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Preliminary Phytochemical Screening, Antioxidant, And Antimicrobial Activities Of Alstonia Scholaris R. Br Leaves Medicinal Plants

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Abstract:

Plants provide valuable medicinal chemicals with significant potential in the pharmaceutical sector. The study aims to determine the phytochemical composition of therapeutic plants and evaluate their antioxidant and antibacterial properties. This study was undertaken with an objective of testing the antimicrobial and antioxidative properties of Alstonia Scholaris leaves and identifying the bioactive compound . Alstonia scholaris R. Br, a part of the Apocynaceae family, is a plant utilised in conventional medicine. The research aims to qualitatively examine and compare the initial phytochemical components present in the Alstonia scholaris leaves. The qualitative Phytochemical screening indicates the existence of flavonoids, phenolic compounds, alkaloids, phenols, and tannins in the leaf extracts. Analysed the antibacterial and antioxidant activities of Alstonia scholaris leaf extracts in vitro. The antibacterial efficacy of extract of leaves was assessed using an alcoholic extract to ascertain the minimal inhibitory concentration (MIC) for both positive and negative pathogens: Staphylococcus aureus, Escherichia coli, using disk diffusion method. Methanolic extract from leaf of Alstonia scholaris showed potential antioxidant activity and antimicrobial activity. Plant extracts' antioxidant activity was assessed by FRAP. The plant extracts exhibited strong antioxidant action against FRAP. The Alstonia scholaris extracts exhibited notable antibacterial activity, with minimum inhibitory concentration (MIC) values ranging from 31.25 to 1000 ug/ml, and demonstrated bactericidal activity against all tested species. The analysed extracts of medicinal plants in our research have the potential to serve as antioxidants, antibacterial agents, and as sources for creating novel pharmaceuticals.

Key word: Alstonia scholaris R., Microbial activity, Antioxidant activity, Agar well diffusion method.

INTRODUCTION:

Alstonia scholaris R.Br. is a plant from the Apocynaceae family commonly utilised in traditional medicine. It has been utilised to address a range of conditions such as arthritis, impotence, injuries, earaches, asthma, leucorrhoea, Canine bites, high body temperature, malignant disease, tumours, jaundice, hepatitis, malaria, skin problems, diarrhoea, and more. The plant is a tree that can grow to heights ranging from 10 to 50 metres. The stem is erect, branching, lignified, and Forest green. Their leaves are solitary, lanceolate in form, tapering at the base and having flat margins with rounded corners. The leaves are pinnate, with dimensions of 10–20 cm by 3–6 cm, a slick upper surface, a stem that is about 1 cm long, and a green tint . This plant is known by various local names such as, chattin, chatium (Bengali), white cheese wood, birrba, milkwood pine, milk wood, milky pine, black board tree, devil's tree, dita bark (English), Chaitan, satni, satwin, saitan-ki-jhad (Hindi), Pulai, Pulai linlin (Malay), and Alstonia (German). The plant contains phytochemical compounds such as flavonoids (isookanin-7-o-alpha-lrhamnopyranoside, Alston side, leucoanthocyanins) and alkaloids (echitamine, echitamine chloride, rhazine, nareline, pseudo akuammigi, scholarine, scholaricine,

dihydrocondylocarpine, 19,20-Z and 19,20-E Valles amines). Moreover, the plant harbours tannins, steroids, saponins, and phenolics.

The information above demonstrated the potential of A. scholaris as a natural medicine source for managing or treating a range of illnesses. The research objectives to Identify the antimicrobial properties. and antioxidant activities of A. Scholaris Foliage extracts. We expect that A. Scholaris extracts will be beneficial as an antioxidant or in treating oxidative stress-related diseases, perhaps preventing, reducing, or managing various ailments, such as cancer.[1]

Since ancient times, Plants have been the basis of traditional medicine globally and continue to offer new possibilities for treating many human illnesses. There has been a significant shift in thinking over the last few decades about the medicinal potential of phytochemicals in ethnopharmacology. Therefore, more research is needed to discover and utilise these phytochemicals as a potential source of new therapeutic substances.[2]

