Large scale screening of commonly used Iranian traditional medicinal plants against urease activity

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Abstract

Background and purpose of the study: H. pylori infection is an important etiologic impetus usually leading to gastric disease and urease enzyme is the most crucial role is to protect the bacteria in the acidic environment of the stomach. Then urease inhibitors would increase sensitivity of the bacteria in acidic medium.

Methods: 137 Iranian traditional medicinal plants were examined against Jack bean urease activity by Berthelot reaction. Each herb was extracted using 50% aqueous methanol. The more effective extracts were further tested and their IC50 values were determined.

Results: 37 plants out of the 137 crude extracts revealed strong urease inhibitory activity (more than 70% inhibition against urease activity at 10 mg/ml concentration). Nine of the whole studied plants crude extracts were found as the most effective with IC50 values less than 500 μg/ml including; Rheum ribes, Sambucus ebulus, Pistachia lentiscus, Myrtus communis, Areca catechu, Citrus aurantifolia, Myristica fragrans, Cinnamomum zeylanicum and Nicotiana tabacum.

Conclusions: The most potent urease inhibitory was observed for Sambucus ebulus and Rheum ribes extracts with IC50 values of 57 and 92 μg/ml, respectively.

Keywords: Urease inhibitor, Iranian traditional medicinal plants, Sambucus ebulus, Rheum ribes, Screening of natural products

Introduction

Ureas (urea amidohydrolases, EC (3.5.1.5) are a group of widespread enzymes in nature, classified as the most proficient enzymes (with proficiency more than 10^{14}), stand as protagonist in biochemistry for several reasons. Urease was the first ureolytic enzyme obtained and named in the late nineteenth century, with landmark significance in enzymology as the first enzyme crystallized (in 1926 by Sumner) to approve the proteinous nature of the enzymes [1]. Also, as ascertained by Dixon et al. in 1975, urease was the first enzyme shown to possess nickel ions in its active site, essential for activity [2]. Since its substrate; urea is pervasively available in nature, urease was important to provide organisms with nitrogen in the form of ammonia for growth [3]. Despite the diversity in the molecular structures of urease, the amino acid sequences of the active sites are principally similar in all of the known them and consequence of this fact is the same catalytic mechanism. The active sites are always located in α subunits and contain the binuclear nickel centre, in which the Ni–Ni distances range from 3.5 to 3.7 Angstrom [4].

Urease as the most characteristic feature of Helicobacter pylori constitutes 5–10% of the bacteria’s proteins. H. pylori a microaerophilic, gram-negative spiral bacterium which was first detected in 1984 by Marshall et al, is one of the most common chronic bacterial pathogens in humans [5]. Approximately more than 50% of people in the world are infected with it, and its prevalence is significantly higher in developing countries in compare with the developed ones. H. pylori infection is an important etiologic impetus usually leading to chronic gastritis, gastro duodenal ulcer and low grade gastric mucosa-associated lymphoid tissue lymphoma. Epidemiological data show
Table 1 Urease inhibitory activity of plants extract at concentration of 10 mg/ml

