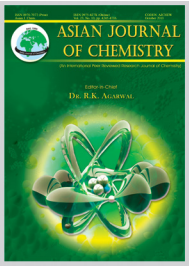
Asian Journal of Chemistry; Vol. 24, No. 4 (2012), 1527-1529

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Urease Inhibitory Activity of Some Iranian Medicinal Plants

A.R. GOHARI[1], F. NABATI[2], S. SAEIDNIA[1,*], M. MALMIR[1] and M. AMANLOU[2]

[1]Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran
[2]Department of Medicinal Chemistry and Drug Design & Development Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding author: Tel/Fax: +98 21 64122330; E-mail: saeidnia_s@tums.ac.ir

(*Received*: 17 February 2011; *Accepted*: 23 November 2011) AJC-10706

Medicinal plants are enriched of phytoconstituents which found to be urease inhibitors. Bacterial ureases are playing an important role in the pathogenesis of many illnesses. Urease inhibitory assays are the noteworthy tools to guide the isolation and identification of promising natural resources for discovery of novel bioactive compounds as the safe and potent antiulcer drugs. In this paper, we report the evaluation of some Iranian medicinal plants for their possible urease inhibitor activity which have not been previously reported. The ethyl acetate and methanol extracts of the plant materials were tested by enzyme assay using Berthelot alkaline phenol-hypochlorite method. The methanol extracts of *Salvia macrosiphon* and *Stachys byzanthina* indicated considerable inhibitory activities 56.35 and 93.12 %, respectively. Apiaceae samples (*Lomatopodium staurophyllum* and *Pimpinella tragioides*) showed no inhibitory activity. All samples of Lamiaceae species, except one, *Lagochilus cabulicus*, showed inhibitory activity in the range of 0.17-93.12 %.

Key Words: Urease inhibitor, *Salvia macrosiphon*, *Stachys byzanthina*, Berthelot alkaline phenol-hypochlorite.

INTRODUCTION

The enzyme urease, (urea amidohydrolase), was crystallized from Jack bean *Canavalia ensiformis*, for the first time[1]. After that time, various urease enzymes have been identified in microorganisms and higher plants even in the soil but there have not been found in the mammalian systems. Usually, urea-splitting bacteria are colonizing in the human body and showing urease activity[2,3]. Abnormal urease activity in the soil can cause an environmental or economic problem by producing a large amount of ammonia and releasing it into the atmosphere[4]. Bacterial ureases are playing an important role in the pathogenesis of many illnesses, such as pyelonephritis, hepatic coma and peptic ulceration[5].

Medicinal plants are enriched of phytoconstituents, such as alkaloids, terpenoids, flavonoids and other polyphenol compounds which indicate a broad range of biological activity. Some of these secondary metabolites have been found to be urease inhibitors. Flurofamide, biscoumarin, triazole and cyclic triketone are the main examples of the synthetic inhibitors even though, quercetin derivatives, datisdirin, gallocatechin, one diterpene polyester and ursolic acid are identified as natural urease inhibitors[6]. Green tea extract was reported to inhibit *Helicobacter pylori* urease which is due to the presence of catechins[7]. Another *H. pylori* urease inhibitor (a protein type) has been reported from *Rubus coreanus*[8]. Datisdirin is a flavone which was isolated from *Datisca cannabina* and reported to be a urease inhibitor[9].

The screening against urease (using urease inhibitory assays) is a noteworthy tool to guide the isolation and identification process of the most active urease inhibitors from medicinal plants which are important and promising natural resources for the discovery of novel bioactive compounds as the safe and potent antiulcer drugs.

In this paper, the screening of some Iranian medicinal plants for their possible urease inhibitor activity is reported.

EXPERIMENTAL

All the plant species were collected from wild growing area of Iran in June and July (2005-2008), during full flowering stages and identified by Dr. A. R. Naghinejad and Mr. Y. Ajani. The voucher herbarium specimens were deposited at the herbarium of Medicinal Plants Research Center housed in Faculty of Pharmacy, Tehran University of Medical Sciences and also herbarium of the Institute of Medicinal Plants, ACECR in Karaj. Plants origins are shown in Table-1.