As a member of the Apocynaceae family, Alstonia scholaris is also referred to as Blackboard tree, Indian devil tree, and White cheese wood in English; in Hindi, Saptparna, and in Sanskrit, Vishwamukha. This tropical tree is endemic to the Indian subcontinent and is evergreen. In India, traditional medical systems such as homoeopathy, Ayurveda, and homoeopathy employ this drug extensively to treat a variety of illnesses. This plant's various parts are utilised in traditional medicine.[3]

Figure No: 1 Alstonia Scholaris Leaves

Material and Method:

Chemicals and Reagents:

For maceration, methanol was procured from a laboratory. The following materials were acquired from laboratories: The ingredients consist of hydrogen chloride, ferric chloride, acetic anhydride, sulphuric acid, chloroform, ammonia, sodium hydroxide, methanol, and sodium carbonate, magnesium powder Agar-agar, Mayer reagent, Muller Hinton broth, and dimethyl sulfoxide (DMSO).

Instruments:

Copper bath, UV-VIS SPECTROSCOPY 119 series

Sample of Alstonia scholaris:

The leaves of Alstonia Scholaris were gathered from the nearby forest in Solapur. There was identification for the leaves. The plant samples that were gathered had an average age of ten years. Subsequently, the samples were washed, dehydrated at ambient conditions, and pulverised. The finely ground leaves were stored in an airtight container until needed.

Extraction:

One hundred grammes of powdered leaves and one hundred millilitres of methanol flasks were cold macerated and mechanical shaking method separately at room temperature for an entire night. Following the filtering of the mixtures, the filtrate was dried in a copper bath, and the concentrated extracts were kept in an airtight container in the freezer.

Figure No :2 Drying Of Filtrate Figure No: 3 Extract

Phytochemical analysis:

Preliminary qualitative screening was conducted utilising modified phytochemical techniques to examine the initial phytochemical composition. Macerate 1 gram of A. Scholaris leaves and stem bark powders separately in 20 ml of methanol at its boiling point for five minutes. After filtering the extracts, the filtrate was concentrated using evaporation. The residue was mixed with one millilitre of water and one millilitre of chloroform, and the mixtures were stirred.

Two layers would have formed after a little period of time. Triterpenoids and steroids were detected in the chloroform layer, whereas flavonoids, phenolics, and saponins were detected in the aqueous layer.[5]

Chemical analyses were conducted following established protocols to determine components.

Flavonoids:

An NaOH solution was added to a 500 µl extract solution, then followed by dilute HCl. The solution transitioned from yellow to colourless, indicating the existence of Flavonoids.

Phenolics

1 millilitre of the aqueous layer was moved to a test tube, and then 3 drops of 1% ferric chloride solution were added. Formation of a blue or green hue indicates the presence of phenolics.

Saponins:

2 ml of the aqueous layer was transferred to a test tube and briefly stirred. The persistent foaming formed after adding a small amount of hydrogen chloride suggests the existence of saponins.

Alkaloids:

Alkaloids were detected using Mayer's reagent. A small amount of the reagent was added to the 500 µl extract solution. The reddish-brown precipitate confirmed the existence of alkaloids.

Phenols and tannins:

FeCl3 was added in small quantities to the test solution, which had a volume of 500 µl. Phenols and tannins were validated by the creation of a blue or blue-green solution (500 µl).[1,2]

Antimicrobial tests:

Test organism:

Escherichia coli, Staphylococcus aureus was used test antimicrobial activity. All the stock cultures were obtained from P.A.H Solapur University.

Preparation of inoculums:

The organisms were cultured in Mueller-Hinton broth at 37°C for 24 hours and collected in the stationary growth phase. Active cultures for the tests were created by transferring cells from stock cultures into Mueller-Hinton (MH) broth. The bacteria were incubated at 37°C without agitation for 24 hours.

