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PHYTOCHEMISTRY ANTI-UROLITHIATIC AND ANTIOXIDANT ACTIVITY OF ALSTONIA SCHOLARIS LINN R. BR.

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ABSTRACT

Plants have served as the foundation for traditional healing practices across the globe for centuries and still offers new possibilities for treatments for various human ailments. In recent years, there has been a significant shift in perspective concerning the ethnopharmacological uses of phytochemical therapies. The present study investigates the phytochemical constitution and antioxidant activity of various solvent (aqueous, butanol and ethyl acetate) extracts of the leaf and bark of Alstonia scholaris. Alstonia scholaris is a member of the Apocynaceae family, which includes around 250 genera and 2000 species of tropical trees, shrubs, and vines. Nearly every part of this plant is utilized in medicinal practices, with the bark exhibiting antihelminthic and astringent properties. It has been employed in the treatment of chronic diarrhea, dysentery, and irregular bowel movements. The aim of the current study was to evaluate the phytochemical composition and determine the antioxidant activity of the solvent extracts derived from the bark and leaves of Alstonia scholaris. The leaves of Alstonia scholaris contain various phytochemical components. The qualitative phytochemical analysis reveals the presence of flavonoids, phenolic compounds, alkaloids, phenols, and tannins in the extracts of the leaves. The antibacterial and antioxidant properties of the leaf extracts from Alstonia scholaris were analysed. The TLC examination of the water extract from the bark, which exhibited the greatest antioxidant activity compared to the other extracts, was also conducted.

KEYWORDS: Alstonia scholaris, Antioxidant, Antihelminthic, Astringent, Phytochemical analysis.

INTRODUCTION

Medicinal plants remain an essential source of diverse bioactive compounds with significant therapeutic possibilities. About 80% of the world's population relies on traditional herbal medicine as their main healthcare resource, primarily drawing from established medicinal practices in China, India, and various regions of Africa. This heavy dependence on plant-based therapies highlights both the historical evolution of indigenous medical systems and the ongoing challenges of accessing conventional healthcare, especially in resource-constrained environments. Traditional Chinese medicine (TCM), Ayurvedic medicine from India, and various African traditional medical systems encompass thousands of years of accumulated wisdom regarding the medicinal properties of plants and their clinical uses.[1] Although herbal medicine saw a decline during the eighteenth and nineteenth centuries, many treatments from traditional healers demonstrated effectiveness and were eventually developed into valuable prescriptions as physicians began to investigate therapeutic agents. William Withering was the first medical practitioner to study a traditional medicine empirically.[2]

Plants have served as a source of medicine for millennia across different cultures globally. Throughout our history, people have depended on plants not just for nourishment but also for healing. Ancient humans became skilled at recognizing plants with healing qualities, and this understanding developed over time. In contemporary medicine, numerous medications that treat ailments such as infections, cardiovascular conditions, and cancer are sourced from plants or their derivatives.[3] For instance, aspirin, which is among the most commonly utilized pain relievers, originates from the bark of willow trees. Herbal medicine, or botanical medicine, also referred to as phytotherapy, entails using various parts of plants (leaves, roots, flowers, etc.) for their therapeutic benefits.[4]

Urolithiasis is the presence of solid, non-metallic minerals within the urinary system. Calcium oxalate stones are the most prevalent type among different kinds of kidney stones. The process of stone formation includes a series of physicochemical processes, starting with the nucleation of crystals, followed by their aggregation, and ultimately leading to their retention in the urinary tract.[5] Kidney stones rank among the most excruciating urological conditions. Renal stones affect 5% to 15% of adults. Epidemiological data show that nephrolithiasis is more prevalent among men (12%) compared to women (6%) and tends to be more common in individuals aged 20 to 40 for both genders. Urinary stones impact 10-12% of the population in developed nations.[6] Over recent years, the occurrence of urinary stones has been on the rise, while the age of first manifestation is decreasing, with a prevalence exceeding 10% and an anticipated recurrence rate of 50%. The condition of stone disease significantly influences healthcare.[7]