| Scientific name                  | Plant family | Common name in English | Common name in Persian | Part used in traditional | Inhibition (%) |
|----------------------------------|--------------|------------------------|------------------------|--------------------------|----------------|
| 1. Abrus precatorius             | Fabaceae     | Paternoster Seed       | Cheshm-e khorus         | Seed                     | 9.21 ± 0.04    |
| 2. Acacia Senegal                | Fabaceae     | Gum Arabic             | Samgh-e arabi          | Gum                      | 12.81 ± 0.09   |
| 3. Acanthophyllum squarosum      | Asparagaceae  | Soap Root              | Chubak                 | Root                      | 14.15 ± 0.02   |
| 4. Alpinia officinarum           | Zingiberaceae| Galangal               | Khulanjan              | Rhizome                  | 41.75 ± 0.05   |
| 5. Althaea officinalis           | Malvaceae    | Hollyhoch              | Khatmi                 | Flower                    | 20.94 ± 0.06   |
| 6. Alyssum homolocarpum          | Brassicaceae  | Madword & Pepper Weed  | Qodume                 | Seed                      | 13.57 ± 0.12   |
| 7. Amaranthus lividus            | Amaranthaceae | Cock’s Comb Seed      | Tokhm-e tajkhoros      | Seed                      | 17.48 ± 0.11   |
| 8. Anethum graveolins            | Apiaceae     | Dill Seed              | Tokhm-e shevid         | Seed                      | 37.50 ± 0.03   |
| 9. Apium graveolens              | Apiaceae     | Celer Seed             | Tokhm-e karafs         | Seed                      | 24.3 ± 0.01    |
| 10. Aquilaria sinensis           | Thymeaceae   | Agarwood               | Udeqamari              | Fruit                     | 32.03 ± 0.08   |
| 11. Arcticum Lappa               | Asteraceae   | Burdock Root           | Bâbââdam               | Root                      | 19.99 ± 0.08   |
| 12. Arca catechu                 | Arecaceae    | Betel Nuts             | Fufel                  | Fruit                     | 96.67 ± 0.01   |
| 13. Artemisia absinthum          | Asteraceae   | Worm Wood              | Afsantin               | Herb                      | 52.50 ± 0.06   |
| 14. Artemisia dracunculus        | Asteraceae   | Tarragon               | Tarkhon                | Leaf                      | 57.53 ± 0.03   |
| 15. Asperugo procumbens          | Boraginaceae | German Madwort         | Bâdranjbuye            | Herb                      | 12.43 ± 0.02   |
| 16. Astragalus arbusculinus      | Fabaceae     | Sarcocolla             | Anzarut                | Gum                       | 17.68 ± 0.06   |
| 17. Astragalus gossypinus        | Fabaceae     | Gum Tragacanth         | Katirâ                 | Gum                       | 1.33 ± 0.02    |
| 18. Bambusa vulgaris             | Poaceae      | Golden Bamboo          | Tabâshir sadaf         | Secretions               | 12.81 ± 0.04   |
| 19. Brassica nigra               | Brassicaceae | Mustard                | Kharde                | Seed                      | 27.63 ± 0.01   |
| 20. Calendula officinalis        | Asteraceae   | Marigold               | Hamishe bahar          | Flower                    | 0.16 ± 0.06    |
| 21. Calendula sp.                | Asteraceae   | Marigold               | Hamishe bahar          | Flower                    | 8.21 ± 0.07    |
| 22. Camelia sinensis             | Theaceae     | Green Tea              | Chây-e sabz            | Leaf                      | 89.40 ± 0.02   |
| 23. Camelia sinensis             | Theaceae     | Green Tea              | Châyeparsefid          | Twig                      | 90.45 ± 0.01   |
| 24. Cannabis sativa              | Cannabaceae  | Hemp Seed              | Shâhâdane              | Seed                      | 9.71 ± 0.02    |
| 25. Capsicum annuum              | Solanaceae   | Red Pepper             | Felfel-e qermez        | Fruit                     | 99.01 ± 0.01   |
| 26. Carthamus tinctorius         | Asteraceae   | Saf                    | Golrang                | Flower                    | 50.78 ± 0.04   |
| 27. Cassia angustifolia          | Fabaceae     | Senna                  | Sena                   | Leaf                      | 3.29 ± 0.03    |
| 28. Celosia cristata             | Amaranthaceae| Cockscomb              | Gol-e halva            | Flower                    | 82.55 ± 0.03   |
| 29. Centaurea sp.                | Asteraceae   | Centaurea              | Gol-e gandom           | Flower                    | 70.33 ± 0.02   |
| 30. Chenopodium botrys           | Amaranthaceae| Lamb’s Quarter         | Dermane-e torki        | Herb                      | 15.13 ± 0.04   |
| 31. Chichorum intybus            | Asteraceae   | Chicory                | Kâsni                  | Herb                      | 40.55 ± 0.04   |
