Pharmacogn. Res., 2023; 15(3):478-483.
https://www.phcogres.com

Original Article

Therapeutic Potential of *Alstonia scholaris* Latex in the Management of Inflammatory Diseases: An *in vitro* Approach

Bapan Banik, Malay Kumar Das*

Department of Pharmaceutical Sciences, Drug Delivery Research Laboratory, Dibrugarh University, Dibrugarh, Assam, INDIA.

ABSTRACT

Background: The different parts of *Alstonia scholaris* (AS) viz. leaf, bark root, and latex are used traditionally to treat pain, swelling, stiffness, immune deficiency, malaria, ulcer, and arthritis. The traditional healers in Dhemaji district of Assam prescribe raw AS latex for topical treatment of inflammatory diseases. The unique feature of this study is that the AS latex has been evaluated scientifically for the first time for its anti-inflammatory properties. **Objectives:** The present investigation evaluates the anti-inflammatory potential of the AS latex in arthritis using *in vitro* assays. **Materials and Methods:** Anti-arthritic potential of AS latex was evaluated using *in vitro* assays of bovine serum albumin (BSA) denaturation, egg albumin denaturation, and human red blood cell (HRBC) membrane stabilization. **Results:** It was observed that the standard diclofenac sodium and the AS latex showed good anti-arthritic potential. In BSA denaturation method, the latex, and diclofenac sodium showed 71.59% and 85.23% inhibition of proteinase enzyme at 250 µg/mL, respectively. In the egg albumin denaturation method, the latex, and standard showed the maximum percentage of inhibition of protein denaturation of 70.42% and 81.32%, respectively, at 250 µg/mL. In the HRBC membrane stabilization model, the latex produced considerable anti-arthritic potential in a concentration–dependent manner. At 250 µg/mL in hypotonic solution, latex, and diclofenac sodium showed 68.77% and 72.12% membrane stabilization, respectively. **Conclusion:** The present investigation proved the antiarthritic activity of AS latex. It may also be used in the management of other inflammatory diseases.

Keywords: *Alstonia scholaris*, Latex, Anti-inflammatory, Anti-arthritic, *in vitro.*

Corresponding author:
Prof. Malay Kumar Das
Department of Pharmaceutical Sciences, Drug Delivery Research Laboratory, Dibrugarh University, Dibrugarh, Assam, INDIA.
E-Mail: mkdps@dibru.ac.in

Received: 24-01-2023;
Revised: 08-03-2023;
Accepted: 28-03-2023.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune and inflammatory disease. It involves the joints and can damage the joints, cartilage, and nearby bone.[1] Approximately 1% of the World's population gets affected by arthritis, especially elderly people. The most important symptoms are pain, swelling, stiffness of joints, and loss of bodily movements. The prime cause of this destructive disease is unknown. However, some genetic, and external factors trigger autoimmune reactions in the body, which directly develop arthritic conditions in the patients. The disease–modifying anti-rheumatic drugs (DMRDs) are the most prescribed treatment once a patient is diagnosed with rheumatoid arthritis. The pain and stiffness in joints of patients with rheumatoid arthritis are generally controlled and modified using a drug regimen of steroidal or non-steroidal anti-inflammatory drugs that persistently persuades harmful consequences. The treatments of rheumatoid arthritis are also quite expensive, which creates a financial burden upon patients.[2,3] Thus, traditionally used herbs by folklore practitioners have strained the attention of researchers around the globe to find an effective, and safe alternative treatment of inflammatory diseases. Herbal medicines reduce the instance of diseases and also uplift the quality of life.

