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ORIGINAL ARTICLE

Phytochemical Screening and in Vitro Anti-Plasmodium Falciparum Activity of Alstonia Scholaris

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Abstract:
Malaria is a major global threat that results in more than 2 million deaths each year. In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. The plant *Alstonia scholaris* has been used in different systems of traditional medicine for the treatment of diseases. The extract of the plant showed pharmacological activities ranging from anti-malarial to anticancer properties. The extract was subjected for pharmacological screening and tested against *P. falciparum,* which exhibited 14 mm zone of inhibition of 200mg/mL. It can be concluded from the present study that the bark and leaf extracts of *A. scholaris* potentiate the antiplasmodial activity against *P. falciparum.*

Keywords: - *Alstonia scholaris, Plasmodium falciparum,* Antimalarial

Introduction

The term Malaria originates from medieval Italian: “Malaria” meaning “Bad air”, the disease was formerly called Auge or Marsh fever due to its association with swamp and marshland [1]. Malaria has infected humans for over fifty thousand years and Plasmodium may have been a human pathogen, close relatives of human malaria parasites remain common in Chimpanzees. Some new evidence suggest that the most virulent strain of human malaria may have originated in gorillas[2].

Malaria is a mosquito-borne infectious disease of humans and other animals caused by Eukaryotic Protists of the genus Plasmodium. This severe disease is largely caused by several species of Plasmodium: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and the simian *Plasmodium knowlesi* transmitted by female Anopheles mosquitoes. The most lethal species is *P. falciparum*, a single-celled parasite found predominantly in Africa[3]. If left untreated, *P. falciparum* causes organ failures (severe malaria) and accumulates in the brain capillaries (cerebral malaria), leading to coma and eventually death.

Alstonia scholaris Linn.,(Family, Apocynaceae) which is popularly known as the "*Saptaparni*" or "*Devil's tree*".It is widely distributed in dried forests of India,

MATERIAL AND METHOD

The current work includes Insilico docking investigations and the in-vitro antimalarial activity of active constituents of *Alstonia scholaris.*

Collection and Identification of Plant: In August 2022, a plant was removed from Oriental University’s Garden in Indore. Plant was identified by Dr. Bhawna Tomar of the Faculty of Agriculture at Oriental University in Indore (M. P.).

Drying of the leaves: The leaves were scrubbed and washed under running water to get rid of any dirt, and then they were dried first in the air and then in a hot air oven at 55 °C until they reached their desired weight.[4]

Extraction methodology: After being ground at a low speed and sieved through a 60-mesh sieve,

the dried plant material was ground into a powder. After being sieved, the material was weighed and hydroalcoholic solvent (absolute ethanol and water in a 3:1 ratio) was applied until the supernatant solvent turned translucent.[5]

Drying of extract: The extract was then filtered through muslin fabric, and dried at 30°C until the weight remained constant, and the result was a reddish-brown hygroscopic powder. and 11.6 % yield was calculated.

Macroscopic examination

Using a basic microscope, morphological examinations were conducted. The shape, apex, base, edge, tastes, and fragrance of the leaves were identified. The flowers were large, bisexual, sub-sessile, fragrant, cream in colour, ebracteate, pedicellate, short pedicels, axillary or terminal, solitary or in pairs (cluster), and 2.5-7.5 cm in diameter. There were no bracts on the blooms. The four sepals that were observed were orbicular, cubbed, puberulous, and flattened at the base. The two outer sepals were slightly shorter than the inner ones. There were four pure white, aromatic petals.[7]

Figure 1. Leaves of A. Scholaris

Phytochemical Screening [8-10] - The extract was screened for the presence of various phytochemical constituents: -

1. Test for alkaloids. A little amount of solvent-free extract was filtered after being agitated with a few drops of diluted hydrochloric acid. The filtrate was examined using the subsequent chemicals.

a. Dragendorff reagent (potassium bismuth iodide) –Orange-brown precipitate

b. Mayer's reagent (mercury potassium iodide) – Cream precipitate

c. Hager's reagent (saturated picric acid) – Yellow precipitate

d. Wagner's reagent (iodine reagent) – Reddish brown precipitate

2. Test for carbohydrates: 5 mL of distilled water was used to dissolve a small amount of the extract, which was then filtered.

a. *Molish test*-Alpha-naphthol solution in alcohol, as indicated by the violet ring at the junction.

b. *Fehling's test:* Sugars are present when a brick-red precipitate forms.

c. *The Barfoed's test*: The presence of monosaccharides is indicated by the formation of a red colour within 2 minutes after the administration of the reagent.

d. *Benedict's test*: The filtrate was heated for two minutes with this reagent. The appearance of decreasing sugars is indicated by the precipitate turning red.