Antimicrobial activity:

An antibacterial activity evaluation was conducted utilising agar well diffusion in an experiment. The test organism was inoculated into MH agar plates, which are specifically designed for bacteria. The standard inoculums were uniformly distributed on the medium's surface. Wells were created in the agar medium and filled with different quantities of Methanol by dissolving it. Three wells were prepared, each with varying concentrations of extract and a standard antibiotic. The plate was cooled for 20 minutes to facilitate dispersion. The plates with bacterial cultures were incubated at 37°C for 21 hours, whereas the plates with bacterial cultures were incubated at room temperature (30-32°C). The antimicrobial efficacy was evaluated by measuring the zone of inhibition against the test organism over a 48-hour period .[6]

Fig no:4 Phytochemical analysis

Determination of minimum inhibitory concentration (MIC):

The extracts were assessed for antibacterial activity by measuring the minimum inhibitory concentration (MIC).The strong antibiotic solutions were mixed with MH broth for bacterial analysis. Different concentrations of the test antimicrobial agents were generated, varying from 5 to 10.The cultures were incubated at 37°C overnight and subsequently diluted with MH broth. The culture was diluted from 1000 ug/ml to 31.25 ug/ml overnight. All tubes, including a control tube without medication. The tubes were incubated at 37°C. Examine the tubes after 24 hours and prolong the incubation period by another 72 hours if needed. A negative control was maintained as well. The Minimum Inhibitory Concentration (MIC) was determined by three assessments for each organism, and the experiment was repeated as needed. The MIC values for a certain isolate were either the same or varied by one dilution.[6]

Antioxidant assay:

FRAP (Ferric reducing antioxidant power Assy)

The Oyaizu method from 1986 was utilised to calculate the decrease in power. Substances with a reduction potential react with potassium ferricyanide to form potassium ferricyanide. The chemical undergoes a reaction with ferric chloride to form a ferric-ferrous complex that shows peak absorbance at 700 nm. Alcoholic extracts at concentrations of 10, 15, 20, 25, and 30 ug/ml were mixed with 1 ml of sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The solution was kept at a temperature of 50 degrees Celsius for 20 minutes. Subsequently, 1ml of a 10% trichloroacetic acid solution by weight/volume was added, and the mixture was centrifuged at 3000 revolutions per minute for 10 minutes. The uppermost layer (1.5 ml) was mixed with 1.5 ml of deionized water and 0.1 ml of 0.1% ferric chloride. The absorbance at 700 nm was measured using a UV-VIS spectrophotometer 119 after 10 minutes.[7]

Percentage increase in reduction power = (Abs test - Abs blank) / Abs blank × 100

Abs test refers to the absorbance of the test solution, while Abs blank is the absorbance of the blank. Reducing Fe[(CN)] to Fe[(CN)6]2 results in the formation of the brilliant Perl's Prussian blue complex, which exhibits notable absorbance at 700 nm as a result of enhanced reducing ability.

RESULTS AND DISCUSSION:

Initial Phytochemical Examination

These days, the study of phytochemicals in plants is becoming more and more important because of the need to understand their bioactivities, particularly their antioxidant qualities, which are utilised to treat a variety of infectious and chronic illnesses. A. Scholaris's leaves contain a variety of phytochemicals, including flavonoids, phenolics, saponins, triterpenoids, steroids, and alkaloids. The findings of a preliminary qualitative screening of these phytochemicals are displayed in Table 1. Steroids, triterpenoids, alkaloids, flavonoids, phenolics, and saponins have been identified in the A. Scholaris plant. This plant can function as an antioxidant due to the presence of flavonoids, phenolics, and alkaloids with antioxidant properties.

Extraction:

Table No: 1. Preliminary Qualitative Screening Results Of Several Phytochemicals From The Leaf And Stem Bark Of A. Scholaris R. Br.

Sr. No	Phytochemical	Leaf
1	Flavonoids	+
2	Phenolics	+
3	Saponins	+
4	Alkaloids	+
5	phenols	+
6	tannins	+

(The symbol + signifies the existence of the compound, whereas the symbol - indicates the lack of the compound.)

Percentage yield

Table no 2 displays the extraction results of A. Scholaris leaves using the maceration method using methanol.

Components of leaves' solubility in certain solvents are arranged as follows: Ethyl acetate > hexane > methanol. Solubility of leaves sample in methanol solvents, it indicates that the sample has more constituents with greater polar characteristics. This aligns with a previous analysis indicating that this plant harbours alkaloids, flavonoids, and phenolics, all of which fall under the category of polar compounds. Therefore, methanol is used as a solvent to extract these components.[1]

Table No 2. Results Of Macerating A. Scholaris Leaves Using Methanol Solvents.

