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Phytochemical composition and urease inhibitory potential of crude extracts and subsequent fractions of *Bauhinia alba*

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Abstract

The nickel-dependent metalloenzyme, urease, is involved in the hydrolysis of urea to carbon dioxide and ammonia and is an important virulence factor in a variety of pathogenic microorganisms such as *Helicobacter pylori*, *Proteus mirabilis* and *Klebsiella pneumoniae*. Overactivity of urease also plays a role in the emergence of gastric ulcers, hepatic encephalopathy, and urolithiasis, and therefore, urease inhibition is an important therapeutic goal. To find safe and natural alternatives to the artificial inhibitors, the given study examined phytochemical composition and urease inhibitory activity of crude extract and solvent-partitioned fractions of *Bauhinia alba* roots. The shade-dried roots were impregnated with ethanol and fractionated with the succession of n-hexane, chloroform, ethyl acetate, methanol and butanol. Phytochemical screening showed that the methanol and butanol fractions were strong in flavonoid, tannin, steroid, and reducing sugars, and nonpolar fractions had low amounts of secondary metabolites. The jack bean urease assay was used to determine the urease inhibitory activity, with thiourea being one of the standard inhibitors. The butanol fraction was the most active (93.76% inhibition; IC_{50} = 29.44 ± 1.09 µg/mL), then the methanol fraction (80.66% inhibition; IC_{50} = 34.77 ± 2.10 µg/mL). There was a direct association between the richness of polar phytoconstituents and urease inhibition, indicating that flavonoids, tannins and steroids are the main contributors to the enzyme inhibition witnessed. The results confirm the application of *Bauhinia alba* as a medicine in ethnomedicine and as a prospective source of natural urease inhibitors against the urease-mediated diseases, including gastritis, peptic ulcers, and urinary infections. The research on the isolation, the description, and the mechanistic analysis of active compounds should be continued to create new therapeutic agents of plant-based origins.

KEYWORDS
Bauhinia alba; phytochemical screening; urease inhibition; flavonoids; natural inhibitors; enzyme inhibition

1.0 INTRODUCTION

Urease (EC 3.5.1.5) is a nickel-dependent metalloenzyme that catalyzes urea hydrolysis into ammonia and carbon dioxide, which is a reaction that is a key step in the metabolism of nitrogen in bacteria, fungi, and plants [1]. Urease has been found to act as a virulence factor in some pathogenic microorganisms despite being physiologically relevant, such as Helicobacter pylori, Proteus mirabilis, Klebsiella pneumoniae and Staphylococcus saprophyticus. Overactivity of urease in these organisms increases the local pH by producing ammonia, stimulating tissue irritation, inflammation, and persistence of microbes in host tissues. Therefore, urease overexpression is firmly associated with the pathogenesis of several clinical diseases, including peptic and duodenal ulcers, gastritis, hepatic encephalopathy, and urolithiasis [2-5]. Furthermore, urease is significant in the agricultural

industry since it enhances the urea-based fertilizer breakdown process, which leads to the loss of nitrogen and alkalization of the soil, hence reducing soil fertility. The urease inhibition has become an essential therapeutic and ecological approach due to its role in the clinical and environmental issues [1].

Even though several synthetic inhibitors have proven to be effective urease inhibitors, including hydroxyurea, acetohydroxamic acid, and thiourea, their application is frequently limited because of their undesirable effects, including teratogenicity, hepatotoxicity, and gastrointestinal discomfort [6-8]. It has necessitated increased interest in the study of natural substitutes, especially medicinal plants, as safer and sustainable sources of bioactive compounds with urease inhibitory properties. The secondary metabolites of plants, such as flavonoids, tannins, terpenoids, alkaloids, steroids, and phenolic acids, have numerous biological effects, in particular, antimicrobial, anti-inflammatory, antioxidant, and anti-enzyme [9,10]. These substances are reported to have an inhibitory effect on urease via the chelation of the nickel ions at the active site of the enzyme, inhibiting the arrival of the substrate, and by causing conformational changes due to the formation of hydrogen bonds and hydrophobic interactions [3,11]. More than that, the qualitative and quantitative composition of plant extracts is greatly affected by the solvent polarity in which phenolic and flavonoid constituents are usually extracted by polar solvents, including methanol and butanol, which are frequently linked to strong urease inhibitory activity [12].

