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Urease Inhibitory Evaluations of Crude Extracts and Various Fractions of *Nicotiana tabacum* L.

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Abstract

Natural products are still a good source for the identification of bioactive compounds that have great therapeutic potential. In the current research, the authors intended to examine the urease inhibitory activity of *Nicotiana tabacum* L. root extracts and solvent fractions in the search of a natural urease inhibitor substitute to synthetic urease inhibitors. Methanol was used as crude extract, and n-hexane, chloroform, ethyl acetate, methanol, and butanol were all used as fractions. The urease inhibitory activity was determined by the use of the indophenol method by using jack bean urease as the source of enzyme and thiourea as the standard inhibitor. The butanol extract was the most potent with 88.01% inhibition and an IC_{50} value of 33.04 +1.35 ug/mL, and then the methanol fraction (82.76% inhibition and an IC_{50} of 38.67 +1.40 ug/mL). The ethyl acetate fraction was moderately active (49.09%), as compared to the hexane and chloroform fractions that had a relatively low level of inhibition (30.32 and 35.87%, respectively). These findings show that solvent polarity has a significant influence on the extraction of bioactive phytoconstituents that give urease inhibition activity, with polar solvents extracting more effective inhibitors. The high activity of the butanol and methanol fractions could be explained by the fact that phenolics, flavonoids, alkaloids, and other secondary metabolites that can chelate nickel ions at the active site of the enzyme were present. These results may indicate that *N. tabacum* root contains an excellent source of natural urease inhibitors that have therapeutic potential in treating urease-related pathologies like peptic ulcers, urease-related infections of the urinary tract, and hepatic disorders. To isolate and characterize the active constituents and investigate their pharmacological significance, further bioassay-directed fractionation, molecular docking, and in vivo investigations are justified.

KEYWORDS :*Nicotiana tabacum*, extract, urease inhibitors, phytochemicals

1.0 INTRODUCTION

Nature has been a rich source of medical agents and has been providing many structurally diverse compounds which have a great potential in therapy. Natural products still maintain an important role in the modern drug discovery, they have brought important influence in the development of pharma to treat infectious, inflammatory, metabolic, and degenerative diseases. Plant-based bioactive secondary metabolite deep-sea exploration is a crucial strategy in the discovery of new lead compounds with particular pharmacological activity [1].

Medicinal plants especially, are also a source of biologically active compounds including alkaloids, flavonoids, terpenoids, phenolics and glycosides that in most cases exhibit good enzyme inhibitory, antioxidant, antimicrobial and anti-inflammatory effects. These phytotransform products of plants are of great interest because of their chemical diversity and biological significance as future therapeutic agents (enzyme-related disorders) [2]. Other studies have shown interest in recent years in the discovery of plant extracts that can inhibit clinically useful enzymes, which provide safer and cheaper replacement to synthetic inhibitors [3-4].

Urease (urea amidohydrolase; EC 3.5.1.5) is one of several therapeutic targets that have attracted significant pharmacological attention due to its central role in the pathogenesis of a number of diseases. Urease accelerates the breakdown of urea into ammonia and carbon dioxide, which triggers the rise of the local pH and consequently causes tissue damage. *Helicobacter pylori*, *Proteus mirabilis* and *Klebsiella pneumoniae* are pathogenic microorganisms that secrete urease as a virulence factor which causes a variety of conditions like peptic ulcer, gastritis and urinary tract infection [5-8]. Thus, the prevention of the urease activity can be discussed as one of the promising methods of treatment of these infections and associated complications.

The search for natural urease inhibitors has expanded considerably in recent years, with numerous reports highlighting the inhibitory potential of plant-derived constituents, particularly polyphenols, alkaloids, and saponins [9-12]. In this context, *Nicotiana tabacum* L. (family: *Solanaceae*), commonly known as tobacco, has been recognized as a chemically rich plant containing a variety of bioactive compounds such as nicotine, anabasine, rutin, chlorogenic acid, and diverse alkaloids [13-14]. Recent phytochemical analyses revealed over 200 identified metabolites in N. tabacum, including terpenoids, phenolic acids, and flavonoids, which are known to modulate enzymatic and microbial activities [15-16]. Beyond its well-known pharmacological and toxicological profiles, *N. tabacum* has demonstrated antimicrobial, antioxidant, and anti-inflammatory activities in several studies, suggesting its potential as a source of enzyme inhibitors [17-20].

