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"Evaluation of the Analgesic Potential of Alstonia Scholaris Extracts Using Eddy's Hot Plate Method"

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ABSTRACT
The present study evaluates the analgesic potential of Alstoniascholaris extracts using Eddy's hot plate method, along with a comprehensive phytochemical and antioxidant profiling. Crude extracts were obtained using petroleum ether and methanol, with methanol yielding a higher percentage (2.46%) than petroleum ether (0.53%), indicating its superior efficiency in extracting polar phytochemicals. Phytochemical screening revealed that the methanolic extract was rich in alkaloids, glycosides, carbohydrates, flavonoids, tannins, and phenolic compounds, whereas the petroleum ether extracts only showed the presence of saponins. Quantitative analysis of the methanolic extract showed significant total phenolic content (up to 70.4 mg/g GAE) and total flavonoid content (up to 51.55 mg/g RE), suggesting strong antioxidant potential. The DPPH radical scavenging assay further confirmed this, with the methanolic extract exhibiting moderate antioxidant activity (IC_{50} = 48.73 µg/ml), though less potent than ascorbic acid (IC_{50} = 21.92 µg/ml). The analgesic activity assessed via the hot plate model demonstrated that Alstoniascholaris extract significantly increased reaction times in a dose-dependent manner. At 400 mg/kg, the extract showed a peak response of 31.92±0.094 seconds at 30 minutes, comparable to the standard analgesic drug pentazocine (10 mg/kg). This suggests that the methanolic extract of Alstoniascholaris possesses notable central analgesic properties, likely due to its phytochemical constituents, supporting its traditional use in pain management and highlighting its potential as a natural analgesic agent.

Keywords: Alstoniascholaris, Analgesic activity, Eddy's hot plate, Methanolic extract, phytochemical screening, DPPH assay, Antioxidant

I. INTRODUCTION

Pain is an unpleasant sensation and emotional experience associated with real or potential tissue damage or described in terms of such damage. The pathophysiology of pain involves two components, peripheral nociception, and central mechanism**(Fong and Schug 2014).**Analgesics are the drugs that selectively relieve pain by acting in the central nervous system (CNS) or on the peripheral pain mechanisms, without appreciably altering consciousness. With the easy availability of analgesics (opioids) and anti-inflammatory drugs (nonsteroidal anti-inflammatory drugs [NSAIDs]) **(Marchand, 2024).**

The medicinal plants have been used in traditional medicine for hundreds of years with a reputation as efficacious remedies although there may not sufficient scientific data to substantiate their efficacy. They serve as therapeutic agents and raw materials for the manufacturer of traditional and modern medicines. These plants are rich sources of bioactive compounds and thus serve as important raw materials for drug production. It has now been established that the plants synthesize and accumulate some secondary metabolites such as alkaloids, glycosides, tannins, and volatile oils that may possess a great potential for biological activity and can be a curative agent in therapeutic purposes **(Djordjevic, 2017).**

Alstoniascholaris Linn. R.Br. belongs to family Apocynaceae, grows throughout India, in deciduous and evergreen forests, also in plains. A. scholaris has a promising place in various ayurvedic preparations to treat whooping cough, malaria, jaundice, gastric complaint, headache, asthma, stomachache, wound, fever **(Khyadeet al., 2014).** The bark is bitter, astringent, acrid, thermogenic, digestive, laxative, anthelmintic, febrifuge, antipyretic, depurative, galactogogue, stomachic, cardiotonic and tonic. It is useful in fever, malarial fever, abdominal disorders, diarrhoea, dysentery, dyspepsia, leprosy, skin diseases, pruritus, tumours, chronic and foul ulcers, asthma, bronchitis, cardiopathy, helminthiasis, agalactia and debility. The plant is a rich source of

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alkaloids, flavonoids, saponins, steroids, reducing sugar and phenolic compounds which witness the ample of medicinal potential of the herb [4]. The evaluation of the anti-inflammatory and analgesic activities in different animal models of extracts, fractions and main alkaloids from the leaf of Alstoniascholaris. Moreover, the in vitro anti-inflammatory evaluation of alkaloids from Alstoniascholaris against COX-1, -2 and 5-LOX was also reported**(Zhao et al., 2016).**

As such, research to discover other alternatives to treat pain is crucial. Many of these herbs with analgesic activity had been used without any adverse effects. The present study aimed to evaluate the analgesic activity of ethanol and aqueous flower extracts of P. alba in animal models.

