International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2019.151.155

Research Article
Cytotoxic and Urease Inhibition Potential of *Moringa peregrina* Seed Ethanolic Extract

[1]Shaymaa Fadhel Abbas Albaayit and [2]Mehmet Ozaslan

[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[2]Department of Biology, University of Gaziantep, Turkey

Abstract

Background and Objective: *Moringa peregrina* have long been used as traditional medicine to treat diseases including fever, ulcer, burns and constipation, antimicrobial and inflammatory throughout the African mainland. The objectives of current study were to evaluate the cytotoxic and urease inhibitory profile of *Moringa peregrina* seed ethanolic extract (MPSE). **Materials and Methods:** Brine shrimp lethality bioassay was used to determine the cytotoxic effect of MPSE treatment at concentrations 1000, 100 and 10 μg mL^{-1}. Urease inhibition was determined by *in vitro* Indophenol inhibition assays. **Results:** It is found that the *Moringa peregrina* seed ethanolic extract was not cytotoxic at 1000 μg mL^{-1} while significantly active with urease inhibitor (81.30±0.31% at 0.2 mg mL^{-1}) with IC_{50} values of 41.0±0.6 μg mL^{-1}. **Conclusion:** These findings suggested that the *Moringa peregrina* seed ethanol extract (MPSE) can be utilized to treat and prevent ulcerative and Urolithiasis diseases through urease inhibition process. Seed extract of *M. peregrina* is a remarkable candidate for further clinical investigation studies to screen out their natural therapeutic potential in ulcerative and urolithiasis diseases.

Key words: *Moringa peregrina*, brine shrimp lethality, urease enzyme, urolithiasis diseases, traditional medicine, indophenol inhibition, seed ethanol extract

Received: October 04, 2018 Accepted: November 09, 2018 Published: December 15, 2018

Citation: Shaymaa Fadhel Abbas Albaayit and Mehmet Ozaslan, 2019. Cytotoxic and urease inhibition potential of *Moringa peregrina* seed ethanolic extract. Int. J. Pharmacol., 15: 151-155.

Corresponding Author: Shaymaa Fadhel Abbas Albaayit, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
Tel: 009647808430086

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Moringa peregrina (Forssk) Fiori is a widespread plant in Africa and mostly along the countries that bordering the Red Sea. In folk medicine, all parts of this plant are used for the treatment of disinfectant, stomach disorder, wound healing and burns, diabetes, headache, malaria, constipation, muscle pain, hypertension, asthma, fever and burns. It is also administered to pregnant women to facilitate the fetus delivery[1-3]. The pharmacological studies have been reported the validation of this plant to be used as anti-inflammatory, anti-microbial, anti-ulcer and anti-oxidant. *Moringa peregrina* seeds oil contains high amounts of oleic acid, linoleic acid, tocopherols, flavonoid and phenolic compounds, which help to reduce microbial infection[4-6]. Thus, the present work was undertaken to emphasis the cytotoxic and urease inhibition effect of *M. peregrina* seed extract, which might explore value natural drug for treatment of different disease as prescribed in folk medicine.

Screening of the cytotoxicity of plant extract can be approximated by *in vivo* Brine shrimp lethality bioassay, which gives information about its safety at a preliminary level[7,8].

Urease is an enzyme responsible for control urea hydrolysis in soil and also performs a significant part in the pathogenesis of *H. pylori,* which involved in many clinical cases such as gastric, hepatic coma and peptic ulcer. Urease is directly engaged with the development of infection induced urolithiasis. Ureases have also contribute in the deactivation of complement, that is an element of host defense mechanism[7,9,10]. Due to the pivotal role of urease enzyme in *H. pylori* pathogenesis, many researches had been focused on searching potent natural compounds having urease inhibition activity. Thus, the present work was undertaken to emphasis the cytotoxic and urease inhibition effect of *M. peregrina* seed extract, which might explore value natural drug for treatment of different disease as prescribed in folk medicine. Therefore, the different fractions of crude extract of *M. peregrina* seeds were prepared and their urease inhibition activity was examined. Togetherly, cytotoxicity of these fractions were also evaluated through brine shrimp lethality assay. This type of research is the base of drug developing and pharmacognosy.

MATERIALS AND METHODS

Collection and extraction of plant material: *Moringa peregrina* seeds were authenticated in 2013 by Dr. Maha Kordofani (Resident Botanist) at the Botany Department, Faculty of Science and University of Khartoum. Fresh seeds were dried in room temperature, powdered and macerated in 1:5 dried plant weights to solvent (ethanol) volume ratio for 3 days. The filtrate was collected and the residues were subjected to further macerate with ethanol. The filtrates were combined and concentrated to dryness under reduced pressure using rotary evaporator at 45-50°C in order to obtain the crude extracts[11].