Sr. No	Sample	Solvent	Percentage %
1	Leaves	Methanol	15

Initial Phytochemical Examination

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ANTIMICROBIAL ACTIVITY

Table 4 displays the results of the antimicrobial test conducted on the methanolic extract of ALSOTONIA SCHOLARIS utilising agar well diffusion assay. The antibacterial activity was assessed by measuring the diameter of the inhibitory zone in millimetres. Two bacterial strains, one gram-positive (S. aureus) and one gram-negative (E. coli), were utilised in this research.

Table No:4 Antimicrobial Activity Of Alstonia Scalarsis On Different Bacterial Species.

Organism	Zones Diameter in mm for respective organism &sample		
	Lower concentration	Higher concentration	Control (DMSO)
E.coli	1.1	1.5	1.4
S.aureus	1.1	1.2	1.2

Determination of MIC

Table 5 displays the results of the minimal inhibitory concentration. The minimum inhibitory concentration (MIC) of A. Scalarsis varied from 31.25 to 1000 ug/ml against all tested microorganisms.

Table no: 5 Minimum inhibitory concentration (MIC) values of A.Scalarsis against various bacterial strains in micrograms per millilitre (ug/ml).

concentration used for both samples was 1000 ug/ml

Concentration of sample	E. coli		S. aureus	
	Lower dose	Higher dose	Lower dose	Higher dose
1000 ug/ml	-	-	-	-
500 ug/ml	-	-	-	-
250 ug/ml	+	+	+	-
125 ug/ml	++	++	++	+
62.5 ug/ml	+++	+++	+++	++
31.25 ug/ml	+++	+++	+++	+++

Note: The minimum inhibitory concentration (MIC) for a lower dose utilising E. coli is 500 ug/ml, and for S. aureus it is also 500 ug/ml.The minimum inhibitory concentration (MIC) of E. coli on AS 400 is 500 ug/ml, and for S. aureus, it is 250 ug/ml.

Ferric reducing antioxidant power (FRAP) assay

TABLE NO:3 A. SCHOLARIS ABSORBANCE

concentration	Absorbance
10	0.077
15	0.488
20	1.147
25	1.202
30	1.305
STD (ascorbic acid)	0.059

The test solution's green hue changes to various shades of green and blue depending on the compound's reducing ability during the assay. Radicals function as antioxidants, transforming the Fe3+/ferricyanide compound in the process into a ferrous form, resulting in the creation of a blue hue (Prussian blue) at a wavelength of 700 nm.

The ferric reducing power assay was conducted on the methanol extract of Alstonia scholaris and compared with ascorbic acid at 700 nm.[4]

Overall antioxidant capacity assessed using the FRAP technique. The Alstonia scholaris species had the highest antioxidant capabilities as determined by the FRAP experiment. Plants from these families showed a greater ability to convert ferric ion (Fe3+) to ferrous ion (Fe2+) than to eliminate free radicals. The maximum antioxidant activity, as determined by the FRAP technique, was displayed by Alstonia scholaris. The plants in the study decreased ferric ion (Fe3+).

The FRAP Assay result showed an increase absorbance.

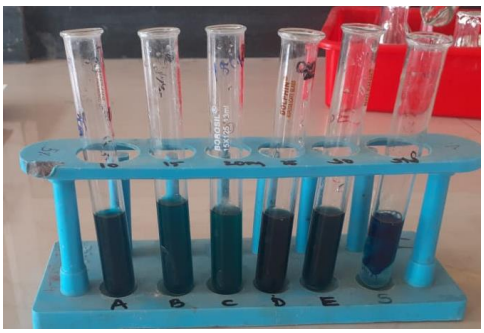

Fig no:4 Antioxidant assay

CONCLUSION:

The results of this study revealed the antimicrobial and antioxidant potential of the studied medicinal plants against drug-resistant pathogens. Additionally, these medicinal plants could be used as a natural source of antioxidants. Further purification and isolation of the bioactive com pounds in these plant extracts would provide possible identification of the mechanism of action and possible lead compounds for the development of new drugs.

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