Alstonia scholaris (AS) (Family: Apocynaceae) is commonly known as the Devil's tree or Dita Bark tree. The different parts of AS viz. leaf, stem, root, and flowers are widely used in folklore practices by traditional healers. Since long time, some communities people of Assam like Missing, Tiwa, Rabha use AS in the management of inflammatory diseases.[4] This is an evergreen plant and is very popular among the tribal populations for its various medicinal values. It grows in the plain area and mountain rainforests of India.[5] *Alstonia scholaris* is rich in different types of bioactive constituents like alkaloids, flavonoids, and phenolic acids.[6] So far, various *in vitro* and *in vivo* pharmacological screenings have been reported on this species. The parts of AS have various medicinal properties like antimicrobial, antiamoebic, antidiarrheal, antihypertensive, antimalarial, febrifuge,

DOI: 10.5530/pres.15.3.049

Publishing Partner : EManuscript Tech. [www.emanuscript.in]

stimulant, hepatoprotective, immunomodulatory, anti-cancer, antiasthmatic, antioxidant, analgesic, anti-inflammatory, anti-fertility, anti-diabetic.[6-9] Amongst them, antimicrobial, anticancer, anti-arthritic, anti-inflammatory, and antiulcer activities were found to be promising.[10] S. Arulmozhi *et al*, 2014 reported that *Alstonia scholaris* is a promising medicinal plant, and is used in the treatment of various diseases including arthritis in folklore medicine.[11] It has been observed from different studies on AS that the anti-inflammatory and anti-arthritic bioactivity of this plant is because of the presence of various alkaloids and flavonoids.[12-14]

Latex is a milky fluid found in 10% of angiosperms. It is mainly a complex emulsion consisting of proteins, alkaloids, starch, sugar, oils, tannins, and resins. Latex is generally sticky, milky substance that colloids after drew off by making an incision in the bark of the plant.

In a field survey in the District of Dhemaji, Assam (India), it was found that the tribal people use the raw latex of *Alstonia scholaris* as a topical lotion in the management of arthritis, and inflammations. This traditional practice was taken into consideration for scientific exploration and the present study was designed to screen the latex for its anti-arthritic and anti-inflammatory potential using *in vitro* assays. To date, various parts of AS have been studied scientifically for anti-inflammatory, and anti-arthritic activities except for its latex.

MATERIALS AND METHODS

Collection of Plant Latex

The fresh latex of *A. scholaris* plant was collected in the month of November 2020 from Dhemaji district in Assam, India (94^0 12' 18" E and 27^0 05' 27" N) at an altitude of 104 meters. The botanical identification of the plant was confirmed by the Department of Herbal Science and Technology, Annadaram Dhekial Phookan College (affiliated to Gauhati University, Guwahati), Nagaon, Assam, where a herbarium was deposited (herbarium number AS/HST/1031). A stainless–steel knife was used to make a longitudinal incision of 4–6 cm length on the bark of the plant and the droplets of latex were allowed to run down and drip into the sterilized container (Figure 1). Upon incision, the resultant latex was collected in sterilized test tubes for further studies of durability, solubility, and pH. Latex was stored at 4°C in the refrigerator until used. The pH of the latex was determined immediately using narrow–range indicator paper and confirmed by a pH meter in the laboratory.

Drugs and Chemicals

Diclofenac sodium, Bovine Serum Albumin (BSA), Di-potassium hydrogen phosphate (K_2HPO_4), and Potassium di-hydrogen phosphate (KH_2PO_4) were procured from Sigma Aldrich Co., St. Louis, USA. All other compounds used for the investigation were freshly prepared and of analytical grade.

Solubility test of Latex

A solubility test for the latex was conducted with hexane, chloroform, methanol, petroleum ether, ethanol, and water. For the solubility test, 1 mL of latex was dissolved in 2 mL of solvents.

In vitro Anti-arthritic Screening

The *in vitro* antiarthritic screening of crude latex was performed using three methods viz. BSA denaturation method, Egg albumin denaturation method, and Human Red Blood cell (HRBC) membrane stabilizing assay.