The Bauhinia genus (family Fabaceae, subfamily Caesalpinioideae) is a group of over 250 species of shrubs, trees, and climbers that are abundantly distributed in the tropical and subtropical areas. They are highly decorative and ethnomedicinal and have been very common in traditional medicine, such as Ayurveda and Unani medicine, to treat diabetes, wounds, ulcers, inflammation, and microbial infections [13-15] The phytochemical studies carried out on Bauhinia variegata, B. racemosa, B. tomentosa, and B. purpurea have shown the presence of various bioactive compounds such as flavonoids (kaempferol, quercetin, rutin), steroids (β-sitosterol, stigmasterol), terpenoids, coumarins, and tannins that are associated with their wide range of pharmacological activities [16]. In spite of these reports, the phytochemical composition and urease inhibitory potential of *Bauhinia alba* have not been studied in detail, which is a significant gap in the existing phytopharmacological research.

The white orchid tree, also known as *Bauhinia alba* , is an evergreen plant that grows in South and Southeast Asia [13]. Historically, the treatment of ulcers, wounds, and microbial infections has been done using different components of this plant. These therapeutic effects are preliminarily reported ethnopharmacological records that attribute these effects to the high concentration of phytoconstituents (flavonoids, tannins, saponins, alkaloids, and phenolic acids) in it [17]. Nevertheless, detailed scientific confirmation of these assertions, especially in relation to the phytochemical profile and enzyme inhibitory potential of *B. alba* roots and its solvent fractions, is unavailable so far. Such properties should be evaluated systematically in order to not only authenticate their ethnomedicinal use but also to find active phytochemicals that can be used as lead compounds in developing new urease inhibitors [18].

Lekmine et al. (2025) state that urease inhibition is a viable treatment approach in reducing the effects of urease-related diseases, especially those related to gastric ulcers

caused by H. pylori and urinary tract infections [19]. Likewise, Leena et al. (2020) have shown that the natural inhibitors have great benefits based on their multi-targeting nature, low toxicity, and synergies caused by complex phytochemical products [20]. Based on the known medicinal interest and the wide range of phytochemical makeup of the *Bauhinia* species, the hypothesis is that *B. alba* roots could contain strong urease inhibitors that could be the reason behind its traditional therapeutic effect.

Thus, the current research aimed at examining the phytochemical composition and the urease inhibitory effect of the crude extract and the sequential solvent fractions (hexane, chloroform, ethyl acetate, methanol, and butanol) of the crude extract of the *Bauhinia alba* roots. The objectives were as follows: (1) conduct qualitative phytochemical screening to assess the key classes of secondary metabolites and (2) determine the in vitro urease inhibitory activity and the IC50 value of each fraction, and (3) establish correlations between the phytochemical profiles and urease inhibitory potential.

This research is expected to offer a scientific explanation of the ethnomedicinal application of *Bauhinia alba* as well as to identify its potential as a natural urease inhibitor to treat urease-related ailments. In addition, the research also highlights the significance of the solvent polarity in the selective extraction of bioactive phytoconstituents and is therefore of great importance in the study of phytopharmacology and the study of enzyme inhibition.

2.0 MATERIALS AND METHODS

2.1 Plant collections

The sampled flowers of *Bauhinia alba* were picked in the botanical garden of the University of Peshawar, Pakistan. Prof. Dr Abdur Rashid, Department of Botany, University of Peshawar, authenticated the plant materials, depositing voucher specimens of the same in the future for further studies.

2.2 Extraction and Fractionation

2.2 The shade-dried roots of *Bauhinia alba* were roughly powdered and macerated in ethanol over a period of five days at ambient temperature and stirred. The prepared extracts were then filtered and concentrated under low pressure in a rotary evaporator at 40 °C in order to get the crude ethanolic extracts. These concentrated extracts were then suspended in distilled water and partitioned with n-hexane, chloroform, ethyl acetate, and methanol in that order, and the respective solvent fractions were obtained. The process of fractionation was performed based on the conventional phytochemical extraction procedures presented by Uddin et al. (2012), Uddin et al. (2011a), Uddin et al. (2011b), and Savithramma et al. (2011) [21-24].

