European Journal of Medicinal Plants
6(4): 223-230, 2015, Article no.EJMP.2015.058
ISSN: 2231-0894

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Anti-urease Activity of *Mimusops elengi* Linn (*Sapotaceae*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HZ and GHR designed the study. Author HZ wrote the protocol and wrote the first draft of the manuscript. Author HS performed the statistical analysis. Author AK managed the analyses of the study, authors AK and ST carried out the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/12240
Editor(s):
(1) Marcello Iriti, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anonymous, Mexico.
(2) Anonymous, Iran.
(3) Anonymous, Iran.
(4) Anonymous, Palestine.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=914&id=13&aid=7970

Original Research Article

Received 24th June 2014
Accepted 24th November 2014
Published 31st January 2015

ABSTRACT

Aim: *Mimusops elengi* L. (*Sapotaceae*) commonly known as Bakul is a small to large evergreen tree found all over the different parts of the world. Literature survey showed no reports on the anti urease activity of the flowers and leaves of *Mimusops elengi*. The present study was therefore envisaged to evaluate the anti urease activity of the methanolic extracts (MFE and MLE) of *M. elengi*.
Study Design: Assessment of anti urease activity and total phenolic contents.
Methodology: For the determination of total phenolic content of *M. elengi* extracts (MFE and MLE) the reaction mixture contained 50% Folin-Ciocalteu reagent (0.5 mL), 20% (w/v) sodium carbonate solution (2.5 mL), and gallic acid solution (1, 2.5, 5, 10, 25, 50, 100 μg/ml) or sample extracts (1.0

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mL). Urease inhibition activity was determined by mixing Urease (Jack bean) method was employed for urease inhibition activity.
Results: From the standard calibration curve of gallic acid the total phenolic contents in extracts (MFE and MLE) was found to be 7.5±0.28 mg/g, 96.8±4.6 mg/g. Urease inhibitory activity of *M. elengi* extracts (MFE and MLE) was comparable with standard Thiourea. The percentage urease inhibition of Thiourea was found to be 98.2%, IC_{50} 88.2 ± 0.01 μg/ml. The maximum urease inhibitory activity was shown by the leaves extract (MLE) i. e, 77.9% (IC_{50} 62.1±1.20 μg/ml) while methanolic extract of flower (MFE) produced 47.6% urease inhibition activity. The total phenolic contents in both methanolic extracts (MFE and MLE) was found to be 7.5±0.28 mg/g, 96.8±4.6 mg/g respectively.
Conclusion: It is, therefore, concluded that this plant can be a sources to isolate some natural urease inhibitory agents.

Keywords: Mimusops elengi Linn; H. pylori; urease inhibition; total phenolic content.

1. INTRODUCTION

Mimusops elengi (*Sapotaceae*) commonly known as Bakul is a small to large evergreen tree found all over the different parts of the world [1]. Bark, seed, flower and leaves of *M. elengi* are used as a remedy in the indigenous system of medicine for centuries. Flowers are to relieve headache used and to produce copious discharge from nose. They are also used as an anti-toxin. The flower extract is helpful not only in heart diseases but also employed as anti diuretic agent in polyuria condition [2-3]. These are also employed for making lotion which is used for wounds and ulcers. Internally bark skin is magnanimous in leucorrhoea, menorrhagia and is also notorious to have antiulcer activity. It is also used as a tonic, febrifuge, as a gargle for odontopathy, inflammation and bleeding of gums. Unripe fruit is utilized as a masticatory and will help to fix loose teeth [4-6]. Leaves are used as an antidote in snakebite. Decoction of seed is used as aphrodisiac, cardio tonic and to treat mouth ulcer. To treat scabies and skin sores latex is applied [7-8]. Saponins, alkaloid, steroids and terpenoids have previously been reported form *M. elengi* [9]. The phytoconstituents reported from the floral part of the plant are volatile oil, D-mannitol, beta-sitosterol and beta-sitosterol-D-glycoside [10-11]. Stem bark contain tannins, spinosterol and taraxerol while leaves contain sterols, reducing sugar, tannins [12].

Several naturally occurring medicinal plants, herbs, and fruit extracts have been shown to possess antimicrobial activity against *H. pylori* i. e, *Artemisia dracunculus* L. (*Asteraceae*), *Carthamus tinctorius* L. (*Asteraceae*), *Apium petroselinum* L. (*Apiaceae*), *Citrus sinensis* L. (*Rutaceae*) and *Punica granatum* L. (*Punicaceae*) have been traditionally used in folk medicine. There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants.