BSA Denaturation Method

Effect of AS latex on heat–induced BSA denaturation was investigated using a method described by S. Chandra *et al* 2012.[15] The reaction mixtures were composed of varying concentrations (50, 100, 150, 200, and 250 µg/mL) of AS latex or reference drug diclofenac sodium (2-[(2,6 dichlorophenyl)amino] benzene acetic acid sodium salt), 1% w/v BSA and phosphate buffered saline (PBS, pH 6.4). The PBS was used as control. The reaction mixtures were incubated at 37°C for 20 min. Subsequently, the temperature was increased to maintain the samples at 70°C for 5 min. After cooling, turbidity was measured at 660 nm using a UV–Visible spectrophotometer (Schimadzu Double Beam UV–2600, Japan). The control embodies 100% protein denaturation. Each test was repeated thrice and the mean absorbance was recorded. The percentage inhibition of BSA denaturation was calculated as stated below:

$$\% \text{ inhibition of BSA denaturation} = 100 \times [\frac{Vt - Vc}{Vc}]$$

Where, Vt = absorbance of the test sample, Vc = absorbance of control

Egg Albumin Denaturation Assay[16]

In this method, fresh eggs of hen were collected from the local market in Dibrugarh Assam. The reaction mixtures were composed of 0.2 mL of egg albumin, 2.8 mL of phosphate buffer solution (pH 6.4) and 2 mL of varying concentrations (50, 100, 150, 200, and 250 µg/mL) of AS latex. Here, 5 mL of double distilled water was taken as control. The mixtures were then incubated at 37 ± 2°C for 15 min and subsequently kept at 65°C for ten minutes. After cooling, their absorbances were measured at 660 nm using a UV–Visible spectrophotometer (Schimadzu Double Beam UV–2600, Japan). Diclofenac sodium at 50, 100, 150, 200, and 250 µg/mL was used as the reference drug and treated similarly for the determination of absorbances. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition of egg albumin denaturation} = 100 \times [\frac{Vt}{Vc} - 1]$$

Where, Vt = Absorbance of the test sample, Vc = Absorbance of control.

Table 1: Nature of collected latex of *A. scholaris*.

Sl. No.	Sample	Solubility	Color	Odor	Consistency	pH
1.	Latex	Soluble in Water	Milky white	Odorless	Slightly sticky	6.8

HRBC Membrane Stabilizing Method

Preparation of blood samples for membrane stabilization assays

The HRBC membrane stabilization method (M. Saleem *et al* 2011 and S. Parvin *et al* 2015) was used to investigate the anti-arthritic and anti-inflammatory potential of *A. scholaris* latex.[17,18] The blood sample was collected from a healthy human volunteer who had not taken any Non-steroidal anti-inflammatory drugs (NSAIDs) for 2 weeks prior to the experiment. Prior consent was taken from the blood donator. The blood sample was mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl). The collected blood samples were kept in a refrigerator at 4°C for 24 hours before use. Then, it was centrifuged at 2500 rpm for 5 min, and the supernatant was removed. The cell suspension was cleaned with sterile saline solution (0.9 % w/v NaCl) and centrifuged at 2500 rpm for 5 min. The cleaning process was repeated three times till the supernatant becomes clear and colorless and the packed cell volume was measured. The cellular component was reconstituted to a 40 % suspension (v/v) with phosphate buffered saline (10 mM, pH 7.4) and was used in the assay.

Hypotonicity–induced haemolysis

Various concentration (50, 100, 150, 200, and 250 µg/mL) of latex were prepared using distilled water. To each concentration, 1 ml of phosphate buffer, 2 ml of hypotonic saline, and 0.5 ml of HRBC suspension were added. The prepared reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm using a UV–Visible spectrophotometer (Schimadzu Double Beam UV–2600, Japan). Diclofenac sodium at different concentrations (50, 100, 150, 200, and 250 µg/mL) was used as reference standard. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following formula:

$$\% \text{ inhibition of haemolysis} = 100 \times [\frac{(ODt - ODc)}{ODc}]$$

Where, ODc= Optical density of hypotonic buffered saline solution, ODt=Optical density of test sample in hypotonic solution.

Statistical Analysis

The results were expressed as Mean±SEM (n=3). They were analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. The level of significance was set at p <0.05.