2.3 Urease Inhibitory Activity

The jack bean urease (EC 3.5.1.5) inhibition assay was used to measure the urease inhibitory potential of the n-hexane, chloroform, ethyl acetate, methanol and butanol fractions of *Bauhinia alba* root extract. The reaction solution contained 25 µL of enzyme solution, 55 µL of phosphate buffer (100 mM, pH 8.2) in which urea was used as the substrate (100 mM), and 5 µL of the test sample. After incubation at 30°C for 15 minutes in a 96-well microplate, the mixture was incubated. The activity of urease was measured spectrophotometrically, according to the quantity of ammonia generated, according to the indophenol technique. Phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (0.5%

w/v NaOH and 0.1% NaOCl containing active chlorine) were added (45µL and 70µL, respectively) to every well in sequence. The incubation period lasted 50 minutes at room temperature, after which the absorbance was recorded at 630 nm in a microplate reader. All experiments were repeated thrice, keeping a final volume of 200µL. Thiourea was taken as the reference inhibitor [25,26]. The degree of urease inhibition was computed by using the equation below:

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

2.4 Statistical Analysis

Results were presented in the form of mean ± SD (n = 3) and subjected to the one-way ANOVA and later to the Tukey test, with a p-value of below 0.05 being the level of statistical significance.

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical Screening of Bauhinia alba Root Fractions

Qualitative phytochemical screening of hexane, chloroform, ethyl acetate, methanol and butanol extracts of *Bauhinia alba* roots showed that secondary metabolites are not evenly distributed throughout the extracts **(Table 1).** Fatty acids were identified in the hexane, chloroform and methanol fractions, which means that there were non-polar lipid constituents present. The abundance of steroids in all fractions indicated that they are soluble in polar and non-polar solvents.

The chloroform, ethyl acetate, methanol and butanol fractions contained flavonoids and tannins, which have antioxidant and enzyme inhibitory abilities and were not found in the non-polar hexane fraction and indicating that the polar solvent extracts were the major sources of these polyphenolic compounds. Conversely, alkoxides and anthraquinones were never detected in any of the fractions, which suggests that the classes of metabolites are absent or below the limit of detection in *B. alba* roots. The presence of reducing sugars was moderate in all the polar fractions (chloroform to butanol), but not in hexane, and the solubility of carbohydrate derivatives as per polarity supported the solubility pattern.

Table-1. Phytochemicals screening test of hexane, chloroform, ethyl acetate, methanol, and butanol fractions of *Bauhinia alba* roots

Secondary metabolites	Hexane	Chloroform	Ethyl Acetate	Methanol	Butanol
Fattyacids	+	+	-	+	-
Steroids	+	+	+	+	+
Flavonoids	-	+	+	+	+
Tannins	-	+	+	+	+
Steroids	+	+	+	+	+
Alkoxides	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Reducing sugars	-	+	+	+	+

Keywords: +=present; -absent

3.2 Urease Inhibitory Activity

As shown in the outcome of the urease inhibition assay of the solvent fractions of *Bauhinia alba* roots, there was a significant enzyme inhibitory potential **(Table 2).** The butanol fraction was the most active in terms of inhibitory activity, using a concentration of 0.2 µg with an inhibition of 93.76% and an *IC50* of 29.44 ± 1.09 µg/mL, which matches that of the standard inhibitor, thiourea (98.18% inhibition; *IC50* = 21.59 ± 0.88 µg/mL).

The methanol fraction was also found to have good inhibitory activity (80.66%; IC50 = 34.77±2.10µg/mL), and the ethyl acetate fraction exhibited moderate inhibitory

activity (48.04%). On the other hand, hexane and chloroform fractions had lower percentages of inhibition percentage of 27.55% and 28.09%, respectively, showing comparatively low urease inhibition.