Extraction process: Aerial parts of the plants were gathered and dried in the shade then reduced to small pieces. Extraction was performed with ethyl acetate and methanol, respectively, employing percolation (72 h) at room temperature. The solvents were removed under reduced pressure and dried by freeze dryer (Christ, Germany).

TABLE-1
INHIBITORY ACTIVITY OF THE ETHYL ACETATE AND METHANOL EXTRACTS FROM IRANIAN MEDICINAL PLANTS AGAINST JACK BEAN UREASE

Plant material	Plant origins (Iran provinces)	Family	Inhibitory activity (%)	
			Methanol extract	Ethyl acetate extract
Salvia limbata	Semnan	Lamiaceae	0.17	ND*
Salvia macrosiphon	Tehran	Lamiaceae	56.35	20.03
Scutellaria tornefortii	Mazandaran	Lamiaceae	<1%	9.43
Stachys byzanthina	Mazandaran	Lamiaceae	93.12	<1%
Salvia hypoleuca	Tehran	Lamiaceae	8.14	15.59
Lomatopodium staurophyllum	Semnan	Apiaceae	0.0	2.62
Pimpinella tragioides	Ghazvin	Apiaceae	0.0	0.0
Satureja bakhtiarica	Chahar-Mahal Bakhtiari	Lamiaceae	<1%	25.69
Lagochilus cabulicus	Tehran	Lamiaceae	0.0	0.0

*ND = Due to insolubility not tested.

Sodium nitroprusside (sodium pentacyanonitrosylferrate III) were purchased from Sigma and Jack beans urease (EC 3.5.1.5) from Merck. All other chemicals were of analytical reagent grade. Distilled water was used in all experiment. Potassium phosphate buffer (100 mM, pH 7.6) was prepared in distillated water. The UV-VIS spectrophotometer (Cecil 9000, UK) was used to measure the optical density of the samples.

Urease assay: Enzyme assay was performed by Berthelot alkaline phenol-hypochlorite method[10]. This method is based on the released ammonia (NH_3) which reacts with hypochlorite (OCl^-) to form a monochloramine. This product then reacts with phenol to form blue-coloured indophenols, whose absorbance is measured at 625 nm. In brief, 10 µL of enzyme solution was incubated with 140 µL urea at final concentration of 25 mM in phosphate buffer solution (100 mM, pH 7.6) for 15 min at 37°C. The liberated ammonia was estimated using mixture of 500 µL of solution A (contained 5 g phenol and 25 mg of sodium nitroprusside) and 500 µL of solution B (contained of 2.5 g sodium hydroxide and 4.2 mL of sodium hypochlorite in 500 mL of distillated water) at 37 °C for 0.5 h and the absorbance was measured at 625 nm with compare to reagent blank (control). All reactions were performed in triplicate and percentages of inhibition were calculated using the formula $[1-(OD_{sample}/OD_{control})] \times 100$.

RESULTS AND DISCUSSION

Inhibitory activity of ethyl acetate and methanol extracts of the tested plants materials at a concentration of 10 mg/mL was listed in Table-1. The methanol extracts of *Salvia macrosiphon* and *Stachys byzanthina* indicated considerable inhibitory activities 56.35 and 93.12 %, respectively. From the urease assay data, it has been found that the aerial part of *Stachys byzanthina* may contains the potent bioactive constituents against Jack bean urease. The assay results in Table-1 indicated that the extracts of both Apiaceae samples (*Lomatopodium staurophyllum* and *Pimpinella tragioides*) showed no inhibitory activity. All samples of Lamiaceae species, except one, *Lagochilus cabulicus*, showed inhibitory activity in the range of 0.17-93.12 %. The ethyl acetate extract of *Salvia limbata* and methanolic extract of *Satureja bakhtiarica* were discarded due to insolubility which may interfere with the assay and give false results as previously demonstrated by Kawai[11].