RESULTS

Physico–chemical Analysis of Crude Latex of *A. scholaris*

The nature of the latex (solubility, color, pH, and odor) is depicted in Table 1.

In vitro Anti-arthritic Screening

The results of anti-arthritic activity of AS latex determined by the BSA denaturation assay and egg albumin denaturation method are shown in Figures 2 and 3, respectively. The results revealed that the AS latex showed significant (p<0.05) amount of inhibition of protein denturation when compared to standard diclofenac sodium at the concentration of 250 µg/mL. The AS latex showed 71.59 ± 1.40 % of inhibition of BSA protein denaturation. On the contrary, the standard diclofenac sodium showed 85.23±1.56 % inhibition of proteinase enzyme at 250 µg/mL (Figure 2). In the egg albumin denaturation method, the latex, and diclofenac sodium showed the maximum inhibition of 70.42 ± 2.38 % and 81.32 ± 1.92 %, respectively, at 250 µg/mL (Figure 3). In these particular assays, it was found that the crude latex had significant anti-inflammatory potency (p<0.05) when compared to the standard drug in respect of inhibition of protein denaturation. In the HRBC membrane stabilization assay, the latex showed considerable anti-arthritic activity in a concentration–dependent manner in the range of 50–250 µg/mL. In this *in vitro* study, the absorbance of hemoglobin was noted. At the concentration of 250 µg/mL, the latex showed 68.77±0.565% inhibition of membrane hemolysis, where as the standard diclofenac sodium exhibited 72.12±0.785% protection of HRBC membrane in

Figure 1: Crude latex collection.

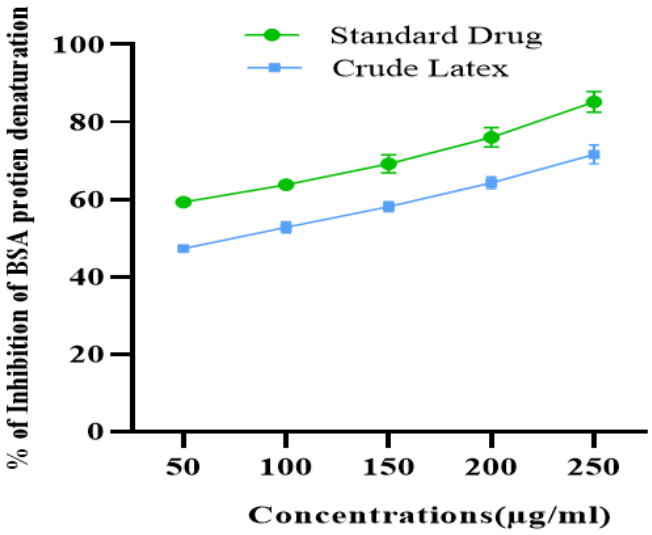

Figure 2: *In vitro* anti-arthritic activity of AS latex in BSA denaturation method (n=3, Mean± SEM).

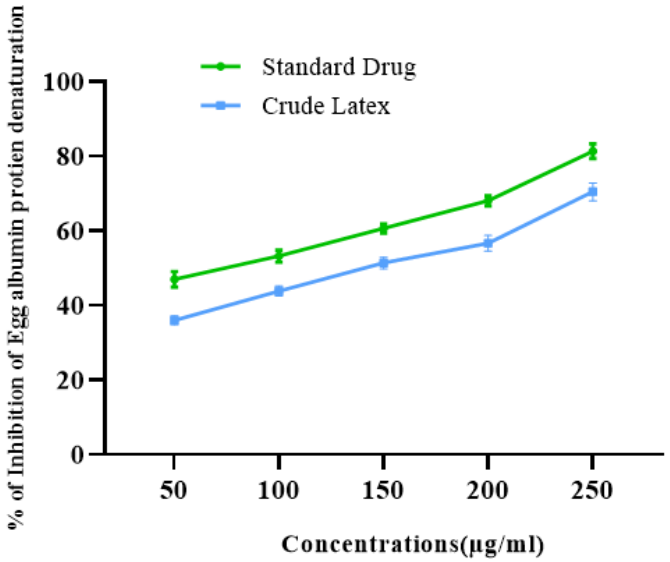

Figure 3: *In vitro* anti-arthritic activity of AS latex in egg albumin denaturation method (n=3, Mean± SEM).

