

In Vitro Anti-Ulcer Potential of *Nardostachys jatamansi* Through Anti-Urease Assay

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ABSTRACT

Background: Drug resistance is the reason for search of new medicines from natural sources. Natural products and plants showed gastro-protective and anti-ulcer effects by either prophylactic (antioxidant) or therapeutic means (anti-urease, etc.). *Nardostachys jatamansi* is used traditionally as gastro-protective. The study aims to evaluate anti-ulcer activity of the methanolic extract of *N. jatamansi* through its anti-urease potential and also appraise its antioxidant activity.

Methodology: Antioxidant activity was evaluated through DPPH radical scavenging assay. Elisa microplate reader was used to estimate DPPH and urease inhibition.

Results: The study results showed that *N. jatamansi* inhibited DPPH, and urease significantly ($p \leq 0.05$). IC₅₀ of *N. jatamansi* against DPPH and urease were 218.5±1.0 µg/mL and 55.9±1.3 µg/mL, respectively.

Conclusion: This study suggests the anti-urease potential of *N. jatamansi*. So, *N. jatamansi* could be used in peptic ulcer treatment. Moreover, this study provides proof of its traditional gastro-protective use.

Keywords: Antiulcer, anti-urease, *Nardostachys jatamansi*.

Authors' Contributions

1 Conception & Study Design, Data Collection, Data Analysis, Drafting, Critical Review.

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INTRODUCTION

Peptic ulcer is usually caused by *H. pylori* whose growth and survival is impossible in acidic media. *H. pylori* is motile, therefore tunnel through and reside in thick mucus layer of alkaline mucus [1]. To protect itself from acidic environment of stomach, *H. pylori* releases urease enzyme that converts urea in to ammonia, and this ammonia protect it from acidic environment of stomach and made its survival possible. In urease negative mutant, its colonization is not possible hence urease activity inhibition is the most suitable treatment option to eradicate it from the body [2]. Triple therapy with proton pump inhibitors and antibiotics are used to treat ulcers with a success rate of 80-90% [3]. Drug resistance due to

inappropriate and wide use of these medicines is a major issue, moreover there are various side effects of these commercially available drugs [4]. Due to these reasons, it is need of the time to find new and less toxic medicines for ulcers from the natural source.

Natural products showed their anti-ulcer activity by different mechanisms, either prophylactic or therapeutic or both. The products that showed prophylaxis showed antioxidant or anti-inflammatory activity while therapeutic agents showed healing effects, anti-secretory or anti-*H. pylori* effects [5]. Urease inhibitors have high efficacy against microorganisms while less harming human cells [6]. So, botanicals with urease inhibitory potential and

antioxidant potential could be used as best anti-ulcer medicines.

Nardostachys jatamansi (Valerianaceae) roots are traditionally used as anti-epileptic, in the treatment of eye diseases, hysteria, heart palpitations, boils and itch, etc. [7]. In Unani system of medicine, Sumbul-ut-teeb (*Nardostachys jatamansi*) has been mentioned as a gastro-protective, hepato-tonic, cardiotoxic, diuretic and analgesic [8]. Proved activities of *N. jatamansi* include antibacterial, antifungal, anti-hyperlipidemic, antioxidant and hepato-protective [9].

In about 80% cases cause of peptic ulcer is *H. pylori* [1], so there is a need to find anti-ulcer medicines which can inhibit the growth and survival of *H. pylori*. This study aims to find anti-ulcer effect of *N. jatamansi* through anti-urease potential.

METHODOLOGY

Plant Material, Bacteria and Chemicals

Nardostachys jatamansi (Balchar) rhizomes were collected from the herbalist and identified by a botanist (Voucher No. 2204/L.S) and placed in I.U.B herbarium. Urease and 2, 2 di phenyl 1, picrylhydrazyl (DPPH) of Sigma Aldrich, Germany were used.

Equipments Used

Synergy HT BioTek® USA microplate reader, Sonicator-Elmasonic, Germany, Rotary evaporator-Heidolph Laboratory, Germany, EZ-Fit enzyme kinetics software.