The appearance of pathogenic confrontation is a natural phenomenon and research toward the advances of new diverse inhibitors has always been at the esteem of pharmaceutical research. Enzyme inhibition has draw great attention of researchers for last couple of decades. The urease (nickel containing) is an enzyme which hydrolyzes urea to produce ammonia and carbon dioxide on further decomposition. Urease occurs in many plants, selected fungi, and a wide variety of prokaryotes [13].

The functioning of microbial ureases depends on their cellular location, regulation, genetic makeup and the relationships between the microbial urease and the well-characterized persuasive range of inhibitors. The urease inhibitors can play an imperative role to contradict the negative role of urease in living organisms. Urease inhibitors are effective against numerous serious infections caused by the secretion of urease by *Helicobacter pylori* which include gastric tract syndromes, urinary tract infections, struvite urolithiasis mainly in dogs and cats [14-15].

Urease enzyme impart as factor in the pathogens liable for kidney stones entailed in urolithiasis that contributes toward the acute pyelonephritis with other urinary tract infection which persuaded arthritis and gastric intestinal infections and ultimately the urease imbalance lead to peptic ulcers [16]. Urease controls the nitrogen contents in the physiological systems, while it provides a protection mechanism against predators and pathogenic organisms [17-19].

The gastric and duodenal ulcers are generally caused by *H. pylori,* which survive and grow in acidic environment. It has been demonstrated that inhibition of urease does not cause gastritis due to difficulties in colonization of *H. pylori* therefore urease inhibition activity has been proposed as a successful strategy to eradicate the organism in the body. In some parts of the World more than 50% of the population is reported to be infected with *H. pylori*. The cases of *H. pylori*-related infections are increasing in the developing countries. Triple therapy, consist of a proton pump inhibitor and any of the two antibiotics such as amoxicillin (AMX), clarithromycin (CLA), metronidazole (MNZ) and tetracycline (TET), is commonly utilized to treat *H. pylori* infections [20-22]. Clinical trials in this aspect have demonstrated an eradication rate of about 80-90% by the use of a relevant triple therapy including AMX [23].

To the best of our knowledge the subject plant *Mimusops elengi* have not been screened yet for their anti *Helicobacter pylori* and urease inhibition activity. The present study was therefore envisaged to evaluate the urease inhibition activity of the flower and leaves extracts of *M. elengi*.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

The flowers and leaves of *Mimusops elengi* Linn were collected from the premises of University of Karachi, Pakistan. These were identified and authenticated by Prof. Dr. Ghazala H. Rizwani, Faculty of Pharmacy, University of Karachi, Pakistan. A voucher specimen (083) was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.

2.2 Preparation of Extracts

The collected plant parts (flower and leaves) were dried separately under shade. 500 gm of flower and leaves were soaked in methanol (250 L) at room temperature separately for seven to ten days. After that, methanol was filter through Whatmann filter paper No. 1 and the extracts obtained were concentrated under reduced pressure and controlled temperature (40°C) on a rotary evaporator (Buchi, Switzerland). The yield of flower extract (MFE) and leaves extract (MLE) were 27.2 g (5.44%) and 60 g (12.0%) respectively.

2.3 Chemicals

The chemical used were, Methanol (Merck, Germany), Folin–Ciocalteu reagent (B.D.H. laboratory supplies, UK), Gallic acid (B.D.H. laboratory supplies, UK), Sodium carbonate (Merck, Germany), Sodium nitroprusside (B.D.H. laboratory supplies, UK), Phosphate buffer (pH 7.4), Sulfanilic acid reagent (B.D.H. laboratory supplies, UK), Sodium hydroxide (B.D.H. laboratory supplies, UK), Thiourea (Sigma-Aldrich, USA), Sodium hypochlorite (Sigma-Aldrich, USA)

2.4 Determination of Total Phenolic Contents

For the determination of total phenolic content of *M. elengi* extracts (MFE and MLE) the reaction mixture contained 50% Folin-Ciocalteu reagent (0.5 mL), 20% (w/v) sodium carbonate solution (2.5 mL), and gallic acid solution (1, 2.5, 5, 10, 25, 50, 100 μg/ml) or sample extracts (1.0 mL). The mixture was placed in the dark for 40 minutes and the absorbance was recorded at 750 nm against a blank with a spectrometer respectively [24]. The total phenolic content was expressed based on gallic acid equivalent (GAE) calculated by the formula

$$C\ (GAE) = \frac{c \times V}{M}$$

Where, C = total content of phenolic compounds in mg/g; c = concentration of gallic acid established from the calibration curve in mg/ml; V = volume of extract in ml; M = weight of plant extract in gm.