Organisms such as E. coli, Proteus species, streptococcus, staphylococcus, pseudomonas, and Ureaplasma urealyticum were connected to infected stones. There are increasing studies that have been documented that the end products of urealysis can damage the glucose layer of the renal urolithial cells thus leading to the bacterial adhesion, biofilm development and mineral encrustation. Therefore, in order to identify and treat the infection causing the stone formation, thorough microbiological studies are reuired.[8] The process of kidney stone formation is intricate and involves numerous physiochemical properties such as supersaturation, nucleation of crystals, precipitation, growth of crystals, aggregation of these crystals, and the retention of stone constituents within tubular cells. Kidney stones can take various forms, including cystine stones, calcium oxalate stones, calcium phosphate stones, struvite stones, and uric acid stones. Nonetheless, it is clear that factors like crystal retention, apoptosis of cells, injury to renal cells, and the influence of certain stone promoters or inhibitors significantly contribute to the development of kidney stones.[9]

A comprehensive investigation is required to examine botanicals as potential alternative or complementary therapies for urolithiasis. In addition, gaining insights into the fundamental pathophysiology, pathogenesis, and genetic factors involved in the development of kidney stones may pave the way for discovering innovative medications and approaches to manage urolithiasis in the near future.[10] Herbal treatments for kidney stones have been recognized for their safety for a long time. Additionally, these herbal remedies effectively eliminate fat from kidney stones without harming the kidneys. These treatments are economical and typically have fewer side effects compared to allopathic medications. It is important to enhance existing herbal formulations through newer technologies to achieve improved effectiveness, synergistic benefits, better patient adherence, and cost-efficiency in treating kidney stones and other urinary issues such as inflammation and complications related to urinary stents.[11]

Alstonia scholaris is an evergreen tree that thrives in the tropical regions of Asia. It can reach heights of up to one hundred meters and produces white flowers. The species is named "scholaris" because its wood has been utilized to make school boards. India and China are known for their traditional healing practices such as Ayurveda, Unani, and Siddha. This plant is part of the Alstonia genus, which has been associated with various ailments including malaria, fever, insomnia, chronic diarrhoea, and rheumatic pain. Alstonia scholaris is incorporated into several Ayurvedic formulations like saptaparnasatyadiyati, saptacchadadivatha, and saptaparnaghanasara, which are used to remedy conditions such as asthma, malaria, cough, jaundice, digestive issues, headaches, and fever.[12]

The current study aims to investigate the cytoprotective and antioxidant properties of bark extract as well as the idea that the phytoconstituents of A. scholaris may prevent or dissolve the development of calcium oxalate crystals in an in vitro model. The study's background is that when cells are subjected to chemical stimuli, free radicals are produced, which causes cell death due to the toxicity of the stimulus. This research examined the potential for antioxidant activity as well as the defensive or protective effect of A. scholaris bark extract against chemical or free radical toxicity and in vitro anti-urolithiasis activity.

AIM AND OBJECTIVES

Aim of study

- The purpose of the current study was to examine the anti-urolithiasis and anti-oxidant properties of the phytochemical components from the plant Alstonia scholaris.

Objectives of the study

- Collection and authentication of Alstonia scholaris.
- Extraction and identification of flavonoids from Alstonia scholaris.
- Isolation and characterization of phytoconstituents of Alstonia scholaris.
- Evaluation of Alstonia scholaris extract for in vitro anti urolithiatic and anti-oxidant activity.

PLANT PROFILE

ALSTONIA SCHOLARIS	
PLANT NAME	Alstonia scholaris
KINGDOM	Plantae
FAMILY	Apocynaceae
SUBTRIBE	Alstoniinae
GENUS	Alstonia
SPECIES	Alstonia scholaris[13]

Figure 1: Alstonia scholaris tree.

Figure 2: Alstonia scholaris flowers.

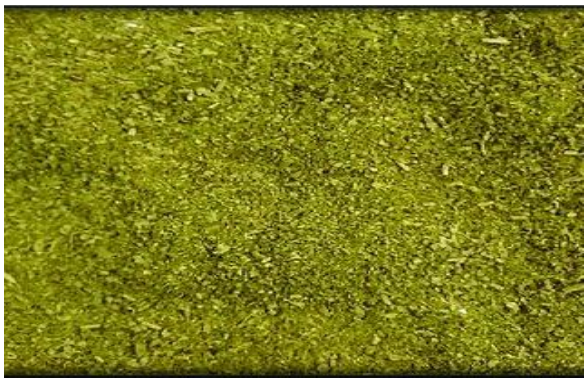

Figure 3: Alstonia scholaris powder.