Preparation of Plant Extract

After authentication of plant by botanist, dried rhizomes were grinded in an electric grinder. The coarsely powdered dried rhizomes were extracted with methanol [10]. From approximately 100.0 g of grinded dried rhizomes of *N. jatamansi*, the extraction yield was approximately 25.0 g of dried extracts. A stock solution of *N. jatamansi* extract (0.5 mg/mL) was prepared and serial dilutions were prepared from the stock solution for antioxidant and anti-urease activities.

Preliminary Phytochemical Screening

The preliminary qualitative phytochemical screening of *N. jatamansi* rhizome extract for flavonoids, saponins, alkaloids, tannins and phenols was carried out [11].

DPPH Radical Scavenging Assay

The scavenging activity of methanolic plant extracts was checked against 2, 2 diphenyl 1, picrylhydrazyl radical by method of Ghouri *et al.* [12]. Ascorbic acid was taken as standard control. 90 µL of DPPH solution (0.1 mM) and 10 µL of tested extract were placed in 96 well microplate. The process was done in triplicate. The reaction mixture was placed for 30 minutes. The absorbance was measured at 517 nm. The percent inhibition was calculated by the following formula:

$$\text{Scavenging activity \%} = [100 - (A_s / A_c)] * 100$$

Where,

A_c = Absorbance of negative control

A_s = Absorbance of sample extract

The sample concentration providing 50% inhibition of DPPH (IC₅₀) was considered by using EZ-Fit5 enzyme kinetics software.

Anti-Urease Assay

The experiment was performed by the methodology [13] which is based on phenol hypochlorite. This method detects the formation of ammonia that is formed by the reaction of urease with urea. A colored complex is formed showing presence of ammonia produced. If enzyme was inhibited, ammonia was not produced thus showing colorless mixture. A total of 85 µL in one well of 96 microplate was taken. Firstly at 37°C, 15 µL of test compound, 15 µL of potassium phosphate buffer (1M, pH: 7), 15 µL of enzyme were incubated for 15 minutes. 40 µL of substrate was added and incubated at 37°C. After incubation absorbance was measured at 630 nm. This was taken as pre read. Then 45 µL of phenol reagent was added to the reaction mixture followed by addition of 70 µL of alkali reagent. Incubate it at 37°C for 1 hour. After incubation absorbance was measured at 630 nm. This was taken as after read. Thiourea was standard control. The procedure was done in triplicate. Absorbance was checked at 530 nm and % inhibition of urease was calculated by the following formula:

$$\% \text{ Urease Inhibition} = [100 - (\text{Absorbance of sample} / \text{Absorbance of control}) * 100]$$

IC₅₀ values were calculated by checking percent inhibition at different concentrations and then use EZ-Fit5 Perrella Scientific Inc. Amherst USA software.

Statistical Analysis

Results were expressed as mean \pm S.E.M (standard error of mean). SPSS was used for statistical analysis of data. One-way ANOVA (analysis of variance) was used for statistical analysis of results. The significance level was $p \leq 0.05$.

and tannins (Table 1). In this study, *N. jatamansi* methanolic extract showed a marked inhibition of DPPH (IC₅₀ 218.5 μ g/mL) as shown in Table 2. Maximum inhibition of DPPH (79%) was with the stock solution of the methanolic extract (0.5 mg/mL in methanol). Methanol (negative control) showed no effect on DPPH inhibition. There is a significant difference between antioxidant activity of *N. jatamansi* extract and standard.

RESULTS

Preliminary phytochemical analysis of extract showed presence of flavonoids, alkaloids, saponins, phenols

Table 1. Qualitative phytochemical analysis of *N. jatamansi* (rhizomes).

| S. No. | Chemical Constituents | Standard | Tests Name | Result of Standard | Result of Sample |
|--------|-----------------------|---------------------------|------------------------|--------------------|------------------|
| 1 | Flavonoids | Orange peel | Sodium hydroxide test | + | + |
| 2 | Saponin | Glycyrrhiza | Froath test | + | + |
| | | | Emulsifying properties | + | + |
| 3 | Phenolic glycosides | | Erdmann's test | + | + |
| 4 | Alkaloids | <i>Nicotiana tobaccum</i> | Mayer's reagent | + | + |
| | | | Wagner's reagent | + | + |
| | | | Hager's reagent | + | + |
| | | | Dragendorff's reagent | + | + |
| 5 | Tannins | Cinnamon | Ferric chloride test | + | + |
| | | | Bromine water | + | + |
| | | | Formalin test | + | + |
| | | | Sodium nitrite test | + | - |

