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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *ALSTONIA SCHOLARIS* AS POTENT ANTIOXIDANT COMPOUND

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ASBTRACT

Alstonia scholaris is a well-known medicinal plant used traditionally for treating various ailments, including infections, respiratory issues, and inflammatory disorders. This study investigates the phytochemical profile and pharmacological activities of plant. Key bioactive compounds such as alkaloids, flavonoids, saponins, and phenolics were identified. Methods included thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and *in vitro* antioxidant assays. The findings highlight the potential of *Alstonia scholaris* as a source of therapeutic agents with significant antioxidant properties.

KEYWORDS: *Medicinal Plant, Alstonia scholaris, Saptaparni, Antioxidant, DPPH.*

INTRODUCTION

In India, the Ayurvedic system of medicine has been practiced for over three millennia. Ancient scholars demonstrated profound knowledge of the medicinal properties of Indian plants. Herbal drugs have gained remarkable prominence in the global healthcare system. Medicinal plants have been used as remedies for human diseases due to their rich therapeutic potential. This efficacy is attributed to bioactive compounds primarily secondary metabolites that exert specific physiological effects on the human body. Among these, alkaloids, tannins, and phenolics are particularly noteworthy for their medicinal properties. Biologically active components derived from plants play a critical role in the treatments of various diseases. Many drugs have been sourced directly or indirectly from plants traditionally used in healthcare.[1, 2]

Alstonia scholaris is an evergreen tropical tree native to Indian sub-continent and South East Asia. This plant is a native of India, Sri Lanka, Pakistan, Nepal, Thailand, Burma, Malaysia, South East Asia, Africa, Northern Australia, Solomon Islands and Southern China. The plant is a large evergreen tree, growing up to 17-20 m in height, with a straight often fluted and buttressed bole, about 110 cm in diameter. Bark is grayish brown, rough, lenticellate abounding, bitter in taste secreting white milky latex. Leaves are 4-7 in a whorl, coriaceous, elliptic-oblong. Flowers are small, greenish white, many in umbellate panicles; corolla tube is short, very strongly scented. Fruits have follicles, 30-60 cm long. Seeds are papillose with brownish hair at each end. It is a beautiful foliage tree (**Figure 1**) with a large canopy. *Alstonia scholaris* is traditionally being used in debility, arthritis, impotence, wounds, asthma, fever, cancer, jaundice, malaria and hepatitis, etc.[3-5]

Figure 1: Plant of *Alstonia scholaris.*

AIM AND OBJECTIVE

- To Perfrom Phytochemical Evaluation of *Alstonia scholaris.*
- To Determine Antioxidant Potency of *Alstonia scholaris.*

MATERIAL AND METHODS

Collection, Identification and Authentication of Plant Material

The Indigenous plants was collected from Indore districts and identified by Head department of *Dravyaguna*, Govt. Asthang Ayurveda College, Indore, (M.P.). The voucher specimens are kept in the herbarium of Govt. Asthang Ayurveda College, Indore, M.P. for future reference.

Scientific Classification of *Alstonia scholaris* R. Br.

- ✓ Kingdom : Plantae
- ✓ Order : Gentianales
- ✓ Family : *Apocynaceae*
- ✓ PlumeriaeSubtribe : Alstoniinae
- ✓ Genus : *Alstonia*
- ✓ Species : S*cholaris*

Preparation of sample for extraction

The plant material was washed off to remove all the aldurants. The freshly washed collected plant materila was air dried in a shade for 7 days at room temperature. Dried plant material was coarsely pulverized to powdered form in a sophisticated instrument, grinder. That after dried plant material was subjetecd for extraction using soxhlet apparatus. Dried extract was further subjected to phytochemical analysis using various chemical tests.