Previous study revealed that the methanol extract of *Stachys byzanthina* showed a high toxicity (LC_{50} = 11 µg/mL) against the larvae of *Artemia salina* but its ethyl acetate extract was moderately cytotoxic (LC_{50} = 118 µg/mL)[12]. Therefore, it is important that the possibly bioactive compound(s) of this species needs cytotoxicity evaluation to prove the safety without toxicity. Both ethyl acetate and methanol extracts of *Salvia macrosiphon* showed moderate urease inhibitory activity and also these extract have not exhibited toxicity in brine shrimp cytotoxicity assay in advance[12].

Thus, the mentioned plant species are two appropriate candidates for isolation and identification of the bioactive compounds against urease enzyme. Recently, we reported the main phenolic compounds of some *Salvia* species[13,14] and it seems that the high content of luteolin and apigenin derivatives and phenolic acids such as rosmarinic acid could be responsible for their inhibitory activity against Jack been urease[15]. It has been reported that some urease inhibitors derived from flavonoid by splitting C-ring, in which 4-(4-hydroxyphenethyl)-phen-1,2,3-triol showed the highest inhibitory activity against *H. pylori* urease[16].

In conclusion, *Salvia macrosiphon* and *Stachys byzanthina* might be candidate for more evaluation based on the bioassay guided fractionation which can lead to isolation and identification of the potent urease inhibitors.

ACKNOWLEDGEMENTS

This research is supported by Tehran University of Medical Sciences & Health Services. The authors wish to thank Mr. Yousef Ajani for his help in plants collection and identification.

REFERENCES

1. R.D. Simoni, R.L. Hill and M. Vaughan, *J. Biol. Chem.*, **277**, e23 (2002).
2. H.L.T. Mobley and R.P. Hausinger, *Microbiol. Rev.*, **53**, 85 (1989).
3. S. Odake, K. Nakahashi, T. Morikawa, S. Takebe and K. Kobashi, *Chem. Pharm. Bull.*, **40**, 2764 (1992).
4. J.M. Bremner and L.A. Douglas, *Soil Biol. Biochem.*, **3**, 297 (1971).
5. B. Krajewska and W. Zaborska, *Bioorg. Chem.*, **35**, 355 (2007).
6. S. Samah, A. Sabry and A. Hamid, Ph.D. Thesis, Graduate School of Life and Environmental Sciences. Osaka Prefecture Universiy, Osaka, Japan (2010).
7. S. Matsubara, H. Shibata, F. Ishikawa, T. Yokokura, M. Takahashi, T. Sugimura and K. Wakabayashi, *Biochem. Biophys. Res. Commun.*, **310**, 715 (2003).

8. S.W. Yang, J.N. Ho, Y.H. Lee, D.H. Sin, B.S. Hong and H.Y. Cho, *J. Korean Soc. Food Sci. Nutr.*, **33**, 767 (2004).
9. M. Ahmad, N. Muhammad, M. Ahmed, M.A. Lodhi, N. Mahjabben, Z. Jehan, Z. Khan, R. Ranjit, F. Shaheen and M.I. Choudhary, *J. Enzyme Inhib. Med. Chem.*, **23**, 386 (2008).
10. M.W. Weatherburn, *Anal. Chem.*, **39**, 971 (1967).
11. A. Kawai, Master Thesis, Applied Life Science Department, Graduate School of Life and Environmental Science, Osaka Prefecture University, Osaka, Japan (2009).
12. A.R. Gohari, S. Saeidnia, M.R. Gohari, F. Moradi-Afrapoli, M. Malmir, M. Yazdanpanah and A. Hadjiakhoondi, *J. Med. Plants*, **29**, 87 (2009).
13. A.R. Gohari, S. Saeidnia, M. Malmir, A. Hadjiakhoondi and Y. Ajani, *Nat. Prod. Res.*, **24**, 1902 (2010).
14. A.R. Gohari, H. Ebrahimi, S. Saeidnia, M. Foruzani, P. Ebrahimi and Y. Ajani, *Iran. J. Pharm. Res.*, **10**, 247 (2011).
15. J.E. Shin, J.M. Kim, E.A. Bae, Y.J. Hyun and D.H. Kim, *Planta Med.*, **71**, 197 (2005).
16. Z.P. Xiao, T.W. Ma, W.C. Fu, X.C. Peng, A.H. Zhang and H.L. Zhu, *Eur. J. Med. Chem.*, **45**, 5064 (2010).