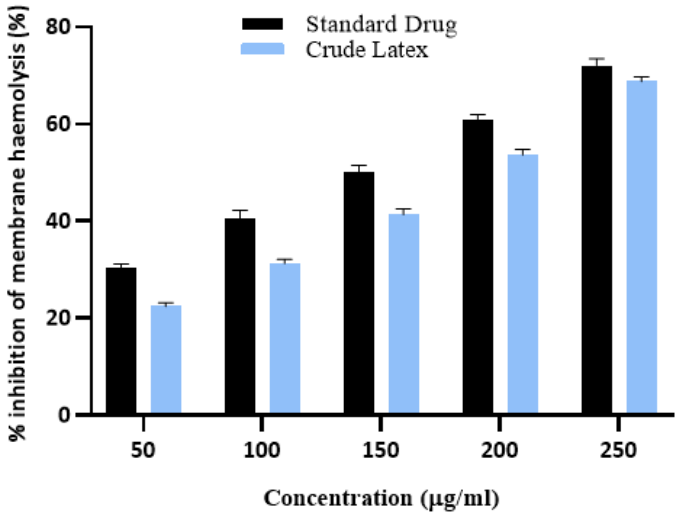

Figure 4: *In vitro* anti-arthritic activity of AS latex in the HRBC membrane stabilizing method (n=3, Mean± SEM).

a hypotonic solution (Figure 4). The AS lates showed almost similar anti-inflammatory potency (p>0.05) when compared to the standard drug in respect of HRBC membrane stabilization.

DISCUSSION

The AS latex was found to be odorless, whitish, and slightly sticky. It was found to be partially soluble in methanol and chloroform. It is soluble in water. The latex was screened for its anti-arthritic activity using various *in vitro* assays viz. inhibition of protein denaturation and HRBC membrane stabilization assays. Various concentrations of AS latex were screened for anti-arthritic potential using the protein denaturation method, where the latex possessed anti-arthritic potential in a dose–dependent manner.

During inflammatory reactions, the proteinase enzyme acts as a very significant role in the expansion of tissue damage.[19] In the present study, the latex showed maximum inhibition of the proteinase enzyme as equal to the inhibition caused by diclofenac sodium (Figures 2, and 3). Due to the denaturation of protein and membrane lysis action in certain arthritic and inflammatory diseases, there might be an increase in the production of autoantigens. The *in vitro* HRBC membrane stabilization assay for anti-inflammatory and anti-arthritic screening becomes very significant as the erythrocyte membrane is analogous to the lysosomal membrane.[20,21] Stabilization of the lysosomal membrane is imperative in restraining the inflammatory reactions by stopping the discharge of components like activated neutrophils, bacterial enzymes, and proteases, which causes additional tissue inflammation, and damage upon extracellular

release. In arthritic and inflammatory conditions non-steroidal anti-inflammatory drugs (NSAIDs) work either by inhibiting the lysosomal enzyme or by alleviating the lysosomal membrane.[22] The release of hemoglobin is due to the lysis of the erythrocyte membrane. In this investigation of HRBC assay, the latex showed a noteworthy anti-inflammatory response in a concentration–dependent manner (Figure 4). The latex was found to be well effective in stabilizing the lysosomal membrane when compared to the standard drug.[23] Based on the results obtained from these three *in vitro* anti-arthritic assays, it can be resolved that the traditional claim of using AS latex in joint paints by elderly people is true, and has scientific validation. The anti-arthritic effect of AS latex might be due to the occurrence of phytoconstituents *viz.* tannin and phenolic content, alkaloids, glycosides, flavonoids, and steroids. When a phytoconstituent is capable of preventing protein denaturation and lysosomal membrane stabilization, it could be considered as an anti-arthritic, and anti-inflammatory drug.[24]