Phytochemical properties

Chromatographic investigations of A. scholaris revealed the presence of several classes of phytochemicals, including alkaloids, monoterpenoids, flavonoids, tannins, triterpenes, sterols, and esters.[14] etc. The HPLC analysis of the methanolic extract demonstrated that A. scholaris includes several phenolic acids such as gallic acid, ellagic acid, catechin, and kaempferol.[15]

Medicinal properties

1. Anti-inflammatory and analgesic activity

In vivo research indicated that a dosage of A. scholaris at 200mg/kg notably decreased pain and mechanical hyperalgesia, while also exhibiting anti-inflammatory analgesic effects.

2. Anti fertility activity

The benzene extract from the bark of A. scholaris was administered orally to Wistar rats for a period of 60 days. Following the administration of the extract, a reduction in the size of the prostate gland, epididymis, and seminal vesicle was noted in the rats. A decline in the production of spermatids and spermatocytes was also observed. Additionally, there was a significant decrease in protein levels and other biochemical parameters associated with the testes and seminal vesicle.[16]

3. Antidiabetic activity

A. scholaris was tested on an animal model of diabetes induced by streptozotocin, and the results showed that A. scholaris considerably lowered blood glucose levels and diminished lipid peroxidation.[17]

4. Antimicrobial activity

The anti-microbial properties of A. scholaris were investigated using various parts of the plant, including stem bark, leaves, and root bark, against both gram-positive and gram-negative bacteria. It has been noted that the phytochemicals found in A. scholaris contribute to its antimicrobial effects.[18]

Phytochemical analysis

Extraction

Preliminary qualitative assessment was carried out using modified phytochemical methods to analyse the initial composition of phytochemicals. Begin by macerating 1 gram of A. Scholaris leaf and stem bark powder separately in 20 ml of methanol at its boiling point for five minutes. Following the filtration of the extracts, the filtrate was concentrated through evaporation. The resulting residue was combined with one milliliter of water and one milliliter of chloroform, and the mixtures were stirred. After some time, two distinct layers would have emerged. The chloroform layer contained triterpenoids and steroids, while the aqueous layer was found to consist of flavonoids, phenolics, and saponins.[19]

Chemical analysis is done for following

- ✓ **Flavonoids:** A NaOH solution was introduced to a 500 µl extract solution, and this was followed by the addition of dilute HCl. The color change from yellow to colorless suggested the presence of flavonoids.
- ✓ **Alkaloids:** Alkaloids were identified with the help of Mayer's reagent. A few drops of the reagent were mixed into the 500 µl extract solution. The presence of a reddish-brown precipitate indicated the presence of alkaloids.
- ✓ **Saponins:** A test tube received 2 ml of the aqueous layer, which was stirred briefly. The lingering foam that appeared upon the addition of a small quantity of hydrogen chloride indicates the presence of saponins.
- ✓ **Phenolics:**1 millilitre of the aqueous layer was transferred to a test tube, followed by the addition of 3 drops of a 1% ferric chloride solution. The development of a blue or green color suggests the presence of phenolics.
- ✓ **Tannins and phenols**: A small amount of FeCl3 was introduced into the test solution, which had a total volume of 500 µl. The presence of phenols and tannins was confirmed by the formation of a blue or blue-green solution (500 µl).[20]

RESULT

Figure 4: Alstonia scholaris Extract.

Figure 5: Phytochemical analysis.

Table 1: Total phenolic contents in the ethanolic bark extract of Alstonia scholaris expressed in terms of mg of Gallic acid equivalent (mg of Gallic acid /g of extract).

Sample	Ethanolic extract
Total polyphenols content (mg Gallic acid/g extract)	3.15 ± 0.15