| 32. Cinchona officinalis         | Rubiaceae    | Cinchona               | Gne gne                | Bark                      | 67.03 ± 0.02   |
| 33. Cinnamonum camphora          | Lauraceae    | Camphre                | Kâfûr                  | Camphor                   | 10.14 ± 0.08   |
| 34. Cinnamonum cassia            | Lauraceae    | Cassia                 | Salikhâ               | Bark                      | 91.19 ± 0.02   |
| 35. Cinnamonum zeylanicum        | Lauraceae    | Cinnamon               | Darchin                | Bark                      | 84.22 ± 0.05   |
| 36. Citrus aurantifolia          | Rutaceae     | Limu Fruit             | Limu ammâni            | Fruit                     | 99.02 ± 0.02   |
| 37. Citrus aurantium             | Rutaceae     | Bitter Orange Peel     | Khâtâl-e närenj        | Rind                      | 1.43 ± 0.05    |
| 38. Citrus bigardia              | Rutaceae     | Orange                 | Gol-e närenj           | Twig                      | 24.31 ± 0.03   |
| 39. Colchicum macrophyllum       | Colchicaceae | Colchicum Cors         | Suranjan               | Corm                      | 9.44 ± 0.08    |
| No. | Plant Name                  | Family         | Part of Plant | Inhibitory Activity |
|-----|----------------------------|----------------|---------------|---------------------|
| 40. | Commiphora molmol         | Burseraceae    | Gum           | 8.22 ± 0.04         |
| 41. | Crataegus microphylla     | Rosaceae       | Flower        | 82.19 ± 0.03        |
| 42. | Curcuma zedoaria          | Zingiberaceae  | Seed          | 4.70 ± 0.06         |
| 43. | Cuscuta epithymum         | Convolvulaceae | Herb          | 9.66 ± 0.01         |
| 44. | Cymbopogon                | Poaceae        | Root          | 14.02 ± 0.03        |
| 45. | Descurainia               | Brassicaceae   | Seed          | 21.81 ± 0.01        |
| 46. | Diploptenia damavendica   | Apiceae        | Gozal         | 12.59 ± 0.06        |
| 47. | Doronicum bracteatum      | Asteraceae     | Darunj-e aqrabi| 10.73 ± 0.01        |
| 48. | Dracaena cinnabari        | Asparagaceae   | Khone siyavosh| 49.49 ± 0.13        |
| 49. | Dracocephalum             | Lamiaceae      | Badrashbi     | 3.95 ± 0.01         |
| 50. | Echinophora platyloba     | Apiaceae       | Khosharize    | 17.48 ± 0.01        |
| 51. | Echium amoenum            | Boraginaceae   | Flower        | 31.66 ± 0.02        |
| 52. | Elaeagnus angustifolia    | Elaeagnaceae   | Senjed        | 4.67 ± 0.14         |
| 53. | Elaeagnus angustifolia    | Elaeagnaceae   | Flower        | 27.45 ± 0.01        |
| 54. | Elettaria cardamomum      | Zingiberaceae  | Hel sabz      | 13.16 ± 0.04        |
| 55. | Elettaria cardamomum      | Zingiberaceae  | Hel sefid     | 6.80 ± 0.07         |
| 56. | Elettaria cardamomum      | Zingiberaceae  | Hel siyah     | 5.78 ± 0.07         |
| 57. | Equisetum arvense         | Equisetaceae   | Dom-e asb     | 52.35 ± 0.05        |
| 58. | Eruca sativa              | Brassicaceae   | Seed          | 13.28 ± 0.05        |
| 59. | Eucalyptus sp.            | Myrtaceae      | Okaliptus     | 47.92 ± 0.01        |
| 60. | Euphorbia sp.             | Euphorbiaceae  | Gav kosh      | 68.94 ± 0.03        |
| 61. | Ferula assa-foetida       | Umbelliferae   | Anqoze        | 34.07 ± 0.04        |
| 62. | Helicteres isora          | Malvaceae      | Screw Tree Pod| 8.18 ± 0.02         |
| 63. | Heracleum persicum        | Apiaceae       | Golpar        | 10.27 ± 0.02        |
| 64. | Hibiscus gossypifolius    | Malvaceae      | Chay-e Makki  | 96.28 ± 0.02        |
| 65. | Humulus lupulus           | Cannabaceae    | Râzak         | 54.85 ± 0.02        |
| 66. | Hypericum perforatum      | Hypericaceae   | St.John’s Wort| 97.99 ± 0.02        |
| 67. | Juglans regia             | Juglandaceae   | Pust-e vasat-e gerdo| 93.62 ± 0.01 |
| 68. | Juglans regia             | Juglandaceae   | Pust-e gerdo  | 1.27 ± 0.06         |
| 69. | Juniperus Sabina          | Cuppressaceae  | Abhal         | 19.63 ± 0.01        |
| 70. | Lactuca sativa            | Asteraceae     | Lettuce       | 2.93 ± 0.04         |
| 71. | Lawsonia inermis          | Lythraceae     | Hana          | 54.00 ± 0.06        |
| 72. | Levisticum officinals     | Apiaceae       | Anjadán romi  | 10.00 ± 0.06        |
| 73. | Linum usitatissimum       | Linaceae       | Tokhm-e katan | 2.71 ± 0.18         |
| 74. | Malabaila secacule        | Apiaceae       | Dogho         | 18.18 ± 0.04        |
| 75. | Malva sylvestris          | Malvaceae      | Gol-e panirak | 14.15 ± 0.05        |
| 76. | Matricaria chamomilla     | Asteraceae     | Bâbon-e shirazi| 87.21 ± 0.01       |
| 77. | Melissa officinalis       | Lamiaceae      | Balm          | 46.22 ± 0.05        |
Table 1 Urease inhibitory activity of plants extract at concentration of 10 mg/ml (Continued)