Chromatographic purification of crude extract

The methanol extract was concentrated by distilling off the solvent and evaporated to dryness. The residue was suspended in water, extracted successively with ethyl acetate and n- butanol and then resulting solutions were concentrated to provide ethyl acetate, n-butanol crude fractions. TLC was done for crude n-butanol and ethyl acetate fractions to find out the probable number of compounds present in sample. The samples were dissolved in a volatile solvent such as methanol. A glass capillary tube was used to apply a small amount of sample solution onto the plate, keeping the sample in as small an area as possible.[1-3]

Coloumn Chrometography

Glass column was packed by wet filling. The slurry of adsorbent (silica gel; 60-120 mesh) was prepared by mixing the adsorbent in the chloroform and used as stationary phase. It was then poured into glass column (60cm x 4.5cm) and allowed to settle. The air entrapped was removed by stirring with glass rod. A small amount of sand was kept atop the column to provide the latter a flat base. Excess of solvent was run off until the level of mobile phase fell to one cm just above the top of the sand layer. Methanol fraction was dissolved in a minimum volume of methanol, adsorbed on silica gel (60-120 mesh), dried and applied on the column to separate possible phytoconstituents.

Selection of mobile phase and separation of the constituents

The combinations of solvent systems developed for TLC were used as mobile phase for column chromatography. Alteration in the composition of the eluting solvent was achieved by adding the second solvent of more polarity gradually to a reservoir of the first. Column was first eluted with methanol to give a crude saponin that showed many spots on silica gel TLC plate with chloroform: methanol (09:01). This mixture was again subjected to column chromatography eluting with chloroform, chloroform-methanol mixtures 95:5, 90:10, 80:20, 50:50 and methanol. Mobile phase was passed with constant flow rate (5ml/min). At uniform interval, the elute were collected and the progress of separation was monitored by thin layer chromatography using solvent system chloroform: methanol (90:10) and iodine vapor as detecting agent.

High-performance thin layer chromatograph (HPTLC)

HPTLC is an analytical technique based on TLC, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds. Some of the enhancements such as the use of higher quality TLC plates with finer particle sizes in the stationary phase which allow better resolution.[1, 2]

Infrared spectroscopy

The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample. Analysis of absorption characteristics reveals details about the molecular structure of the sample.

In-Vitro Anti-Oxidant Activity (DPPH free radical scavenging activity)

Required quantity of ascorbic acid was dissolved in methanol to give the concentrations of 50, 100, 250, 500, 750.µg/ml. Stock solutions of samples were prepared by dissolving 10mg of dried methanolic extract in 10ml of methanol to give concentration of 1mg/ml. 4.3mg of DPPH was dissolved in 3.3ml methanol: it was protected from light by covering the test tubes with aluminum foil.

Protocol for estimation of DPPH scavenging activity

50µl DPPH Stock solution was added to 3ml methanol and absorbance was taken immediately at 516 nm for control reading. Different volume levels of test sample (50, 100, 250, 500, 750 µg/ml) were screened and made 200 µl of each dose level by dilution with methanol. Diluted with methanol with up to 3 ml. 50 µl DPPH solution was added to each test tube. Absorbance was taken at 516 nm in UV- Visible spectrophotometer after 20 minutes of incubation period using methanol as blank. The percentage reduction was calculated using following equation.[6-8]

$$Percentage Inhibition = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

RESULT AND DISCUSSION

The present study was to demonstrate the phytochemistry and pharmacology activity of selected medicinal plant. The powder was extracted consecutively with solvents of increasing polarity namely chloroform, petroleum ether and methanol in Soxhlet apparatus.

Phytochemical Analysis

Alkaloids, flavonoids, carbohydrates, saponins, tannins, steroids were present in methanol extract of plant as depicted in **Table 1**.

Table 1: Phytochemical investigation of methanolic extract of *Alstonia schalaris.*

S. No.	Phytochemical Constituent	Tests	Pesent or Absent in Methanol Extract
01.	Alkaloids	Mayer's reagent Test	+
		Wagner's reagent Test	+
02.	Flavonoids	Alkaline reagent Test	+
		Zinc HCl Test	+
		Shinod's Test	+
03.	Saponins	Frothformation Test	+
		Heamolytic Test	+
04.	Tannins	$FeCl_3$ Test	+
05.	Fats	Saponification Test	_
06.	Steroids	Liebermann Burchard test	+

TLC

The Rf value of methanol extract of *Alstonia scholaris* with solvent chloroform: methanol (90:10) was found to be 0.44 as depicted in **Figure 2**.