Brine shrimp (*Artemia salina*) lethality bioassay: In an empty quadrilateral plastic plate which is filled with the artificial seawater (sea salt- 38 g L^{-1} distilled water, pH 7.4), Brine shrimp eggs were hatched. About 50 mg of eggs were sprinkled in the hatching tray (22×32 cm). One side of the hatching tray, where eggs were sprinkled and protect from light, whereas lamp was placed on another side without eggs so that larvae after growth will come out to wards the light partition. Test sample were dissolved (20 mg/2 mL) in methanol and from these solutions were transferred 5, 50 and 500 µL in triplicate such that the concentration would be 10, 100 and 1000 µg mL^{-1}, respectively and allowed for drying overnight. About 10 larvas which got matured after 48 h of hatching were placed in each vial by using a Pasteur pipette and these vials were filled with 5 mL sea water. Incubation was done for 24 h at 25-27°C under illumination. By the next day, numbers of live and dead larvae were counted and analysis of data was done from the graph[8,12].

Urease inhibition (indophenol inhibition) assay: The *in vitro* urease inhibition assay was carried out spectrophotometric in 96-well plate. About 25 µL urease enzyme (1 U/well) and 5 µL of test compounds (0.2 µg mL^{-1}) were incubated at 30°C for 15 min. After that substrate urea 55 µL (100 mM) was added and re-incubated at 30°C for 15 min. After incubation, 45 µL of phenol (0.005% w/v sodium nitroprusside and 1% w/v phenol) and 70 µL of alkali reagents (0.1% sodium hypochlorite and 0.5% w/v sodium hydroxide) were added. The plate was again incubated for 50 min at 30°C. Urease activity was periodically measured with continuous urea hydrolysis and ammonia production. The rapid change in absorbance (optical density, OD) was monitored at 630 nm on ELISA plate reader (Spectra Max M2, Molecular Devices, CA, (USA)[13]. Mean±SD had been used to analysis data.

RESULTS

Cytotoxicity: Brine shrimp lethality bioassay was used for cytotoxic evaluation of ethanol extract of the seed of *M. perigrena*. The result showed that MPSE extract has no

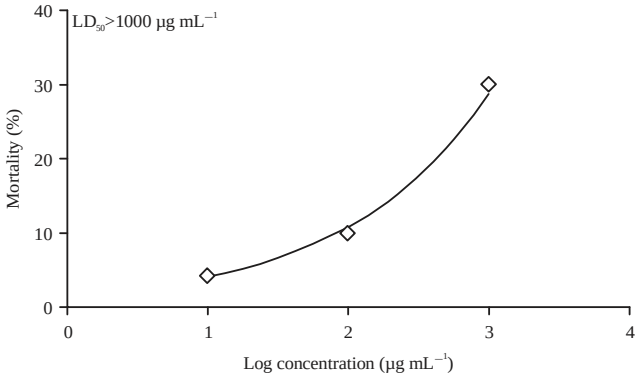

Fig. 1: Toxic effects of the *M. peregrina* ethanol extract after 24 h using brine shrimp lethality bioassay

Table 1: Percentage of urease inhibition of the *M. peregrina* ethanol extract

Concentration (mg mL^{-1})	Inhibition (%)
0.2	81.30±0.31
0.1	69.30±0.30
0.05	52.00±0.11
0.025	45.00±0.10

significant effect on cytotoxic activity. In the Fig. 1, data shown that percentage mortality of brine shrimp larvae after treatment with different concentrations 1000, 100 and 10 µg mL^{-1} (3,2 and1 µg mL^{-1} log concentrations) was found to be 30, 10 and 3% respectively. This result indicated that LD_{50} value of this extract is higher than 1000 µg mL^{-1} indicating the non-cytotoxic nature of MPSE extract even at high.

Urease inhibition assay: In present study, the enzyme inhibition study on the MPSE was carried out against ureases. The ethanol extract of the plant exhibited highest urease inhibition (81.30±0.31%) with IC_{50} values of 41.0±0.6 µg mL^{-1} at 0.2 mg mL^{-1} as shown in Table 1.