| No. | Taxonomy                          | Plant Part          | Plant Name                  | Genus                  | Species                  | Activity (IC50) ± SD (mg/ml) |
|-----|-----------------------------------|---------------------|-----------------------------|------------------------|--------------------------|------------------------------|
| 78  | Mentha spicata                    | Lamiaceae Mint      | NaAna                       | Mint                   | Mentha spicata            | 93.89 ± 0.01                 |
| 79  | Myristica fragrans                | Myristicaceae Nutmeg| Joz-e buya                  | Nutmeg                 | Myristica fragrans        | 78.19 ± 0.01                 |
| 80  | Myrtus communis                   | Myrtaceae Myrtle    | Murd                        | Myrtle                 | Myrtus communis           | 72.99 ± 0.01                 |
| 81  | Nasturtium officinale             | Brassicaceae Watercress| Boolagoti                   | Watercress             | Nasturtium officinale     | 74.00 ± 0.03                 |
| 82  | Nerium oleander                   | Apocynaceae Nerium  | Gol-e Kharzahre             | Nerium                 | Nerium oleander           | 84.62 ± 0.01                 |
| 83  | Nicotiana Tabacum                 | Solanaceae Tobacco | Tutun                       | Tobacco                | Nicotiana Tabacum         | 52.77 ± 0.03                 |
| 84  | Nicotiana tabacum                 | Solanaceae Tobacco  | Tutun                       | Stem                   | Nicotiana tabacum         | 75.26 ± 0.05                 |
| 85  | Nymphaea alba                     | Nymphaeaceae White Lotus| Goel-e nilofar              | White Lotus            | Nymphaea alba             | 97.86 ± 0.01                 |
| 86  | Ocimum basilicum                  | Lamiaceae Basil     | Reyhan-e banafsh            | Leaf                   | Ocimum basilicum          | 19.61 ± 0.05                 |
| 87  | Ocimum basilicum                  | Lamiaceae Basil     | Reyhan-e sabz               | Leaf                   | Ocimum basilicum          | 0.41 ± 0.01                  |
| 88  | Oenothera biennis                 | Onagraceae Evening Star| Goel-e maghrebi            | Evening Star           | Oenothera biennis        | 3.95 ± 0.04                  |
| 89  | Olea europaea                     | Oleaceae Olive Leaf | Barg-e zyton                | Leaf                   | Olea europaea             | 72.30 ± 0.01                 |
| 90  | Orchis latifolia                  | Orchidaceae Oriental Salp| SaAlab-e panji             | Root                   | Orchis latifolia          | 18.90 ± 0.02                 |
| 91  | Orchis mascula                    | Orchidaceae Male Orch| SaAlab-e qolvei             | Root                   | Orchis mascula            | 3.16 ± 0.04                  |
| 92  | Papaver Rhoeas                    | Papaveraceae Corn Poppy| Goel-e shagheyegh           | Corn Poppy             | Papaver Rhoeas            | 27.25 ± 0.12                 |
| 93  | Papaver Rhoeas                    | Papaveraceae Corn Poppy| Goel-e shagheyegh           | Corn Poppy             | Papaver Rhoeas            | 97.50 ± 0.01                 |
| 94  | Papaver somnifenum                | Papaveraceae Opium Poppy| Khashkhash                   | Opium Poppy            | Papaver somnifenum        | 4.79 ± 0.03                  |
| 95  | Papaver somnifenum                | Papaveraceae Opium Poppy| Khashkhash                   | Opium Poppy            | Papaver somnifenum        | 35.95 ± 0.02                 |
| 96  | Passiflora caerulea               | Passifloraceae Passion Flower| Goel-e saAty                | Passion Flower         | Passiflora caerulea      | 46.90 ± 0.008                |
| 97  | Pelargonium graveolens            | Geraniaceae Geranium| Barg-e atr                  | Geranium               | Pelargonium graveolens    | 92.19 ± 0.01                 |
| 98  | Pelargonium graveolens            | Geraniaceae Rose Pelargonium| Goel-e atr                 | Rose Pelargonium       | Pelargonium graveolens    | 96.87 ± 0.02                 |
| 99  | Pterocarpus rubra                 | Fabaceae Mukwa      | Sandal-e sorkh              | Bark                   | Pterocarpus rubra         | 91.75 ± 0.01                 |
| 100 | Petrosemum hortense               | Apiaceae Parsley Seed| Tokhm-e jafari              | Seed                   | Petrosemum hortense      | 50.35 ± 0.03                 |
| 101 | Pistacia lentiscus                | Anacardiaceae Lentisk Pistache| Mastaki                    | Lentisk Pistache      | Pistacia lentiscus       | 92.37 ± 0.01                 |
| 102 | Pistacia vera                     | Anacardiaceae Pistachio Nut Shell| Pust-e peste               | Pistachio Nut Shell    | Pistacia vera            | 97.71 ± 0.01                 |
| 103 | Plantago major                    | Plantaginaceae Great Plantain| Barhang                    | Great Plantain        | Plantago major           | 4.69 ± 0.11                  |
| 104 | Polyergus officinalis             | Fomítospсидеae White Agaric| Ghariqun                    | White Agaric           | Polyergus officinalis     | 19.97 ± 0.06                 |
| 105 | Portulaca oleracea                | Portulacaceae Common Purslane| Tokhm-e khorfe             | Common Purslane       | Portulaca oleracea       | 7.19 ± 0.03                  |
| 106 | Prunus persica                    | Rosaceae Peach      | Barge-e holo                | Fruit                 | Prunus persica           | 9.47 ± 0.11                  |
| 107 | Punica granatum                   | Lythraceae Pomegranate Flower| Golnar                    | Pomegranate Flower    | Punica granatum          | 99.90 ± 0.01                 |
| 108 | Punica granatum                   | Lythraceae Pomegranate| Golnar                    | Pomegranate           | Punica granatum          | 99.90 ± 0.01                 |
| 109 | Quercus infectoria                | Fagaceae Oak Gall    | Qolqaf                     | Oak Gall               | Quercus infectoria       | 53.97 ± 0.02                 |
| 110 | Quercus infectoria                | Fagaceae Oak Fruit Hull| Jaft                        | Oak Fruit Hull        | Quercus infectoria       | 98.84 ± 0.02                 |
| 111 | Rheum nobile                      | Polygonaceae Rhubarb| Rivas                      | Root                  | Rheum nobile             | 98.93 ± 0.01                 |
| 112 | Rosa centifolia                   | Rosaceae Damask Rose| Goel-e sorkh              | Damask Rose            | Rosa centifolia          | 97.51 ± 0.01                 |
| 113 | Rosa foetida                      | Rosaceae Rosa Lutea  | Goel-e zard                | Rosa Lutea             | Rosa foetida             | 89.19 ± 0.023                |
| 114 | Rosmarinus angustifolia           | Lamiaceae Pine Rosemary| Rozmany-e aklilaljabal    | Pine Rosemary          | Rosmarinus angustifolia  | 22.51 ± 0.02                 |
| 115 | Rubia tinctoria                   | Rubiaceae Madder Root| Ronas                     | Root                  | Rubia tinctoria          | 37.31 ± 0.02                 |
| 116 | Ruta graveolens                   | Rutaceae Rue        | Sobad                      | Leaf                  | Ruta graveolens          | 27.91 ± 0.04                 |
| 117 | Saccharum officinarum             | Poaceae Sugar Cane  | Shekar-e sorkh             | Sugar Cane            | Saccharum officinarum    | 35.04 ± 0.06                 |
| 118 | Salix aegyptiaca                 | Salicaceae Aegyption Willow| Bidmeshk                | Flower                | Salix aegyptiaca        | 17.05 ± 0.02                 |
| 119 | Salix sp.                         | Salicaceae Whitewillow| Pust-e bid                 | Bark                  | Salix sp.               | 38.10 ± 0.01                 |
that high *H. pylori* infection rate, result in the incidence of gastric cancer and adenocarcinoma [6,7]. Urease catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide, and the most crucial role is to protect the bacteria in the acidic environment of the stomach [8]. It has been also reported that ammonia and monochloramine, which is a reaction product of ammonia and hypochlorous acid, exhibit potent toxicity in gastric epithelium [9]. Moreover, it has been demonstrated that *H. pylori* lacking urease activity are incapable of causing infection in animal models. Thus, it is most likely that urease is essential for bacterial colonization and perhaps the pathogenesis of related disease in vivo.