II. MATERIAL AND METHODS

2.1 Chemical

Glacial acetic acid, sodium nitroprusside, sodium hydroxide, and ammonia were procured from Merck, a well-known provider of high-quality laboratory chemicals. Petroleum ether, utilized during the extraction process, was obtained from Research Lab, while concentrated sulfuric acid (H_2SO_4) was supplied by Fizmerck. Ethanol, crucial for extraction and analytical testing, was purchased from Molychem. Additionally, reagents including 95% alcohol, concentrated hydrochloric acid (HCl), and chloroform, used in various phytochemical procedures, were sourced from Clorofiltind. Magnesium, essential for confirmatory assays such as flavonoid detection, was acquired from Himedia.

2.2 Plant collection

A total of 300 grams of the medicinal plant Alstoniascholaris was collected for the study. After thorough washing, the material was air-dried in the shade at room temperature for 3 days, followed by oven drying at 45°C to ensure complete removal of moisture. To preserve its quality and prevent any contamination or deterioration, the dried sample was stored in airtight glass containers under cool, dry conditions.

Plant Authentication: The botanical identity and authenticity of Alstoniascholaris were verified by a qualified plant taxonomist, ensuring accurate selection and use of the traditional medicinal plant in the study.

2.3 Extraction

In the present investigation, the extraction of plant material was performed using a Soxhlet apparatus following the continuous hot percolation method. Finely powdered Alstoniascholariswas loaded into the thimble of the Soxhlet extractor. The initial extraction was carried out using petroleum ether, a non-polar solvent, at a controlled temperature of 60°C to remove lipophilic constituents. After the petroleum ether extraction, the plant residue (marc) was air-dried and subjected to a second extraction using methanol to isolate polar components**(Goel and BR 2021).**

The dried extracts were then weighed, and the percentage yield of each extract was calculated using the standard formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

The prepared extracts were labelled for future use and stored in an airtight container after being examined for organoleptic characteristics (percentage yield, color, and odor**)**

2.4 Quantitative Phytochemical Estimation

Quantitative analysis was performed to estimate the levels of major phytoconstituents in the extracts, including phenolics, flavonoids, alkaloids, tannins, and saponins.

2.4.1 TPC (Total Phenolic Content)

The total phenolic content of Alstoniascholar is extract was determined using the Folin–Ciocalteu assay. In this method, 0.2 mL of the extract was mixed with 2.5 mL of Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution. The volume was adjusted to 7 mL with distilled water. The mixture was allowed to stand at room temperature for 2 hours. Absorbance was then measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid was used as the standard, with calibration curves prepared at concentrations of 20, 40, 60, 80, and 100 µg/mL. The results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of extract. The Folin–Ciocalteu reagent reacts with polyphenols and other reducing agents to produce a blue-coloured complex, the intensity of which correlates with the phenolic content (**Azmiret al., 2013).**

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2.4.2 TFC (Total Flavonoid Content)

The total flavonoid content of Alstoniascholaris extract was estimated using the aluminum chloride colorimetric method. Briefly, 0.5 mL of the extract was mixed with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite. After standing for 6 minutes, 0.15 mL of 10% aluminum chloride was added, followed by an additional 6-minute incubation. Then, 2 mL of 4% sodium hydroxide was added, and the solution was mixed thoroughly. The absorbance of the resulting pink solution was measured at 510 nm using a UV-Vis spectrophotometer. A calibration curve was constructed using Rutin as the standard at concentrations of 20, 40, 60, 80, and 100 µg/mL. The flavonoid content was calculated from the standard curve and expressed as milligrams of Rutin equivalent per gram of dry extract (mg RE/g) (**Babbaret al., 2011).**

2.3 In vitro antioxidant activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl)

The antioxidant activity of Alstoniascholaris extract was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A stock solution of the extract (1 mg/mL) was prepared in methanol, and working concentrations ranging from 20 to 100 µg/mL were obtained. To 1 mL of each concentration, 2 mL of 0.1 mM DPPH solution was added. The mixtures were vortexed and incubated in the dark at room temperature for 30 minutes. Absorbance was then measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu 1700). A control solution containing only 3 mL of 0.1 mM DPPH in methanol was treated similarly. Methanol served as the blank. The decrease in absorbance indicated the scavenging potential of the extract, reflecting its antioxidant activity (**Yadav et al., 2011).**

$$\text{Percentage Inhibition } (\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