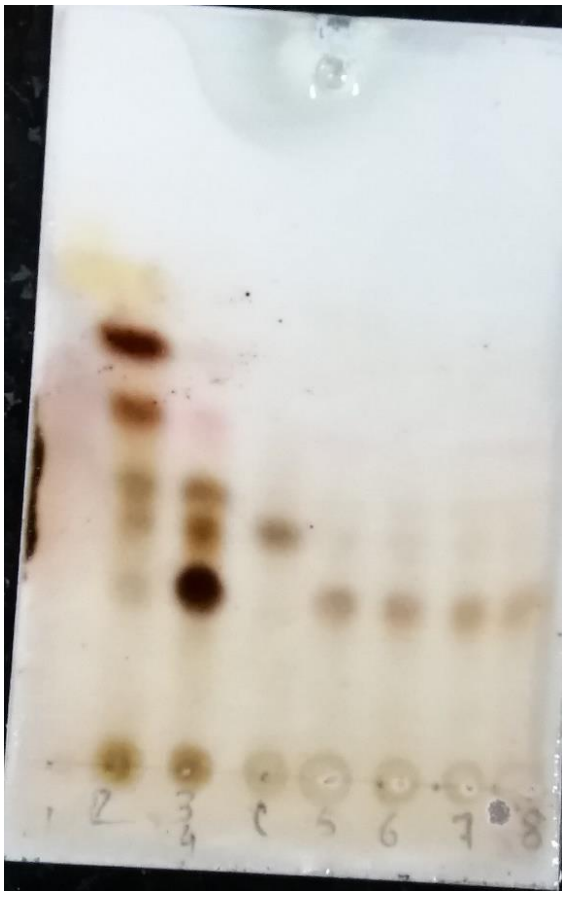

Figure 6: TLC analysis of ethanolic bark extract of Alstonia scholaris L. Chloroform: methanol: water (9:1:1).

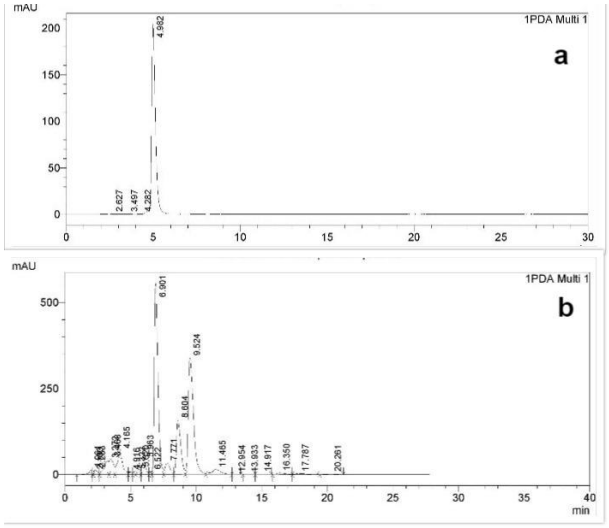

Figure 5: High performance liquid chromatography profile of a) Gallic acid and b) ethanolic extract of Alstonia scholaris (254nm). Mobile phase methanol: water (95:5), 1ml/min flow rate.

CONCLUSION

Alstonia scholaris Linn. R.Br. is part of the Apocynaceae family and is indigenous to India. This species thrives in both deciduous and evergreen forests as well as in lowland areas. The bark of A. scholaris exhibits a wide range of medicinal properties, including being bitter, astringent, thermogenic, laxative, antipyretic, anthelmintic, galactogogue, and cardiotonic, which makes it useful for treating fever, malarial fever, and various abdominal disorders.

Flavonoids are recognized for their antioxidant properties and are found in various plants, exhibiting a wide range of chemical and biological activities, including the ability to scavenge free radicals. Phenolic compounds are well-known for their antioxidant effects and their contribution to human well-being. In this research, the total phenolic content was measured using the Folin–Ciocalteu method, with gallic acid serving as a standard. The bark extract of Alstonia scholaris displayed the highest phenol content. From the current investigation, we conclude that the initial phytochemical analysis of Alstonia scholaris L. revealed the presence of alkaloids, flavonoids, proteins, saponins, terpenoids, phytosterols, carbohydrates, and fatty acids. This study indicates that the ethanolic extract of Alstonia scholaris bark exhibits antioxidant properties, which could aid in the prevention of various diseases related to oxidative stress. Furthermore, the current research provides evidence for the existence of bioactive compounds such as flavonoids and phenols, warranting further efforts to isolate and understand the bioactive principles responsible for the antioxidant activity currently being investigated. An in-vitro study on calcium oxalate crystallization inhibition was conducted. This study concludes that the ethanol extracts of Alstonia scholaris L. effectively inhibit the crystallization of calcium oxalate. The findings of this study indicate that the ethanol extracts of Alstonia scholaris L. demonstrated significant antioxidant and anti-urolithiatic activity. Future research may focus on identifying the phytoconstituents and conducting in vivo studies.

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