World Health Organization (WHO) has categorized *H. pylori* as a class 1 carcinogen [10]. Fortunately, its eradication with antibiotics can result in ulcer healing, prevent peptic ulcer recurrence and reduce the prevalence of gastric cancer in high-risk populations. However, it is not always successful because of its resistance to one or more antibiotics and other factors such as poor patient compliance, undesirable side effects of the drugs and significant cost of combination therapy [11]. Wolle et al. reported that approximately 20% of the patients undergoing antibiotics therapy would experience therapeutic failure [12]. 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 anti-*H. pylori* agents that can hopefully eradicate the invasion and presence of survived *H. pylori* strains to avoid relapse of gastric ulcer. Hence, a considerable variety of studies involving tests for medicinal plants showing antimicrobial activity and discrepant susceptibility test results are available due to variations in the methods and conditions used for its susceptibility testing.

One of the best sources of new substances to treat *H. pylori* is natural products and their derivatives [13]. Variety of techniques such as synthesizing [6], and also molecular modeling and virtual screening methods [14,15] have been applied to find possible urease inhibitors. The biological activity of plant-derived substances may be considered as a source of new anti-*H. pylori* drugs come from different classes of compounds and are characterized by the diversity of their structures. Therefore, almost all traditional Iranian herbal medicines that are used as remedies and sold as medicines to manage different diseases were screened to discover possible plant-derived urease inhibitors.