DISCUSSION

The uses of herbal plants as a source of folk medicines in primary health care have become popular globally as a safe drug because of their nature source. Many traditional plants have been shown to possess excellent medicinal properties against various diseases. *Moringa* species have been widely uses for their nutritional and medical values in many countries. However, *M. peregrina* seeds have been reported to be widely used in traditional medicine including disinfectant, stomach disorder, wound healing and burns, only a few scientific studies exist on its therapeutic efficacy and mechanism of action[1,2,14,15]. Brine shrimp lethality bioassay is a rapid and inexpensive assay was used to evaluate cytotoxic activities of any substances[16,17]. In this study, the acute cytotoxicity assay of MPSE on Brine shrimp showed that plant extract did not possess cytotoxic effect on Brine shrimp since the viability is more than 70% at concentrations 1000 mg mL^{-1}, which declared that safe for use as a natural therapeutic against microbial infections.

The significance of urease inhibition assay is because of their large usage in bacterial urease therapy of pathogenic activity of *H. pylori* alike peptic ulcer and stone emergence and control of urea hydrolysis because of nitrogen loss in urea fertilizer that used in soil[18]. Based on the urease inhibition assay, results confirmed that administration of *M. peregrina* seeds is one of the remarkable safety agent for treatment of ulcer related diseases. The traditional use of the genus *Moringa perigrena* also targeted on disinfectant and stomach disorder[1,2,19]. Additionally, increasing bacterial resistance, paid attention of the researchers to find other modes of therapy like urease inhibition[7]. From the outcome of enzyme inhibition studies, it is concluded that MPSE showed enzyme inhibitory activity of 81%. The *M. perigrena* has been reported to contain high amounts of oleic acid, linoleic acid, tocopherols and phenolic compounds, which are attributed to the enzyme inhibition and antimicrobial properties of extract[6,20-23].

The outcome is in agreement with reported by Fahey *et al.*[24], who proved that *Moringa* tree has an inactivation potencies effect against the secretion of urease. The results of urease inhibition assay of plant, *Moringa peregrina* authenticate the conventional use for cure of ulcer. These results will lead the scientists to do work on the isolation unique urease inhibitors for increase of the activity.

CONCLUSION AND RECOMMENDATION

The ethanolic extract of *M. peregrina* seeds was not toxic against brine shrimp eggs (LD_{50} more than 1 mg mL^{-1}), which act as inhibitors of urease enzyme that could normalize the conditions created by bacteria infection. The above cited outcomes signified in this study will advantage forth coming studies of *Moringa peregrina* seeds as they will be interesting to be used as safe treatment for pathogenic microbial infections like ulcer-related bacteria and inflammation that can cause chronic diseases.

SIGNIFICANCE STATEMENT

This study discover the urease inhibition activity of ethanolic extract of seeds of *M. peregrina* that can be

beneficial for treating *Helicobacter pylori* infected patient by inhibiting urease enzyme and thus delaying the progression of peptic ulcer. This study will help the researcher to uncover the potent compound inside the seeds, having strong urease inhibition activity which could be novel in structure. Thus this plant can be enlisted in the urease inhibiting capacity plants.

ACKNOWLEDGMENT

The authors are grateful to NAM-ICCBS (International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan) for fellowship award to Shaymaa Fadhel Abaas Albaayit.