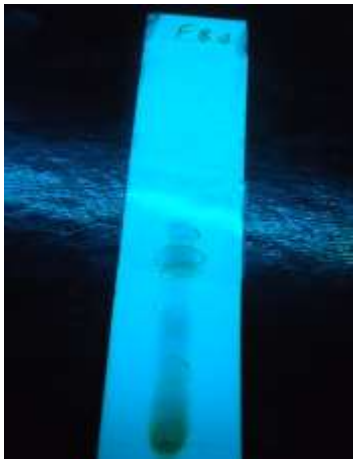

Figure 2: TLC plate of methanol fraction of *Alstonia scholaris.*

High-performance thin layer chromatograph (HPTLC)

The retention time of various constituents of *Alstonia scholaris* were found to be 17.463 (Alkaloids), 39.731 (Flavanoids), 25.054 (Phenol), 28.865 (Terpenoids), 31.538 (Steroids) and 35.314 (Saponins), as depicted in **Figure 2.**

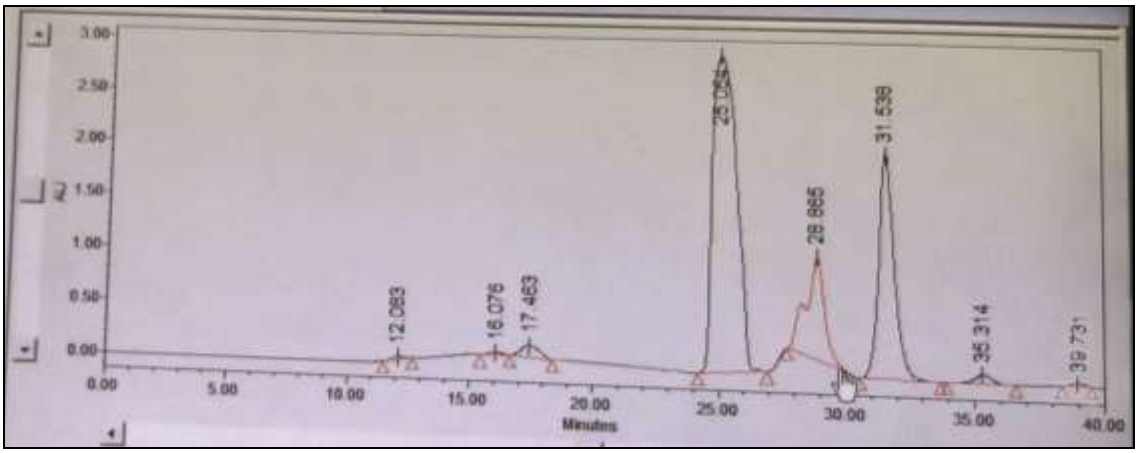

Figure 2: HPTLC of *Alstonia scholaris.*

Infra-red Spectroscopy

Infra-red spectroscopy revealed various peaks of functional groups present in isolated compound of *Alstonia scholaris* as mentioned in **Table 2** and **Figure 3**.

Table 2: IR Spectroscopy of *Alstonia scholaris.*

Wave Length cm^{-1}	Observation/Functional group
3474.5	- O-H stretch
2926	– C-H stretch of aromatic ring
1622	– C=C stretch
1478	– O-H deformation vibration
1274	– C-O carbonyl stretch

