of *Helicobacter pylori* urease and catalase at low pH. *Gut* (1997) 40: 25-30.
(5) Clyne M, Labigne A and Drumm B. *Helicobacter pylori* requires an acidic environment to survive in the presence of urea. *Infect. Immun*. (1995) 63: 1669-1673.
(6) Mandell R, Douglas G and Bennett JE. *Principles and Practices of infectious Diseases.* 4th ed., Churchill Livingstone, New York (1995) 1956-1964.
(7) Ahmad V, Hussain J, Hussain H, Jassbi AR, Ullah F, Lodhi MA, Yasin A and Iqbal M. First natural urease inhibitor from *Euphorbia decipiens. Chem. Pharm. Bull*. (2003) 51: 719-723.
(8) Ciurli S, Benini S, Rypniewski WR, Wilson KS, Miletti S and Mangani S. The complex of *Bacillus pasteurii* urease with acetohydroxamate anion from X-ray data at 1.55 Å resolutions. *J. Coord. Chem. Rev*. (1999) 190: 331-355.
(9) Nabati F, Mojab F, Habibi-Rezaei M, Bagherzadeh K, Amanlou M and Yousefi B. Large scale screening of commonly used Iranian traditional medicinal plants against urease activity. *Daru, J. Pharm. Sci.* (2012) 20: 72-81.
(10) Biglar M, Soltani K, Bazl R and Amanlou M. A preliminary investigation of the jack-bean urease inhibition by randomly selected traditional used herbal medicine. *Iran. J. Pharm. Res.* (2012) 11: 831-837.
(11) Heinrich M, Barnes J, Gibbons S and Williamson EM. *Fundamentals of Pharmacognosy and Phytotherapy.* Churchill Livingstone, Edinburgh (2004) 200-210.
(12) Golbabaei S, Bazl R, Golestanian S, Nabati F, Bahrampour Omrany Z, Yousefi B, Hajiaghaee R, Rezazadeh S and Amanlou M. Urease inhibitory activities of β-boswellic acid derivatives. *Daru, J. Pharm. Sci.* (2013) 21: 2-7.

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