REFERENCES

1. Senthilkumar, A., N. Karuvantevida, L. Rastrelli, S.S. Kurup and A.J. Cheruth, 2018. Traditional uses, pharmacological efficacy and phytochemistry of *Moringa peregrina* (Forssk.) Fiori.-A review. Front. Pharmacol., Vol. 9. 10.3389/fphar.2018.00465.
2. El-Hak, H.N.G., A.R.A. Moustafa and S.R. Mansour, 2018. Toxic effect of *Moringa peregrina* seeds on histological and biochemical analyses of adult male Albino rats. Toxicol. Rep., 5: 38-45.
3. Al-Majali, I.S., S.A. Al-Oran, M.R. Hassuneh, H.N. Al-Qaralleh and W.A. Rayyan *et al*., 2017. Immunomodulatory effect of *Moringa peregrine* leaves, *ex vivo* and *in vivo* study. Cent. Eur. J. Immunol., 42: 231-238.
4. Koheil, M.A., M.A. Hussein, S.M. Othman and A. El-Haddad, 2011. Anti-inflammatory and antioxidant activities of *Moringa peregrina* seeds. Free Radicals Antioxid., 1: 49-61.
5. Selvakumar, D. and P. Natarajan, 2008. Hepato-protective activity of *Moringa oleifera* Lam leaves in carbon tetrachloride induced hepato-toxicity in albino rats. Pharmacogn. Mag., 4: 97-98.
6. Elbatran, S.A., O.M. Abdel-Salam, K.A. Abdelshfeek, N.M. Nazif, S.I. Ismail and F.M. Hammouda, 2005. Phytochemical and pharmacological investigations on *Moringa peregrine* (Forssk) Fiori. Natl. Prod. Sci., 11: 199-206.
7. Ghaffari, M.A., B.A. Chaudhary, M. Uzair and K. Ashfaq, 2016. Cytotoxic, α-chymotrypsin and urease inhibition activities of the plant *Heliotropium dasycarpum* L. Afr. J. Tradit. Complement. Altern. Med., 13: 194-198.
8. Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med., 45: 31-34.
9. Ismail, A., J. Hussain, K. Arif-ullah, A.L. Khan and L. Ali *et al*., 2012. Antibacterial, antifungal, cytotoxic, phytotoxic, insecticidal and enzyme inhibitory activities of Geranium wallichianum. Evidence-Based Complementary Alter. Med., 10.1155/2012/305906.
10. Pervez, H., M.S. Iqbal, M.Y. Tahir, F.U.H. Nasim, M.I. Choudhary and K.M. Khan, 2008. *In vitro* cytotoxic, antibacterial, antifungal and urease inhibitory activities of some N^4-substituted isatin-3-thiosemicarbazones. J. Enzyme Inhib. Med. Chem., 23: 848-854.
11. Albaayit, S.F.A., Y. Abba, R. Abdullah and N. Abdullah, 2014. Evaluation of antioxidant activity and acute toxicity of *Clausena excavate* leaves extract. Evidence-Based Complement. Alternat. Med., Vol. 2014. 10.1155/2014/975450.
12. Geran, R.I., 1972. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother. Rep., 3: 17-27.
13. Martin, P.R. and R.P. Hausinger, 1992. Site-directed mutagenesis of the active site cysteine in *Klebsiella aerogenes* urease. J. Biol. Chem., 267: 20024-20027.
14. Lalas, S., O. Gortzi, V. Athanasiadis, J. Tsaknis and I. Chinou, 2012. Determination of antimicrobial activity and resistance to oxidation of *Moringa peregrine* seed oil. Molecules, 17: 2330-2334.
15. Padayachee, B. and H. Baijnath, 2012. An overview of the medicinal importance of Moringaceae. J. Med. Plants Res., 6: 5831-5839.
16. Ateeq-ur-Rehman, A. Mannan, S. Inayatullah, M.Z. Akhtar, M. Qayyum and B. Mirza, 2009. Biological evaluation of wild thyme (*Thymus serpyllum*. Pharm. Biol., 47: 628-633.
17. Ahmad, M.S., M. Hussain, M. Hanif, S. Ali and B. Mirza, 2007. Synthesis, chemical characterization and biological screening for cytotoxicity and antitumor activity of organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropenoic acid. Molecules, 12: 2348-2363.
18. Upadhyay, L.S.B., 2012. Urease inhibitors: A review. Indian J. Biotechnol., 11: 381-388.
19. Verma, V.K., N. Singh, P. Saxena and R. Singh, 2012. Anti-ulcer and antioxidant activity of *Moringa oleifera* (Lam) leaves against aspirin and ethanol induced gastric ulcer in rats. Int. Res. J. Pharm., 2: 46-57.
20. Saleh, N.M., M.I. Mabrouk, M.M. Salem-Bekhit and E.H. Hafez, 2017. Challenge of *Moringa peregrine* Forssk as an antimicrobial agent against multi-drug-resistant *Salmonella* sp. Biotechnol. Biotechnol. Equip., 31: 380-386.
21. Zahid, H., G.H. Rizwani, H. Shareef and S.T. Ali, 2014. Antioxidant and urease inhibition activity of methanol extract of *Hibiscus schizopetalus* (Mast) Hook. J. Pharmacogn. Phytochem., 2: 07-11.

22. Cheenpracha, S., E.J. Park, B. Rostama, J.M. Pezzuto and L.C. Chang, 2010. Inhibition of Nitric Oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells by the norsesterterpene peroxide, epimuqubilin A. Mar. Drugs, 8: 429-437.
23. El-Alfy, T.S., S.M. Ezzat, A.K. Hegazy, A.M.M. Amer and G.M. Kamel, 2011. Isolation of biologically active constituents from *Moringa peregrina* (Forssk.) Fiori. (Family: Moringaceae) growing in Egypt. Pharmacogn. Mag., 7: 109-115.
24. Fahey, J.W., K.K. Stephenson, K.L. Wade and P. Talalay, 2013. Urease from *Helicobacter pylori* is inactivated by sulforaphane and other isothiocyanates. Biochem. Biophys. Res. Commun., 435: 1-7.