Table-2. Urease inhibitory activity of hexane, chloroform, ethyl acetate, methanol, and butanol fractions of *Bauhinia alba* roots

Extract/standard	Concentration (µg)	Percentage inhibition (%)	*IC50*
Hexane	0.2	27.55	-
Chloroform	0.2	28.09	-
Ethyl Acetate	0.2	48.04	-
Methanol	0.2	80.66	34.77±2.10
Butanol	0.2	93.76	29.44±1.09
Thiourea	0.2	98.18	21.59±0.88

The high activity of the polar fractions (methanol and butanol) is associated with the high concentration of flavonoids, tannins and steroids, which phytochemical screening shows and therefore indicates that they might be the crucial components involved in the urease inhibition detected. The findings point to the potential of the *Bauhinia alba* root extracts and the butanol fraction of the extract in particular as promising natural sources of urease inhibitors, and that further investigations can be extended to exploit the root extracts as potential urease inhibitors in the management of urease-related diseases such as peptic ulcers and urinary tract infections.

4.0 DISCUSSION

Medical plants are still an invaluable source of bioactive compounds that have influenced contemporary pharmacology. Natural products are biologically selective in that they have a tremendous structural diversity that allows them to interact with biological targets like enzymes, receptors, and nucleic acids. They have a proven therapeutic use, particularly in the treatment of metabolic, inflammatory, and infectious diseases [9,18]. Enzymes are among the biomolecular targets of their large number, and play an important role in regulating the course of disease and are targets of drug discovery. In that regard, urease enzyme inhibition, which is engaged in the pathophysiological and environmental mechanisms, has become an important pharmacological approach [2,4].

Urease is an enzyme that is nickel-dependent, which catalyzes the hydrolysis of urea into carbon dioxide and ammonia; a reaction crucial to nitrogen metabolism but harmful when out of control [1]. Urease is also a virulence factor in a number of pathogenic microorganisms, *such as Helicobacter pylori, Proteus mirabilis, Klebsiella pneumoniae*, and *Staphylococcus saprophyticus*, which can survive in acidic conditions by keeping the local pH elevated due to the release of ammonia [3,5]. The result of this hyperactivity is inflammation and tissue damage, which causes diseases like gastritis, peptic ulcers, hepatic encephalopathy, and urolithiasis. Even though synthetic inhibitors, such as acetohydroxamic acid and thiourea, are present, usage is restricted due to toxicity, hepatotoxicity, and gastrointestinal side effects [6,7]. It has led to the global effort to explore medicinal plants as less toxic, naturally derived, enzyme inhibitors with fewer side effects and higher biocompatibility.

The current research determined the phytochemical profile and urease inhibitory activity of the crude extract and solvent-partitioned fractions of *Bauhinia alba* roots. It was found that screening of phytochemicals contained an unequal distribution of secondary metabolites across the fractions, where polarity was a determining factor in the extraction profile. The reducing sugars, tannins, and steroids, along with the flavonoids, were bright in the methanol

and butanol fractions and relatively lower in the hexane and chloroform fractions. The obtained results are in line with other research studies showing that polar solvents yield a greater percentage of phenolic and flavonoid compounds that, in many respects, cause antioxidant and enzyme inhibitory effects [12]. These compounds have been identified to disrupt the catalysis of enzymes in many ways, such as chelation (removal) of key metal ions such as nickel, entry of substrates, or conformational alteration of the enzyme structure [3,11].

The urease inhibition test demonstrated that *B. alba* has great inhibitory ability in its polar fractions, especially in the butanol and methanol extracts. Butanol fraction showed maximum inhibition (93.76% inhibition, IC50 = 29.44±1.09) and then the methanol fraction (80.66% inhibition, *IC50* = 34.77±2.10). These findings can be compared to the conventional inhibitor thiourea, which exhibited an *IC50* of 21.59±0.88 µg/mL. The ethyl acetate fraction had moderate activity, and the nonpolar hexane and chloroform fractions had little activity, which implies that the active phytoconstituents that inhibit urease are mostly concentrated in the polar fractions. This tendency agrees with the identified phytochemical composition, which suggests that flavonoids, tannins, and steroids may have significant roles in the inhibition mechanism. Such data can be compared with those of other researchers [4] who reported that polyphenolic compounds could hardly be dissociated from urease active sites, which are involved in enzyme inactivation through chelation by metals and hydrogen bonds.

