Screening of 20 Commonly Used Iranian Traditional Medicinal Plants Against Urease

Mahmood Biglar*, Hessameddin Sufi, Kowsar Bagherzadeh, Massoud Amanlou and Faraz Mojab*

*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Science Research Center, Tehran University of Medical Sciences, Tehran, Iran. \( ^{\text{b}} \)Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

Infection with \textit{Helicobacter pylori} is the most common cause of stomach and duodenal ulcers. About more than 80% of people are infected with \textit{H. pylori} in developing countries. \textit{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 \textit{H. pylori}. Inhibitory activity of 20 traditional medicinal plants were examined and evaluated against Jack bean urease activity by Berthelot reaction to obtain natural sources of urease inhibitors. Each herb was extracted using 80% aqueous methanol, then tested its IC\textsubscript{50} value was determined. Eight of the whole 20 studied plants crude extracts were found the most effective with IC\textsubscript{50} values of less than 100 µg/mL including \textit{Laurus nobilis}, \textit{Zingiber officinale}, \textit{Nigella sativa}, \textit{Angelica archangelica}, \textit{Acorus calamus}, \textit{Allium sativum}, \textit{Curcuma longa}, and \textit{Citrus aurantium} extracts, from which most potent urease inhibitory was observed for \textit{Zingiber officinale}, \textit{Laurus nobilis}, and \textit{Nigella sativa} with IC\textsubscript{50} values of 48.54, 48.69 and 59.10 µg/mL, respectively.

Keywords: Urease; \textit{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 extracellular 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 \textit{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 \textit{H. pylori} infection (6, 7).
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±1°C) 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 spectrophotometric 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 nitroprussid 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 ºC 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:

\[ I(\%) = 100 - \frac{100 \times (T/C)}{ } \]

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.

IC₅₀ 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. IC₅₀ 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*, *Cucuruma longa*, and *Citrus aurantium* extracts have shown inhibitory activity with IC₅₀ values of less than 500 µg/mL(Table 1). Further examinations and IC₅₀ 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 *Acorus calamus* (88.77 µg/mL), respectively (Table 2).

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

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### Table 1. Urease inhibitory activity of plants extract.

| No. | Scientific Name      | Family         | Common Name (English) | Part used | IC₅₀ (μg/mL) |
|-----|----------------------|----------------|-----------------------|-----------|--------------|
| 1   | *Achillea millefolium* | Compositae     | Yarrow, Milfoil       | Flower    | 774.82       |
| 2   | *Acorus calamus*      | Araceae        | Sweet flag            | Root      | 88.77        |
| 3   | *Allium sativum*      | Liliaceae      | 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   | *Datura stramonium*   | 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 lupulas*     | Cannabinaceae  | Hops                  | Twig      | 651.91       |
| 13  | *Hyssopus officinalis* | Labiatae     | Hyssop                | Herb      | 703.12       |
| 14  | *Laurus nobilis*      | Lamiaceae      | 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       |

### Table 2. IC₅₀ (μg/mL) and medicinal uses of most active plants.

| Scientific name         | Effects and medicinal uses                           | IC₅₀ (μg/mL) |
|-------------------------|------------------------------------------------------|--------------|
| 1                       | *Zingiber officinale*                                | 48.54        |
| 2                       | *Laurus nobilis*                                     | 48.69        |
| 3                       | *Nigella sativa*                                     | 59.10        |
| 4                       | *Angelica archangelica a*                            | 64.03        |
| 5                       | *Acorus calamus*                                     | 88.77        |
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