### Methods

#### Materials

Sodium nitroprusside (sodium pentacyanonitrosyloferrate III) and urease (EC 3.5.1.5) from Jack beans were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical reagent grade from Merck. Deionized water was used in all experiments. Potassium

| Plant Name | Scientific Name | Family | Part | Potency (Remarks) |
|------------|-----------------|--------|------|------------------|
| 120. | *Salvia hydrangea* | Lamiaceae | Mountain Sage | Gol-e arune | Flower | 91.09 ± 0.01 |
| 121. | *Salvia macrosiphon* | Lamiaceae | Wild Sage Seeds | Thokhm-e marv | Seed | 2.86 ± 0.04 |
| 122. | *Sambucus ebulus* | Adoxaceae | Dwarf Elder | Tarásit | Fruit | 99.70 ± 0.01 |
| 123. | *Santalum album* | Santalaceae | Sandalwood | Sandal-e sefid | Bark | 58.69 ± 0.02 |
| 124. | *Satureja hortensis* | Lamiaceae | Savory Seed | Tokhm-e marze | Seed | 35.77 ± 0.04 |
| 125. | *Scrophularia striata* | Scrophulariaceae | Striata Figwort | Mokhalace | Stem & Flower | 16.47 ± 0.05 |
| 126. | *Sinapis alba* | Brassicaceae | White Mustard | Khardal-e sefid | Seed | 39.77 ± 0.06 |
| 127. | *Spinacia oleracea* | Amaranthaceae | Spinach Seed | Tokhm-e esfenaj | Seed | 19.76 ± 0.04 |
| 128. | *Taraxacum sp.* | Asteraceae | Dandelion | Ghasedak | Flower | 14.83 ± 0.01 |
| 129. | *Thymus kotschyanus* | Lamiaceae | Kotschyan Thyme | Avishan | Herb | 17.94 ± 0.01 |
| 130. | *Tilia platyphyllos* | Malvaceae | Linden | Zirfun | Leaf & Flower | 25.79 ± 0.01 |
| 131. | *Trigonella foenum-graecum* | Fabaceae | Fenugreek Seed | Tokhm-e shanbalile | Seed | 44.02 ± 0.02 |
| 132. | *Triticum sativum* | Poaceae | Wheat | Sabos-e ghandom | Husk | 16.14 ± 0.04 |
| 133. | *Tussilago farfara* | Asteraceae | Coltrs-foot | Pakhari | Herb | 69.08 ± 0.01 |
| 134. | *Veratrum album* | Melanthiaceae | White Hellebore | Kharbogh | Leaf | 96.85 ± 0.06 |
| 135. | *Verbascum georgicum* | Scrophulariaceae | Mullein | Dom-e gav | Leaf | 30.40 ± 0.03 |
| 136. | *Verbascum sp.* | Scrophulariaceae | Mullein | Marg-e mâhi | Fruit | 0.82 ± 0.05 |
| 137. | *Ziziphus vulgaris* | Rhamnaceae | Jujube | Annâb | Fruit | 26.34 ± 0.01 |
| 138. | *Hydroxyurea* | ———— | ———— | ———— | Reference compound | 100 ± 0.01 |
Table 2 IC50 and medicinal uses of most active plants