These observations are further supported by comparative literature about related *Bauhinia* species. *B. variegata*, *B. racemosa*, and *B. purpurea* extracts have been shown to have antioxidant, antimicrobial and enzyme inhibitory properties due to their high concentration of flavonoids, including quercetin, kaempferol and rutin [13,16]. The current results therefore confirm *B. alba* as a pharmacologically active species and have therapeutic potential. Its good inhibition of urea stipulates that *B. alba* may be a good natural source of antiurease agents that can be used to cure urease-related diseases like gastric ulcers and urinary tract infections. Moreover, as flavonoids and tannins are both reported to have antioxidant and anti-inflammatory effects, their coexistence in *B. alba* could have synergistic gastroprotective effects, which could be helpful in addition to urease inhibition [19,20].

The mechanism through which *B. alba* fractions inhibit the effect of the enzyme might be through a number of interactions with the active site of the enzyme. The active configuration of urease may be destroyed by the effect of phenolic hydroxyl groups in flavonoids and tannins that chelate nickel ions that are important in catalyzing the hydrolysis of urea [11]. In addition, the compounds can interact with the amino acid residues around the compound via hydrogen bonds and hydrophobically, which causes a lack of conformational stability in the enzyme structure. These kinds of mechanisms explain the high level of inhibition observed by the butanol fraction, which is probably concentrated with such active polyphenolic constituents.

In addition to enzyme inhibition, there is a potential pharmacology beyond enzyme inhibition as indicated by the phytochemical diversity of *B. alba*. *H. pylori*-induced gastric damage can be reduced by the

synergetic effects of the strong antioxidant and antimicrobial activity of flavonoids and tannins with urease inhibition [17,20]. These are the two mechanisms that add therapeutic benefit to the use of *B. alba*; it is used traditionally to treat gastrointestinal and infectious diseases.

The further research must be aimed at isolating and characterizing the bioactive compounds involved in the observed urease inhibition using the latest chromatographic and spectroscopic methods, e.g. HPLC, LC-MS, and NMR. The computational studies, such as molecular docking and dynamics simulations, may also be used to gain a deeper understanding of the binding mechanism between the urease active site and *B. alba*-derived phytochemicals. To ensure efficacy and safety, in vivo studies on disease models relevant to the disease, e.g. *H. pylori*-induced ulceration, will be necessary. In addition, the investigation of possible synergistic effects of *B. alba* extracts and traditional urease inhibitors can create new possibilities in combination treatment with high activity and lower toxicity.

To sum it up, the current research has shown that *Bauhinia alba* is a good source of natural urease inhibitor, and butanol and methanol extracts of the plant have high levels of urease inhibition, which is associated with the high concentrations of phytochemical substances. The findings not only indicate the traditional usage of the plant in medicine but also indicate that the plant may be used in the future in the development of safer and more effective phytotherapeutic agents against urease-mediated illnesses. The investigation supports the increasing significance of natural products in the research on enzyme inhibitors and motivates future investigations to transform *B. alba* beyond preliminary phytochemical screening to the identification of lead compounds to develop them into therapeutic agents..

5.0 CONCLUSION

The current research shows that the roots of *Bauhinia alba* contain a high urease-inhibitory activity, mostly in the methanol and butanol fractions that contain flavonoids, tannins, and steroids. The prominent inhibition rate was similar to that of the standard thiourea, and this indicates that these polar phytoconstituents are the determining components of the enzyme inhibition. The results confirm the modern scientific usefulness of the use of *B. alba* in the treatment of gastric and urinary diseases and emphasize its future as an independent source of natural and safe, effective urease inhibitors. It is suggested that future research on the isolation, structural characterization and in vivo studies of active compounds will be required to come up with new plant-based therapeutic agents against urease-related diseases.

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