2.4 Acute Toxicity Study

The acute oral toxicity of the test substance was assessed following OECD Guideline 423 (Acute Toxic Class Method). Groups of three animals of the same sex received a single oral dose at one of the fixed levels (5, 50, 300, or 2000 mg/kg body weight). Animals were observed for signs of toxicity and mortality for 14 days. Based on the observed effects, dosing for subsequent groups was adjusted up or down to determine the substance's toxicity classification. This stepwise approach minimizes animal use while providing reliable toxicity data **(Madhu et al., 2016).**

2.5 Experimental work

Animals Protocol

- **IAEC Approval** All animal experiments were approved by Institutional Animal Ethics Committee (IAEC).
- **Animal used**
- **Weight** 250±60 gm
- **Strain** Wistar rat
- **Sex** Either
- **Housing Condition-** Animals were housed in a group of six inseparate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly.

Procedure

The rats were randomly divided into 4 groups, each consisting of six animals. The first group served as the negative control and received normal saline (10 mL/kg). The second and third groups were treated with Alstoniascholaris extract, while the fourth group received the standard analgesic drug pentazocine at a dose of 10 mg/kg body weight. All treatments were administered via intraperitoneal injection. The analgesic activity of the extract was evaluated using the Hot Plate method **(Chandran et al., 2020).**

2.6 Hot plate method

The analgesic activity of the extract was evaluated using the hot plate method. Rats were placed individually on a hot plate maintained at 55°C inside a restrainer. The reaction time, or latency period, was recorded as the time (in seconds) taken for the rats to respond to the thermal stimulus by either licking their paws or jumping. Measurements were taken prior to treatment (0 min) and at 15-, 30, 45, 60 and 125 minutes after-administration. To prevent tissue damage, a cutoff time of 45 seconds was set; any reaction time exceeding this was considered indicative of maximum analgesia. The maximum possible analgesia (MPA) was then calculated using the formula**: (Patel et al., 2016).**

$$\text{MPA } (\%) = \frac{\text{Reaction time (treatment)} - \text{Reaction time (saline)}}{15\,\text{sec} - \text{Reaction time (saline)}} \times 100$$

Experimental design

Rats (n=6) were randomized into following groups:

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Group 1- Negative control (saline 10mL/kg)
Group 2-Alstoniascholaris extract treated group (200mg/kg)
Group 3-Alstoniascholaris extract treated group (400mg/kg)
Group 4- Treated with standard drug pentazocine (10 mg/kg)

Figure 1: Normal rat dosing

III. RESULT AND DISCUSSION

3.1 Percentage Yield

Table1: Percentage Yield of crude extracts of Alstoniascholaris extract

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Alstoniascholaris	Pet ether	300	1.60	0.53%
	Methanol	250	6.15	2.46%

Methanol gave a higher yield (2.46%) compared to petroleum ether (0.53%), indicating that methanol is more effective in extracting a broader range of polar phytochemicals from the plant.

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.4	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Flavonoids**		
4.1	Shinoda's Test	Absent	Present
5.	**Tannin and Phenolic Compounds**		
5.1	Ferric Chloride test	Absent	Present
5.2	Gelatin Test	Absent	Present
5.3	Lead Acetate Test	Absent	Present
6.	**Saponin**		
6.1	Froth Test	Present	Present