Although plant-generated bioactive compounds hold great therapeutic potential, additional research is yet to be done to discover and define a set of specific inhibitors that can be used in the treatment of enzymes such as urease that are also critical in the pathogenesis of various infections. Although the anti-urease activity of plant extracts has been reported by different studies, the mechanism, effect and

safety of these natural anti-urease inhibitors have not been properly studied. In particular, the inhibitory effect of *Nicotiana tabacum* (tobacco) is not adequately examined, although this chemical is rich and has bioactive properties.

This research paper is aimed at exploring the urease inhibitory potential of *Nicotiana tabacum*, as well as to examine the possibility of *Nicotiana tabacum* as a source of natural plant-based enzyme inhibitors. Our proposed study aims to determine which bioactive compounds of *Nicotiana tabacum* have potent urease inhibitory properties, which would be considered as alternative urease inhibitor to current inhibitors that are synthetic, safer, and cheaper. The results of the present research can be used to proceed in designing novel treatment approaches to deal with the infections related to urease-producing pathogens.

2.0 MATERIALS AND METHODS

2.1 Plant Collections and Extraction

In the Swabi District, Khyber Pakhtunkhwa (KPK), Pakistan, four native types of Nicotiana tabacum were sampled on local farmers, and these were SN1, SN2, SPG28, and K399. The following samples were sent to the Department of Chemistry to the University of Swabi, for further handling and the voucher sample (NO.UOS-BOT/120) was deposited in the Department of Botany, in the same university.

The leaves of the four varieties of *N. tabacum* were cut off the stems, washed and dried in the air in the shade during a period of two weeks. The plant material was dried and ground into a fine powder of 2 kg which was subsequently subjected to further analyses [20-21]. One hundred grams of the powdered leaf of each variety was put into a 1 L volumetric flask, 800 mL of methanol and the mixture was left to macerate in seven days. The mixture was then filtered using Whatman Grade 1 filter paper, and the resulting filtrate concentration was done under reduced pressure to extract the crude extracts. These extracts were stored at -20°C in order to use them later. The chemicals, reagents and solvents used in the process were of analytical grade and obtained by Honeywell-Fluka (Charlotte, NC, USA), Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2 Urease Inhibitory Activity

The urease inhibitory activity of the n-hexane, chloroform, ethyl acetate, methanol, and butanol fractions of Calotropis procera extract was evaluated using the jack bean urease (EC 3.5.1.5) inhibition assay. The reaction mixture was prepared in a 96-well microplate and consisted of 25 µL of enzyme solution, 55 µL of phosphate buffer (100 mM, pH 8.2) containing urea as the substrate (100 mM), and 5 µL of the test sample. The mixture was incubated at 30 °C for 15 minutes.

The enzymatic activity was determined spectrophotometrically by measuring the amount of ammonia produced using the indophenol method. After the initial incubation, 45 µL of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% NaOCl containing active chlorine) were added sequentially to each well. The plates were incubated for an additional 50 minutes at room temperature, and the absorbance was then recorded at

630 nm using a microplate reader.

All experiments were carried out in triplicate, maintaining a final reaction volume of 200 µL per well. Thiourea was used as the reference standard inhibitor [22–23]. The percentage of urease inhibition was calculated using the following equation:

$$\text{Percent effect} = 100 - \frac{OD\ testwell}{OD\ control} \times 100$$

2.3 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) based on three independent replicates (n = 3). The results were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to evaluate significant differences among treatment means. A p-value less than 0.05 was considered statistically significant.