3. Test for glycosides - The following tests were performed on the hydrolysate, which was produced by hydrolyzing a tiny quantity of the extract by heating it in diluted hydrochloric acid for a short period of time.

a. *Liebermann-Burchard test* - A chloroform solution of the hydrolysate was treated with acetic anhydride and sulphuric acid for the Liebermann-Burchard test. A blue or blue-green hue forms when steroidal saponins are present, while a red, pink, or violet colour forms when triterpenoid saponins are present.

b. *Legal's test* - The hydrolysate was dissolved in pyridine before being mixed with a sodium nitroprusside solution to make it alkaline. Heart glycosides are present when pink or red coloration forms.

c. *Borntrager's test* - The hydrolysate was mixed with an organic solvent, such as ether or chloroform, and the mixture was shaken. Shaking was done before applying an ammonia solution to the organic layer. Anthraquinone glycosides are indicated by the appearance of a pink tint.

4. Test for saponin - In a graduated cylinder, 1 mL of the extract was diluted with 20 mL of water and agitated for 15 minutes. The presence of saponins is indicated by a 1 cm layer of foam.

5. Test for phenolic compounds and tannin - The following reagents were used to examine a small sample of the extract after it had been diluted with water.

a. Diluted $FeCl_3$ solution (5%)- Phenolic compounds are present when the solution is intensely blue, green, red, or purple in colour. The presence of tannins is indicated by the emergence of a violet tint.

b. Precipitate shows a positive tannin test result in the case of a 1% solution of gelatin containing 10% NaCl.

c. Lead acetate solution (10%) - Produces a phenolic compound precipitate that is buff in color.

6. Test for proteins and amino acid - The extract was examined using the following chemicals.

a. *Millon's reagen*t - A red tint appearing in Millon's reagent denotes a positive test result.

b. *Biuret's test* – Formation of a violet color indicatesthe test is positive.

7. Test for gum and mucilage – 1 ml of extract was gently added to around 25 ml of alcohol while being constantly stirred as part of a test for gums and mucin. Precipitate formation suggests the presence of mucilage and gums.

8. Test for flavonoids - A small amount of magnesium ribbon was added to the extract's alcohol solution, and then powerful hydrochloric acid was added drop by drop. Red to crimson colour changes suggest flavonols, crimson to magenta colour changes indicate flavanones, and green to blue colour changes indicate the test is positive.

All Phytochemical test observations and results were observed in **Table 1.**

Ash Value

To evaluate the quality and purity of the crude pharmaceuticals, ash values are utilized. For the entire plant, the amounts of total ash, water-soluble ash, acid-insoluble ash, and sulfated ash were measured. The amount of alcohol and water-soluble components was calculated using extractive values for alcohol and water. [10]

Evaluation of Anti-Plasmodial Activity

The plant extract was subjected to anti-*Plasmodium falciparum* activity.

Method: Well-plate method

Strain used: *Plasmodium falciparum strain*(collected from local pathology lab)

Preparation of medium: Five nutrient agar plates were prepared using nutrient broth powder (3.25 gm.) and agar powder (3.75 gm.) and then sterilized by autoclaving under standard conditions of 121^{o}C temperature & 15 p.s.i for 30 minutes. After cooling and solidification, these plates were then supplemented with fresh uninfected human blood in the form of a layer covering the whole plate.

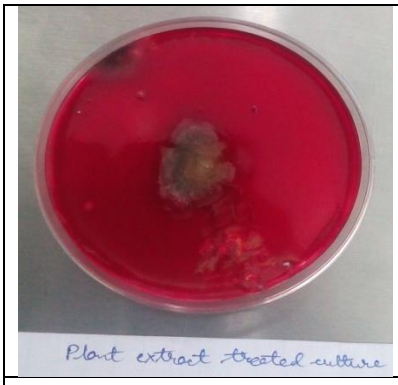
Test solutions: The dried plant extract was used to make test solutions with concentrations of 50 mg/ml,100 mg/ml & 200 mg/ml in dimethyl sulphoxide (DMSO).

Standard solution: Standard solution of reference drug chloroquine was made at 5mg/ml concentration using a marketed formulation (Lariago®500 mg chloroquine tab. by IPCA

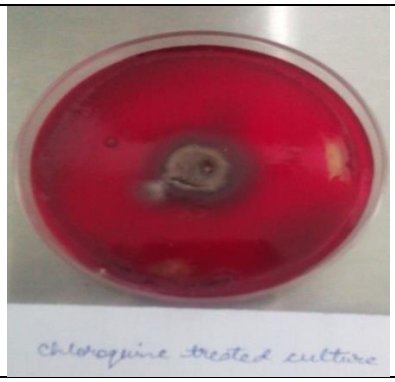
Control: Pure dimethyl sulphoxide (DMSO)

Inoculation of parasite: All the plates were seeded with a *Plasmodium falciparum* (+ive) blood sample (obtained from pathology, Indore) at the periphery using an inoculation loop.