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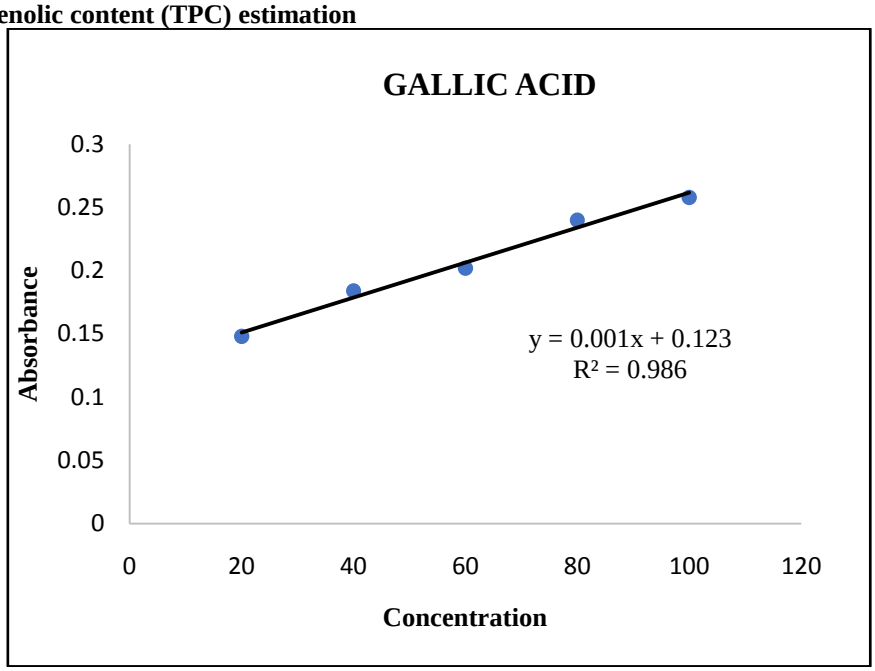
7.	**Test for Triterpenoids and Steroids**		
7.1	Salkowski's test	Absent	Absent
7.2	Libbermann-Burchard's test	Absent	Absent

Phytochemical screening (Table 4) revealed that the methanolic extract of Alstoniascholaris contains a wide range of phytochemicals, including alkaloids, glycosides, carbohydrates, flavonoids, tannins, and phenolic compounds. These results indicate that methanol, due to its polarity, is more effective in extracting diverse bioactive compounds from the plant.

3.3 Quantitative Analysis

3.3.1 Total Phenolic content (TPC) estimation



Graph 1: Represent standard curve of Gallic acid

- **Total Phenolic Content in extract**

Table 3: Total Phenolic Content in extract of Alstoniascholaris

Absorbance	**TPC in mg/gm equivalent of Gallic Acid**
0.142	70.4 mg/gm
0.190	
0.250	

The presence of significant phenolic content suggests antioxidant potential, as phenolic compounds are known for their free radical scavenging activity.

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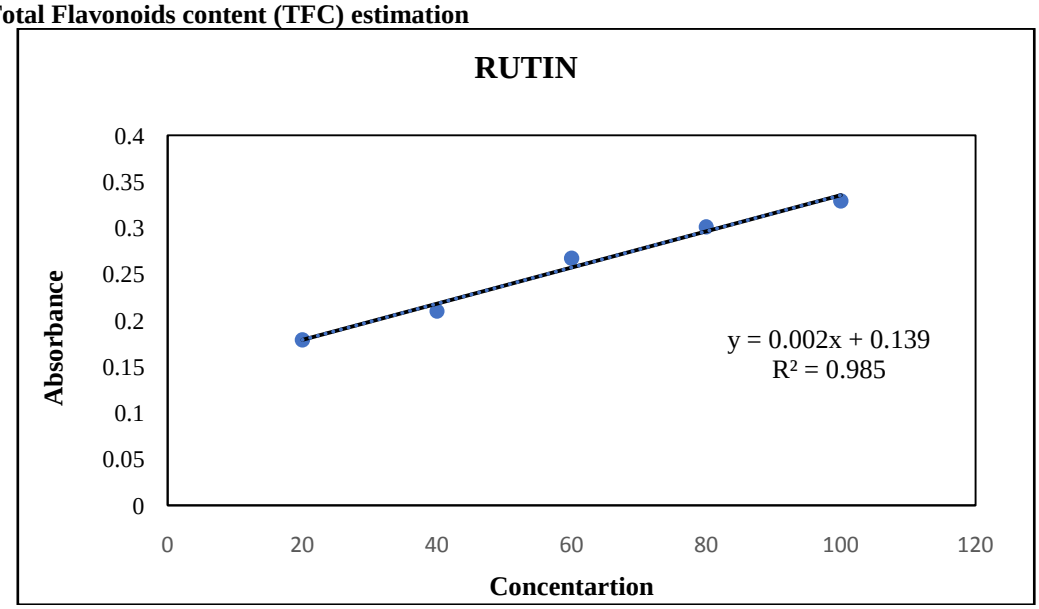
3.3.2 Total Flavonoids content (TFC) estimation



Graph 2: represent standard curve of Rutin

- **Total Flavonoid Content in extract**

Table 4: Total Flavonoid Content in extract of Alstoniascholaris

Absorbance	TFC in mg/gm equivalent of Rutin
0.191	51.55 mg/gm
0.235	
0.304	

The Total Flavonoid Content (TFC) of Alstoniascholaris extract was estimated using a Rutin standard curve. One sample showed a TFC of 51.55 mg/g, while others with higher absorbance values (0.235 and 0.304) indicate even greater flavonoid content.