2.5 Urease Inhibition Activity

Urease inhibitory activity of the extracts of *M. elengi* (MFE, MLE) was determined according to the reported protocol [25-26]. 25 μl of Urease (Jack bean) solution was mixed with the 5 μl each extracts (500 μg) and incubated at 30°C for 15 min. Aliquots were taken and immediately transferred to assay mixtures containing urea (100 mM) in buffer (40 μl) and re incubated for 30 minutes in 96 well plate. 50 μl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μl of alkali reagent (0.5% w/v sodium hydroxide NaOH and 0.1% sodium

hypochlorite NaOCl) were added to wells. Increase in absorbance was measured after 50 min at 630 nm against blank (Spectramax Plus 384 Molecular Device, USA). Final volume of reaction is 200 µl at pH 8.2. All reactions were performed in triplicates. Thiourea was used as positive control. The percentage inhibitions were determined by formula

$$\text{Urease Inhibition } (\%) = 100 - (OD_{\text{test well}}/OD_{\text{control}}) \times 100$$

To calculate the IC_{50} value for each sample, % inhibition was plotted verses the concentration of the samples and a regression curve was established. IC_{50} value is the concentration of the given sample to inhibit the activity of urease by 50%.

2.6 Statistical Analysis

The results are expressed as Mean ± SEM. The data were analyzed by ANOVA followed by LSD multiple comparison tests using SPSS software No. 20. A level of $P < 0.05$ was considered as statistically significant.

2.7 Determination of IC_{50} values

IC_{50} values (half maximal inhibition concentration) were determined by investigating the MFE, MLE extracts inhibitory activity to positive control employing spectrophotometric measurement. IC_{50} values were obtained from curves obtained by linear regression.

3. RESULTS

3.1 Total Phenolic Content

In the determination of total phenolic contents, the absorbance for various dilutions (1, 2.5, 5, 10, 25, 50, and 100 µg/ml) of gallic acid with Folin - Ciocalteu and sodium carbonate were found as 0.02, 0.10, 0.11, 0.14, 0.28, 0.54 and 0.97 nm. By plotting concentration and absorbance standard curve for gallic acid was obtained with standard curve equation; $y = 0.0093x+0.0516$, $R^2 = 0.9954$, presented in Fig. 1. From the standard calibration curve of gallic acid the total phenolic contents in extracts (MFE and MLE) was found to be 7.5±0.28 mg/g, 96.8±4.6 mg/g respectively showed in Fig. 2.

3.2 Urease Inhibition Activity

Urease inhibitory activity of *M. elengi* was depicted in Fig. 3. The activity was comparable to that of Thiourea which was used as standard. The percentage urease inhibition of standard Thiourea was 98.2%, IC_{50} 88.2±0.01 µg/ml. The maximum urease inhibitory activity was shown by the leaves extract i. e, 77.9% (IC_{50} 62.1±1.20 µg/ml) while MFE produced 47.6% urease inhibition activity. IC_{50} value of MFE was not calculated because of the low efficacy i.e., less than 50% inhibition. Standard (Thiourea) and extracts (MLE) IC_{50} was obtained by plotting percentage inhibition and concentration of standard and extracts respectively (Fig. 4).