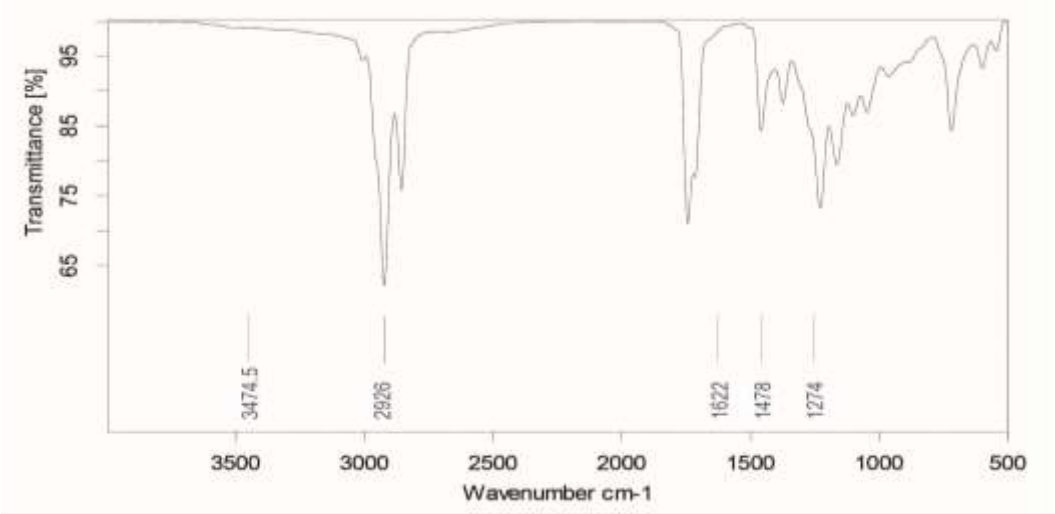

Figure 3: IR spectrum of isolated compound from *Alstonia scholaris.*

***In-Vitro* Anti-Oxidant Activity (DPPH free radical scavenging activity)**

The DPPH (2, 2-diphenyl-1-picryl hydrazyl radical) assay was performed to estimate *In-vitro* anti oxidant activity of sample using ascorbic acid as a standard compound. **Figure 4** represents regression lines of standard ascorbic acid and extracts from which IC_{50} values were calculated. **Table 3** represents the percentage inhibition of standard ascorbic acid and extracts at different concentrations. The methanolic extract of *Alstonia schloraris* showed IC_{50} value 485.63µg/ml.

Table 3: % Inhibition of DPPH free radical by methanolic extract of *Alstonia schlolaris.*

S. No	Conc. (µg/ml)	% Inhibition
01.	50	43.55556±0.67334
02.	100	45.3333±0.68722
03.	250	47.11111±0.71010
04.	500	49.18519±0.77421
05.	750	53.77778±0.82131
IC_{50} Value		485.63 µg/ml

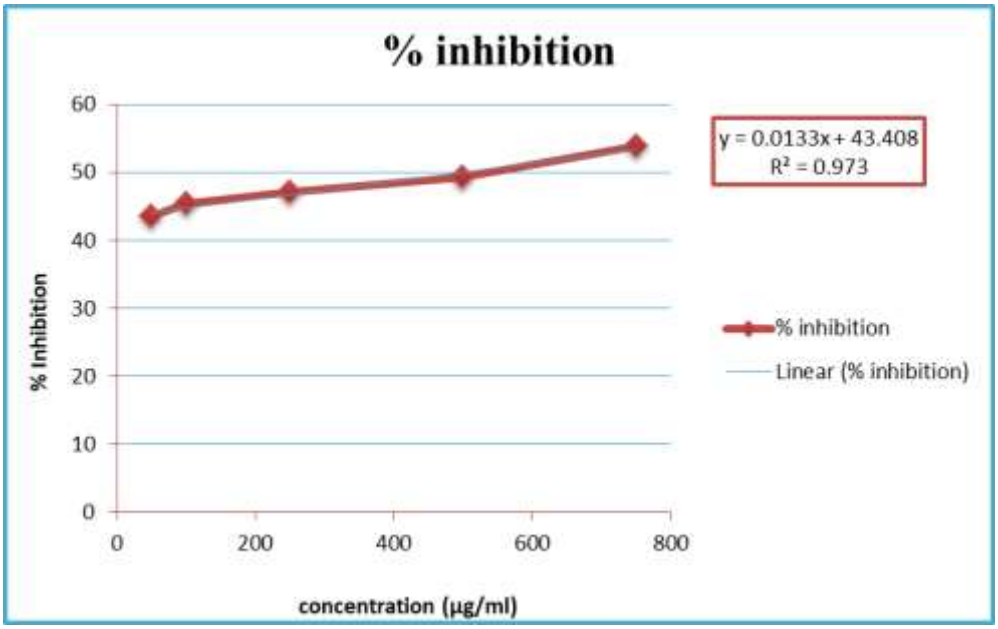

Figure 4: % DPPH inhibition curve of methanolic extract of *Alstonia schlolaris*.

The presence of alkaloids, phenolic compounds, tannins, flavonoids have been associated with various degrees of antioxidant activities. Therefore, antioxidant activity was observed in this study may be due to the presence of phenolic compounds and flavonoids in selected plant extract.[7, 8]

CONCLUSION

The results of phytochemical analysis showed presence of flavonoids, terpenoids, alkaloids, tannins and phenolic compounds in methanolic extract of plant material. *Alstonia scholaris* extract was rich in flavonoids and alkaloids. These contituents can be considered responsible for antioxidant property of plant extract.

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