3.4 In vitro Antioxidant Assays

In the present study, the in vitro antioxidant activity of Alstoniascholaris extracts was assessed using the DPPH radical scavenging assay. The results of the assay, indicating the free radical scavenging potential of the extracts, are summarized in Tables.

3.4.1 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 5: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.482	51.313
40	0.434	56.161
60	0.344	65.252
80	0.283	71.414
100	0.144	85.454
Control	0.990	
IC50	21.92	

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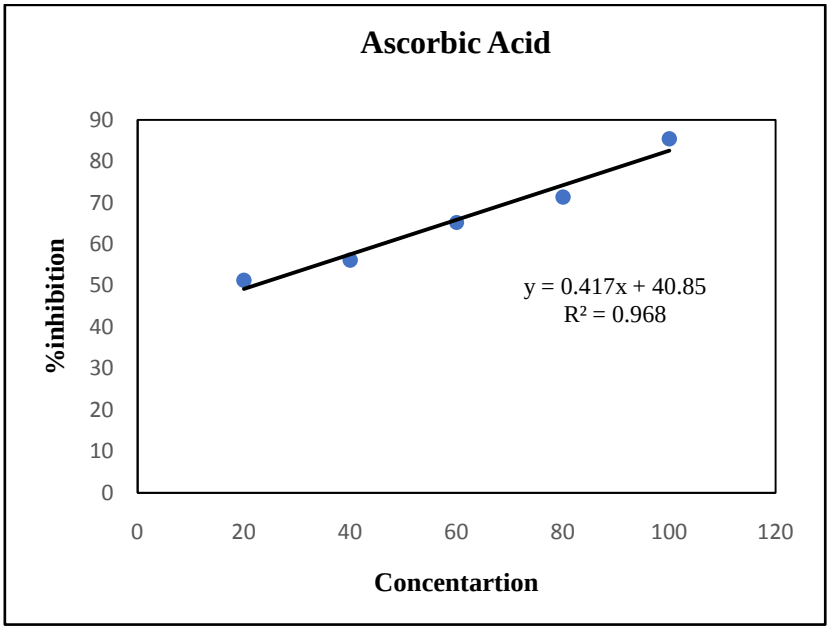
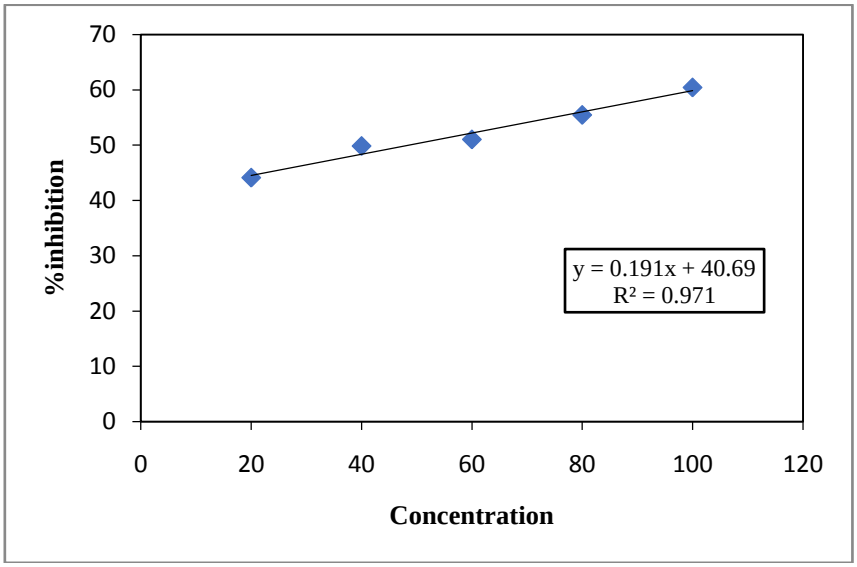


Graph 3: DPPH radical scavenging activity of Std. Ascorbic acid

Table 6: DPPH radical scavenging activity of methanol extract of Alstoniascholaris

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.518	44.108
40	0.464	49.837
60	0.453	51.027
80	0.412	55.459
100	0.366	60.432
Control	0.925	
IC50	48.73	