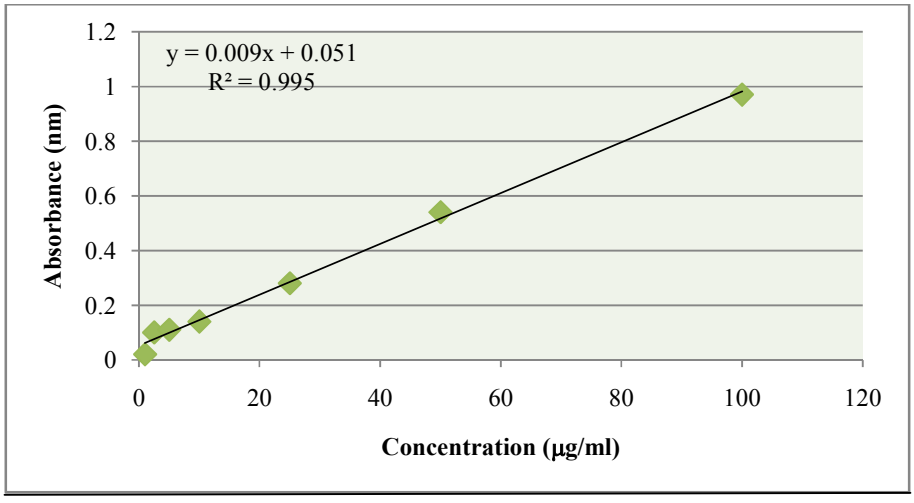

Fig. 1. Calibration curve for gallic acid

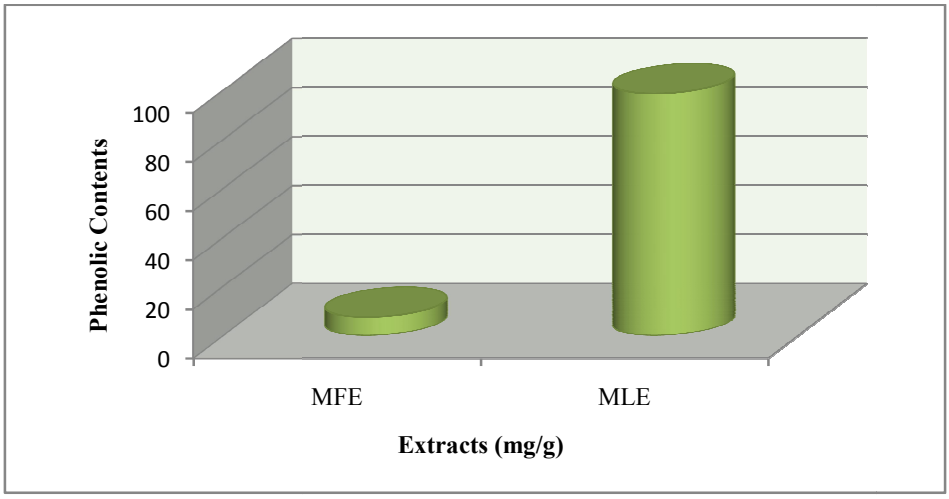

Fig. 2. Total phenolic contents of *M. elengi* Linn

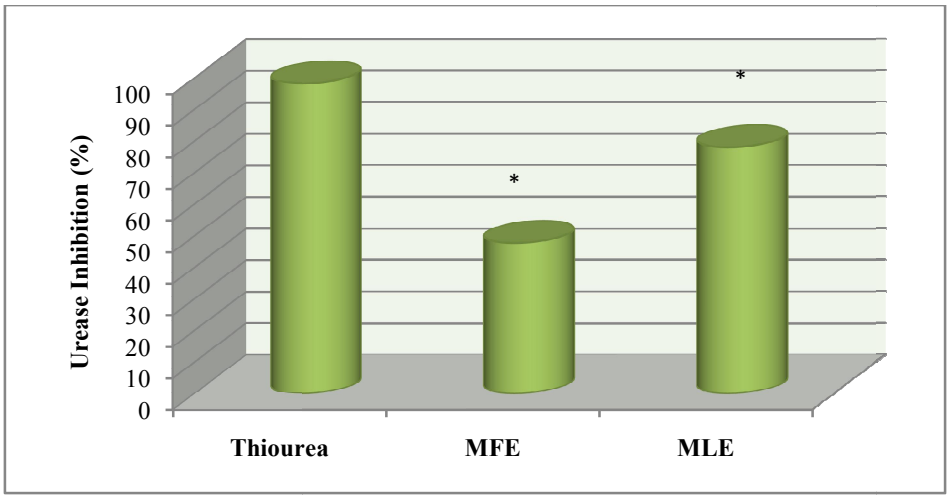

Fig. 3. Urease inhibition activity of Thiourea and *M. elengi* extracts (MFE, MLE)

4. DISCUSSION

Natural products have been increasingly considered as an important source of innovative therapeutics which could be adjunctive or even alternative to the currently used antimicrobial agent [27]. The present study, investigated the phenolic content of flower and leaves of *M. elengi* (MFE and MLE). From the results, it was found that high content of phenol was present in the MLE while comparatively low phenolic content found in MFE. Phenolic are bioactive compounds and a diverse group of secondary metabolites generally present in higher plants [28]. The Folin-Ciocalteu reagent is used for the crude estimation of the phenolic compounds present in an extract of medicinal plants. Polyphenols could scavenge reactive chemical species as well as reduce oxidative damage resulting from excessive light exposure. Some plant polyphenols are important components of diets of both human and animal and they are safe to be consumed [29]. Ascorbic acid, α-tocopherol, carotenoids, amino acids, peptides, proteins, flavonoids and other phenolic compounds referred as food antioxidants which play a significant role as dietary antioxidants [30]. Natural antioxidants obtained from the medicinal plants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory activities [31].