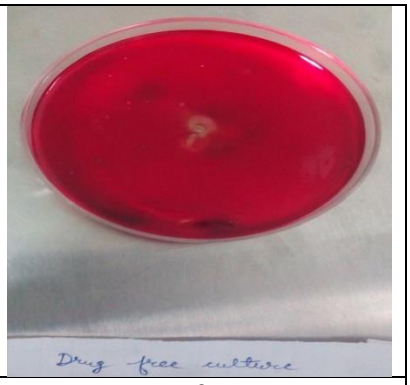
Method: A well of about 3 mm. diameter was bored at the Centre. Three plates contained test solutions at different concentrations, One other plate contained standard drug solution and the last one contained pure DMSO (control). The plates were then incubated at 37^{o}C for 48 hrs and then observed through a microscope for the presence of parasites in different regions of the plates. Using this information, the zone of inhibition was calculated for all the plates.



Extract treated culture (200 mg/ml)



Chloroquine-treated culture (5 mg/ml)



Control (drug-free)

Figure:2. Zone of inhibition against plasmodium strain

Observations

The diameter of the circular zone of inhibition was measured using a zone measurement scale following the incubation period, and the zone of inhibition was compared to the standard chloroquine (5mg/ml). Each reading was recorded in triplicate, and one-way ANOVA was used; observations were expressed as the mean standard error. In Table 3, all observations were compiled.

Result & Discussion

Phytochemical Test

Preliminary phytochemical analysis showed the presence of flavonoids, proteins, sugars, steroids, alkaloids, glycosides, and tannins like phytoconstituents in extracts of *Mesua Ferrea* and result are shown in **Table 1**.

Table 1 **Qualitative chemical test of A. scholaris**

S. No.	Phytochemical test for	Result
1.	Carbohydrates	(+)
2.	Starch	(-)
3.	Reducing sugars	(-)
4.	Alkaloids	(+)
5.	Flavonoids	(+)
6.	Saponins	(+)
7.	Steroids	(+)
8.	Proteins	(-)

(+) = Present; (-) = Absent

ASH Values

Ash values were used to determine the quality and purity of the crude drug. Total ash and acid insoluble ash were determined. Acetone, alcohol, and water-soluble extractive values were determined to find out the amount of acetone, water and alcohol-soluble components. Results are shown in **Table 2**

Table 2 :- Determination of ash values (% w/w) of *A. scholaris*

S. No.	Ash Value	Percentage
1	Total ash	6.30 w/w %
2	Water soluble ash	2.31 w/w %
3	Acid insoluble ash	2.30 w/w %

Antiplasmodial Activity

In order to develop the antimalarial activity of A. scholaris, a study was undertaken to evaluate the in vitro antimalarial activity of A. scholaris with

the *P. falciparum* strain as the drug target. All data was revealed in the table and figure. It was also observed that Activity is directly proportional to concentration. As the concentration of the extract was increased, it showed greater potency for P. falciparum as compared to the standard. The zone of inhibition was expressed as the distance from the bore. (in mm). It was revealed from Table 3 and Figure 3 that activity is diretly proportional to concentration at 200mg/ml 14.00 mm was observed.

Table – 3. Showing Zone of Inhibit at different concentration again p. falciparum

S. No	Concentartion	Zone of Inhibition (mm)
1	50 mg/ml	3.00±0.94
2	100 mg/ml	8.33±0.88
3	200 mg/ml	14.00±0.57
4	Standard (5mg/ml)	26.19±0.58
5	Control	0.00±0.00

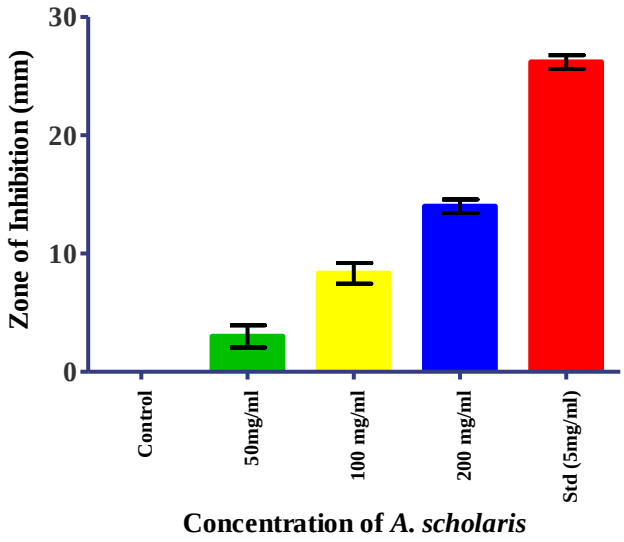


Figure 3 – Graph showing Zone of inhibition against P. Falciparum

Conclusion

The results revealed that the hydroalcoholic leaf extract of the plant Alstonia scholaris has inhibitory activity against Plasmodium falciparum which is evident from the dose-dependent zone of inhibition data discussed above. Therefore, the plant has scope to be used in the future as a source of potent antimalarial. In conclusion, more research is required for developing a potential natural plant product used as an anti-plasmodium effect.

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