Graph 4: Represents the Percentage Inhibition Vs Concentration of extract of Alstoniascholaris

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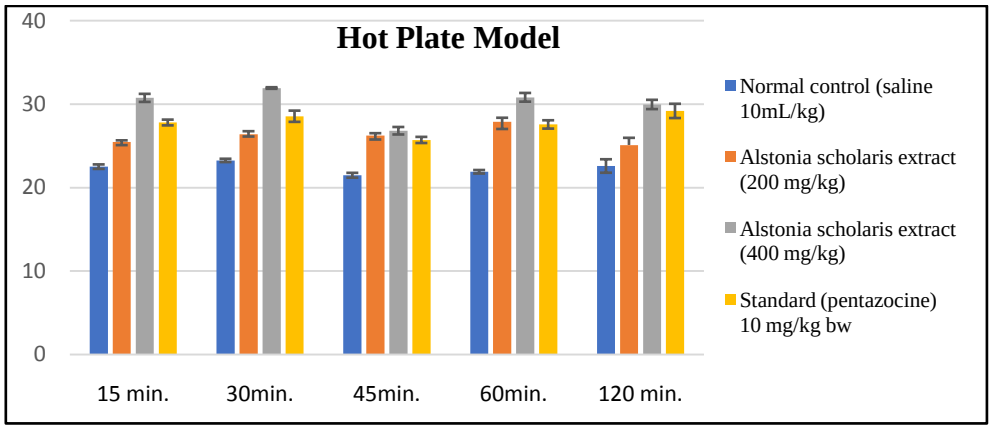
The DPPH assay revealed that the methanolic extract of Alstoniascholaris exhibits moderate antioxidant activity, with a maximum inhibition of 60.43% at 100 µg/ml and an IC_{50} value of 48.73 µg/ml. In comparison, ascorbic acid showed stronger activity with 85.45% inhibition and an IC_{50} of 21.92 µg/ml.

3.5 Hot plate model

Figure 2: Hot plate model

Table 71: Hot plate model

(Min)	15 min.	30min.	45min.	60min.	120 min.
Normal control (saline 10mL/kg)	22.50±0.27	23.25±0.199	21.47±0.29	21.89± 0.209	22.58±0.808
Alstoniascholarisex tract (200 mg/kg)	25.45±0.202	26.38±0.369	26.24±0.269	27.87±0.488	25.10±0.857
Alstoniascholarisex tract (400 mg/kg)	30.74± 0.482	31.92±0.094	26.805±0.449	30.81± 0.523	29.95± 0.556
Standard (pentazocine) 10 mg/kg bw	27.79±0.0345	28.54±0.673	25.70±0.368	27.56±0.500	29.18±0.855



Graph 5: Hot plate model

The hot plate model was employed to assess the central analgesic effect of Alstoniascholaris extract, which measures pain response through increased reaction time to a thermal stimulus. In contrast, both doses of Alstoniascholaris extract (200 mg/kg and 400 mg/kg) significantly prolonged the reaction time at all intervals, suggesting effective analgesic activity.

The 200 mg/kg dose showed moderate activity, with reaction times ranging from 25.45±0.202 to 27.87±0.488 seconds. Notably, the 400 mg/kg dose exhibited a more substantial and sustained effect, with peak latency times of

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31.92±0.094 at 30 minutes and 30.81±0.523 at 60 minutes, which were comparable to the standard drug pentazocine (10 mg/kg). The effectiveness of the extract at a higher dose indicates a dose-dependent response.

IV. CONCLUSION

The findings from this study indicate that the methanolic extract of Alstoniascholaris possesses significant analgesic activity as evidenced by increased latency in the hot plate model. This analgesic effect is likely attributed to the rich presence of phytochemicals such as alkaloids, flavonoids, tannins, and phenolics, which may act synergistically to modulate pain pathways centrally. The extract's moderate antioxidant activity further supports its therapeutic potential, as oxidative stress is often linked to inflammation and pain.

Overall, the study validates the traditional use of Alstoniascholaris for pain relief and suggests that it could be a promising natural source for analgesic agents. Further research is warranted to isolate and characterize the specific active compounds responsible for the analgesic effect and to explore their precise mechanisms of action. Additionally, studies on safety, toxicity, and clinical efficacy will be essential before considering its development into a standardized herbal formulation.

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