Table 2. DPPH radical scavenging by *N. jatamansi* (rhizomes) methanolic extracts.

| Tested Items | % age inhibition of DPPH (0.5 mg/mL) | IC ₅₀ (μ g/mL) |
|--------------------------|--------------------------------------|--------------------------------|
| <i>N. jatamansi</i> | 79 \pm 0.5 ^b | 218.5 \pm 1.0 |
| Ascorbic acid (standard) | 93 \pm 0.12 ^a | 22.7 \pm 0.001 |

Superscripts showed that mean \pm standard error of mean (S.E.M.) with different superscript are significantly different ($p \leq 0.05$)

Table 3. Urease inhibition by *N. jatamansi* (rhizomes) methanolic extracts.

| Tested Items | % age inhibition of Urease (0.5 mg/mL) | IC ₅₀ (μ g/mL) |
|---------------------|--|--------------------------------|
| <i>N. jatamansi</i> | 84.7 \pm 0.5 ^b | 55.9 \pm 1.3 |
| Thiourea (standard) | 89.2 \pm 0.7 ^a | 19.0 \pm 0.08 |

Table 3 described the results of urease inhibitory assay. *N. jatamansi* methanolic extract showed a striking inhibition of urease (IC₅₀ 55.9 µg/mL). Stock solution showed 84% inhibition of urease while methanol, negative control, showed no inhibition.

DISCUSSION

Drugs from plants and natural sources are preferred due to their biological friendliness than synthetic compounds [14]. Secondary metabolites from natural sources would become the potential source for drug discovery [15]. It is noteworthy to find drugs with multiple targets instead of single targeting drugs [16].

In this study, *in vitro* antioxidant and anti-urease activities of methanolic extract of *N. jatamansi* rhizomes were determined. Methanol was selected for extraction because it exhibited highest efficacy to absorb the various phytoconstituents. Although methanol is a polar solvent, with polarity index of 5.1, however, due to its amphiphilic nature it efficiently absorbs most of the non-polar phytoconstituents [17].

Antioxidant activity can be done through several mechanisms and different assays are used to assess antioxidant activity. Extracts are usually the mixture of dozen of compounds with different functional groups, chemical behavior and polarity thus could lead to different results [18]. The use of DPPH to measure antioxidant activity is a simple and rapid method. Moreover, it can be used for both liquid as well as solid substances and is not specific for particular antioxidant component, thus measure the total antioxidant capacity of sample. Flavonoids and phenolic compounds have antioxidant potential and thus helpful in preventing degenerative disorders [19]. The results of present study presented the striking inhibition of urease. Many polyphenols exhibited marked inhibitory activity against *H. pylori* urease [20]. The phytochemical evaluation of this extract showed the presence of phenols and flavonoids in *N. jatamansi* methanolic extract, which might be the possible reason lying behind antioxidant and anti-urease potential of this extract. To find mechanism of action of urease inhibition, enzyme kinetic studies should be carried out. A previous study [11] described the carbonic anhydrase inhibition of *N. jatamansi*. Carbonic anhydrase inhibitors are also used for peptic ulcer treatment. The present study showed inhibition of urease that also suggests its possible use in treatment of peptic ulcer. In this way, *N. jatamansi*

may be a multi-directional drug of choice in treatment of peptic ulcer. Moreover, gastro-protective effect of *N. jatamansi* is also appraised in this study.

CONCLUSION

In conclusion, *N. jatamansi* rhizome methanolic extract showed marked inhibition of urease and DPPH thus could be used as an anti-ulcer medicine. So this study suggests gastro-protective of *N. jatamansi* through its *in vitro* anti-ulcer effect. However, further research is required to isolate compounds that are purely responsible for their antioxidant and anti-urease activities.

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