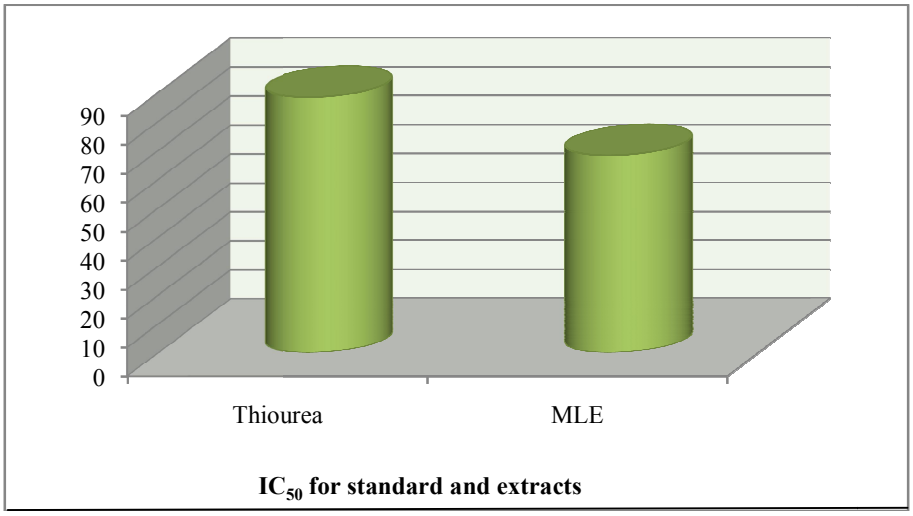

Fig. 4. IC_{50} values for standard and extracts of MLE in Urease inhibition activity

In addition, he results for the assessment of urease inhibitory activity of the extracts (MFE and MLE) it was found that maximum urease inhibitory activity was showed by the leaves extract (MLE) as compare to the flower extract at the same concentration. WHO recognized *H. pylori* are as class 1 carcinogen. Consequently efforts are being made worldwide for its suppression through the application of several therapies. It is well accepted that urease enzyme released by *H. pylori* protects it from the acidic environment of the stomach. The activity of urease can be controlled by the use of urease inhibitors [32]. Urease inhibitors can play a significant role in the abolition of infection caused by urease-producing bacteria [33-34]. Treatment of *H. pylori* with synthetic compounds is related with several problems (high pretreatment cost, pretreatment bacterial resistance and adverse side effects). Therefore, searching of some safer urease inhibitors derived from medicinal plants is becoming important as an alternate therapy against *H. pylori* based infections.

The mechanisms whereby *H. pylori* significantly increase the risk of gastric carcinoma are clearer for intestinal-type gastric cancers. The development of this type is marked by a slow progression, starting with *H. pylori* infection and then, subsequently development to the chronic active gastritis. And this chronic active gastritis may in turn progress into atrophic gastritis and intestinal metaplasia. In certain individuals, the metaplastic epithelium undergoes further genomic and phenotypic changes, resulting in gastric dysplasia and finally in adenocarcinoma [35].

The increasing development of antibiotic resistance against synthetic drugs is a worldwide concern. The utilization of medicinal plants and their chemical constituents may have possible benefits as *H. pylori* inhibiting agents for addressing such problems [36-37].

The findings of the present study provide a scientific support towards the traditional uses of *M. elengi* in stomach related diseases. Further bioactivity-directed assay are still required to formulate suitable natural pharmaceuticals to combat *H. pylori* infections. So far nothing has been reported on urease inhibition activity of *M. elengi*.

5. CONCLUSION

This is the first account of mention investigate on *Mimusops elengi* Linn in Pakistan. Our results clearly revealed a strong urease inhibitory effect of *M. elengi* (MFE, MLE) which provoked further to perform a comprehensive isolation and characterization of active constitutes from this species as potent urease inhibitory agent.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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