| Scientific name | Effects & medicinal uses                                                        | IC50 (μg/ml) | Std. Error | log IC50 |
|-----------------|---------------------------------------------------------------------------------|--------------|------------|----------|
| 1. A. catechu   | Anthelmintic, gastric tonic                                                      | 216          | 0.01       |          |
| 2. C. cristata  | Styptic, depurative, sedative, constipating, antibacterial, febrifuge,          | 6175         | 0.68       |          |
| 3. C. annuum    | Anti flatulence, gout, gastric tonic, paralysis                                  | 751          | 0.14       |          |
| 4. C. aurantifolia | Appetitive, anti-flatulence, analgesic                                          | 432          | 0.06       |          |
| 5. C. cassia    | Gastric tonic, anti-spasmodic, anti-flatulence                                  | 867          | 0.05       |          |
| 6. C. microphylla | Anti-flatulence, gastric tonic                                                  | 665          | 0.14       |          |
| 7. C. sinensis  | Anti-bacterial, anti-diarrhea, diuretic, astringent, reduce cholesterol          | 579          | 0.04       |          |
| 8. C. zeylanicum | Gastric tonic, anti-flatulence, ‘anti-spasmodic’                                | 361          | 0.02       |          |
| 9. C. sinensis  | Anti-diarrhea, diuretic, astringent, anti-bacterial, reduce cholesterol          | 1314         | 0.04       |          |
| 10. C. microphylla | Anti-inflammatory, astringent, emmenagogue, sedative                          | 5152         | 0.05       |          |
| 11. H. gossypifolius | Analgesic, anti-tussive, demulcent, diuretic, febrifuge, highly emollient, slightly laxative and odontalgic, anti-inflammations and laryngitis, | 819          | 0.01       |          |
| 12. H. perforatum | Astringent, analgesic, anti-inflammatory, anti-anxiety aphrodisiac              | 3509         | 0.10       |          |
| 13. J. regia    | Anti-inflammatory, astringent, anti-spasmodic                                   | 1271         | 0.08       |          |
| 14. M. chamomilla | Anti-inflammation, appetitive, and aids digestion and sleep, acts as a diuretic and nerve tonic. | 3188         | 0.02       |          |
| 15. M. fragrans | Anti-flatulence appetitive ‘anti-spasmodic’ antisepic, analgesic, anti-inflammatory | 215          | 0.15       |          |
| 16. M. spicata  | Analgesic, ‘anti-spasmodic, anti-flatulence’                                    | 7822         | 0.17       |          |
| 17. M. communis | Antiseptic, disinfectant, expectorant, deodorizer                              | 170          | 0.04       |          |
| 18. N. officinale | Diuretic, expectorant, purgative, hypoglycemic, odontalgic, stimulant, tonic and stomachic | 2055         | 0.19       |          |
| 19. N. alba     | Astringent, antiseptic, anesthetic, aphrodisiac, sedative, used for gastrointestinal disorders and jaundice | 820          | 0.19       |          |
| 20. N. Oleander | Dermatitis, abscesses, eczema, psoriasis, sores, warts, corns, ringworm, scabies, herpes, skin cancer, asthma, dysmenorrheal, epilepsy, malaria, | 9877         | 0.26       |          |
| 21. N. tabacum  | Anti-spasmodic, diuretic, sedative, sialagogue                                 | 473          | 0.15       |          |
| 22. O. europea  | Hypotensive, diuretic, hypoglycemic                                             | 2857         | 0.06       |          |
| 23. P. granatum(Exlnd) | Hypoglycemic, anti-cancer, anthelmintic                                        | 1484         | 0.10       |          |
| 24. P. granatum(Exlnd) | Hypoglycemic, anti-cancer, anthelmintic                                        | 1331         | 0.11       |          |
| 25. P. graveolens | Anti-inflammatory, antiseptic, aromatherapy, astringent, anti-cancer, sedative | 976          | 0.03       |          |
| 26. P. graveolens | Analgesic, anti-Bacterial, anti-Depressant, anti-inflammatory, antiseptic, astringent, diuretic, insect repellent, refreshing, relaxing, sedative, styptic, tonic | 1242         | 0.14       |          |
| 27. P. lentiscus | Antibacterial                                                                   | 121          | 0.03       |          |
| 28. P. Rhoes    | Anodyne, emmenagogue, emollient, expectorant, hypnotic, sedative, tonic         | 5636         | 0.04       |          |
| 29. P. rubra    | Astringent, tonic                                                               | 930          | 0.06       |          |
| 30. P. vera     | Aphrodisiac, anti-anxiety                                                      | 4687         | 0.12       |          |
| 31. Q. infectoria | Gingivitis, infectoria, anti-diabetic, anti-tremorone, local anesthetic, antiviral, antibacterial, antifungal. | 1214         | 0.12       |          |
| 32. R. centifolia | Anti-inflammatory, antispasmodic, aphrodisiac, astringent, depurative, laxative, analgesic, appetitive | 544          | 0.07       |          |
| 33. R. foetida  | Heart diseases, digestive, skin diseases, muscular pains, anti-parasite         | 2441         | 0.19       |          |
| 34. R. ribes    | Appetitive, astringent, anti bacteria, anti depressive and used to treat diabetes, hemorrhoids, ulcer, diarrhea | 92           | 0.06       |          |
| 35. S. hydrangea | anti-flatulence, astringent, anti-spasmodic                                    | 2960         | 0.11       |          |
| 36. S. ebulus   | Anti-inflammatory, antinociceptive, anti-cancer, anti-angiogenic, anti-oxidative | 57           | 0.05       |          |
| 37. V. album    | Analgesic, anthelmintic, cathartic, emetic, expectorant, hypnotic               | 1037         | 0.07       |          |
| 38. hydroxyurea |                                                                   | 37           | 0.02       |          |
phosphate buffer (100 mM), pH 7.6 was prepared in distilled water.

The studied plants were collected from local medicinal herb shops, Tehran, Iran (June 2010) 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 Sciences.

**Extract preparation**

10 g of air-dried and powdered plant material was extracted in 10 ml, 50:50 methanol: water at room temperature for 24 hrs. The resulting liquid extract was filtered and concentrated to dryness under reduced pressure. The dry extracts were stored at -20°C till used [16].

**Determination of urease activity**

All extracts were tested for urease inhibitory at concentration of 10 mg/ml by the modified spectrophotometric method developed by Berthelot reaction [17]. For herbal extracts that were proven to exert significant inhibition and also for positive controls, inhibitory assays were performed. The plant extracts were tested in a concentration range of 0 to 10 mg/ml. Hydroxyurea was used as standard inhibitor.