3.0 RESULTS AND DISCUSSION

The inhibitory effect of different solvent fractions of the Nicotiana tabacum roots on urease was measured using thiourea as the standard inhibitor and the findings are in Table 1. The best inhibitory effect was observed with the butanol extract which presented the highest inhibition at 88.01 percent with the IC_{50} value of 33.04 ± 1.35 µg/mL. The next fraction was the methanol fraction and it was found to inhibit 82.76 with an IC_{50} of 38.67 ± 1.40 µg/mL. The ethyl acetate (49.09 percent) fraction showed moderate urease inhibition, but in contrast, the hexane (30.32 percent) and chloroform (35.87 percent) fractions had a lower activity when compared to the same concentration (0.2 µg/mL). The most active standard inhibitor was thiourea with the highest inhibition value of 98.23 percent and the IC_{50} of 21.03 ± 1.00 µg/mL.

Table 1: Urease screening of hexane, chloroform, ethyl acetate, methanol, and butanol fractions of Nicotiana tabacum roots

Extract/standard	Concentration (µg/mL)	% inhibition	IC_{50}
Hexane	0.2	30.32	-
Chloroform	0.2	35,87	-
Ethyl Acetate	0.2	49.09	-
Methanol	0.2	82.76	38.67±1.40
Butanol	0.2	88.01	33.04±1.35
Thiourea	0.2	98.23	21.03±1.00

These results indicate that the solvent polarity used had a large effect on the urease inhibitory capacity of N. tabacum root fractions. The polar solvents like methanol and butanol were found to be more effective in extracting bioactive constituents that would inhibit the urease activity than the non polar solvents like hexane and chloroform. It can be explained by the fact that polar solvents are more soluble to phenolic compounds, alkaloids, flavonoids, and other secondary products, which are suspected to be the contributors of urease inhibition [24].

Urease is a metalloenzyme (containing nickel) that catalyses urea breakdown into carbon dioxide and ammonia resulting in elevated pH and ammonia toxicity. Possible pathological conditions linked to excessive urease activity include peptic ulcers, hepatic encephalopathy, and UTI [25]. Consequently, the phytonanolytic urease inhibitors found in plants have

pharmacological significance due to the creation of alternative therapy with fewer side effects.

The high inhibitory value recorded in the butanol and methanol fraction of N. tabacum is an indication of high concentration of bioactive phytochemicals that bind with the active site of the enzyme (hydrogen bonding or chelation with nickel ions in the catalytic site). In earlier research, it was found that nicotine-derived phenolic acid, alkaloid, and flavonoid enzyme inhibitors due to similar mechanisms achieved a significant level of enzyme inhibition [26-28]. Such compounds are probably more concentrated in the methanolic and butanolic extracts and lead to strong inhibition.

Relatively, the moderate activity exhibited by ethyl acetate fraction and weak activity by the non-polar fractions (hexane and chloroform) are compared with the tendencies of solvent polarity in the literature of other urease inhibition analyses in plants [29-30]. The findings all indicate that N. tabacum root extracts, especially the butanol and methanol fractions, have promising prospects of development of natural urease inhibitors, which may act as safer alternatives to the synthetic urease inhibitors such as thiourea.

4.0 CONCLUSION

The present study demonstrated that the Nicotiana tabacum root possesses significant urease inhibitory potential, particularly in its polar solvent fractions. Among all tested extracts, the butanol fraction exhibited the highest activity with an IC_{50} of 33.04 ± 1.35 µg/mL, followed closely by the methanol fraction (IC_{50} = 38.67 ± 1.40 µg/mL), indicating that bioactive constituents with urease inhibitory properties are more soluble in polar solvents. The results highlight the promising potential of N. tabacum roots as a natural source of urease inhibitors, offering a safer and potentially more biocompatible alternative to synthetic agents like thiourea.

These findings provide a scientific basis for further bioassay-guided fractionation and phytochemical investigations aimed at isolating and characterizing the active compounds responsible for urease inhibition. Future studies integrating molecular docking and in vivo validation could elucidate the mechanism of enzyme binding and therapeutic relevance of these compounds in the management of urease-related pathologies such as peptic ulcers, urinary tract infections, and hepatic disorders

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