The solution assay mixture consisted of urea (30 mM) and (100 μl) crud extract with a total value of 950 μl. The reactions were initiated by the addition of 50 μl of urease enzyme solution in phosphate buffer (100 mM, pH 7.6, 1 mg/ml). Urease activity was determined by measuring ammonia concentration after 15 minutes of enzymatic reaction. The ammonia was determined using 500 μl of solution A (contained 5.0 g phenol and 25 mg of sodium nitroprusside in 500 ml of distilled water) and 500 μl of solution B (contained of 2.5 g sodium hydroxide and 4.2 ml of sodium hypochlorite 5% in 500 ml of distilled water) at 37°C for 30 minutes. The absorbance was read at 625 nm. Activity of uninhibited urease was designated as the control activity of 100%.

**Data processing**

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

\[
I(\%) = \left[1 - \frac{T}{C}\right] \times 100
\]

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.

**Determination of IC\(_{50}\) values**

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**

Medicinal plants as an appropriate and renewable source of active chemical compounds can be used as templates to discover new lead compounds. Doxorubicin, vincristine, and taxol, are examples of these herbal compounds which are clinically applied. According to the literature, 50% of commercially presented medicines in 1985 was from herbal origins [18]. Gastrointestinal diseases, especially gastric, duodenal and peptic ulcer, arise from different factors, in particular microbial agent \(H. pylori\).
Common multi-drug therapies not only have side effects, but are also expensive. On the other hand, the probability of drug resistance occurrence and disease retrogression is quite concerning. Already reported studies have shown that herbal compounds have the ability to prevent this microbe. Among the studied herbal essences and extracts, many did not exceed the study level due to production limits, toxicity and impossibility of drug form preparation. Majority of the researches have focused on ways to inhibit the bacteria growth or its elimination from the culture, while a few has particularly concerned inhibition of urease enzyme which is responsible for the bacteria defense system against the stomach very acidic medium.

Specific inhibition or reduction of urease enzyme activity would result in an increased sensitivity of the bacteria in acidic medium and therefore it's natural elimination by stomach acidic condition or the body immune system.

In the presented study, urease enzyme inhibition potency of 137 herbal extracts was investigated from which 37 extracts have shown inhibitory activity up to more than 70% in the concentration of 10 mg/ml (Table 1). Further examinations and IC50 determination revealed that Sambucus ebulus, Rheum ribes, Pistacia lentiscus, Myrtus communis, Myristica fragrans, Aerea catechu, Cinnamomum zeylanicum, Citrus aurantifolia and Nicotiana tabacum extracts inhibit urease enzyme in concentrations less than 500 μg/ml. It should also be mentioned that C. zeylanicum, M. chamomilla and M. spicata are already used as gastrointestinal remedies and this research has proved that these herbs can inhibit urease activity and prevent gastric upsets. Names of the studied plants and the 37 final more active extracts are presented in Table 2. As it is shown, the most potent urease inhibitory was observed for S. ebulus and R. ribes with IC50 values less than 100 μg/ml.

S. ebulus (Figure 1) is a native perennial herb of the Adoxaceae family [19]. It has been prescribed in traditional medicines for the treatment of inflammatory reactions, such as hemorrhoid, bites and sore-throat. In addition, S. ebulus has been shown to have anti-inflammatory, antinociceptive, anti-cancer, anti-angiogenic and anti-oxidative activities. Ebulitin, ebulin 1, flavonoid, anthocyanin and other components have been isolated from S. ebulus and identified as active ingredients of biological and pharmacological activities [20]. The anti-H. pylori effect of the S. ebulus extract was observed by using the agar dilution method [13].

R. ribes (Figure 2) is a hardy perennial, cultivated in some temperate countries for its edible red leaf stalks [21]. It is used to treat diabetes, hemorrhoids, ulcer, diarrhea, and expectorant activity reported. The efficacy and safety of a hydroalcoholic extract of R. ribes in treatment of mild to moderate major depression disorder has been investigated and the observations show some anti depressive effects. The methanolic extract of R. ribes have demonstrated anti-ulcer activity comparable with standard drugs cimetidine [22].

According to strong inhibitory activity of the herbs presented in Table 2, simultaneous application of theses herbs and the medicines prescribed in gastrointestinal disease therapies would fasten the treatment. Additionally, isolation of active compounds and further investigation of each isolated compound against urease activity would lead to new chemical structures which may have the potency to inhibit urease activity.

Competing interests
There are no other conflicts of interest related to this publication.

Authors’ contributions
All authors contributed to the concept and design, making and analysis of data, drafting, revising and final approval. MA and BY are responsible for the study registration. FN and KB carried out plant extraction and enzymatic tests and drafted manuscript. MHR, FN, MA and BY participated in collection and/or assembly of data, data analysis, interpretation and manuscript writing. All authors read and approved the final manuscript.

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