CONCLUSION

Alstonia scholaris (Devil's tree) is widespread in India, especially in Assam. This plant has significant traditional uses for its anti-inflammatory, anti-arthritic, antiulcer, anticancer, antioxidant activity. The present investigation confirms that the AS latex have good potential for inhibition of protein denaturation and significant membrane stabilizing properties. Thus, it justifies the traditional claim of using AS latex as an anti-arthritic drug. Further *in vivo* studies and molecular mechanisms involved in the antiarthritic activity of the latex are warranted to develop it as an alternative herbal therapy in the management of arthritis and other inflammatory diseases.

ACKNOWLEDGEMENT

The authors are thankful to the Assam Science Technology and Environmental Council (ASTEC), Govt. of Assam, Guwahati for financial assistance. The authors are also thankful to the Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India, and the Department of Herbal Science, and Technology, ADP College, Nagaon, Assam, India for providing the platform to conduct this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RA: Rheumatoid arthritis, **AS:** *Alstonia scholaris*; **BSA:** Bovine serum albumin; **HRBC:** Human red blood cell; **DMRDs:** Disease–modifying anti-rheumatic drugs; **NSAID:** Non-steroidal anti-inflammatory drugs, **OD:** Optical density.

SUMMARY

Alstonia scholaris (AS) is a very popular and widely used medicinal plant in the Indian subcontinent. The different parts of this plant viz. leaf, bark root, and latex are in folklore used since Ayurvedic times. This plant is having traditional claims of curing pain, swelling, stiffness, immune deficiency, malaria, ulcer, cancer, fever, and arthritis. The AS latex was screened for its antiarthritic and anti-inflammatory properties, which is backed by traditional claims in the Dhemaji district of Assam, India. *In vitro* assays *viz.* BSA denaturation, egg albumin denaturation, and human red blood cell (HRBC) membrane stabilization were carried out to determine the anti-arthritic, and anti-inflammatory properties of AS latex. It was observed that the standard diclofenac sodium and the AS latex showed good anti-arthritic anti-inflammatory potential. In the HRBC membrane stabilization model, the latex produced good protection of the HRBC membrane stability in a hypotonic solution. The findings of this study give a scientific base to the traditional claim of using AS latex in the management of arthritic and other inflammatory symptoms. Considering the findings and results of *in vitro* studies, the latex could be subjected to *in vivo* studies along with chemical characterization, and molecular docking studies to validate its use as an anti-arthritic drug.

REFERENCES

1. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Braz J Pharm Sci. 2016;52(3):493-507. doi: 10.1590/s1984-82502016000300015.
2. Singh G, Ramey DR, Morfeld D, Shi H, Hatoum HT, Fries JF. Gastrointestinal tract complications of nonsteroidal anti-inflammatory drug treatment in rheumatoid arthritis- A prospective observational cohort study. Arch Intern Med. 1996;156(14):1530-6, PMID 8687261.
3. Gaffo A, Saag KG, Curtis JR. Treatment of rheumatoid arthritis. Am J Health Syst Pharm. 2006;63(24):2451-65. doi: 10.2146/ajhp050514, PMID 17158693.
4. Channa S, Dar A, Ahmed S. Evaluation of *Alstonia scholaris* leaves for broncho-vasodilatory activity. J ethnopharmacology. 2005;97(3):469-76. doi: 10.1016/j.jep.2004.12.009.
5. Dey A. *Alstonia scholaris* R.Br. (Apocynaceae): phytochemistry and pharmacology: a concise review. J Appl Pharm Sci. 2011;1(6):51-7.
6. Kaushik D, Rana A, Kaushik P, Sharma N. *Alstonia scholaris*: it's Phytochemistry and pharmacology. Chron Young Sci. 2011;2(2):71-8. doi: 10.4103/2229-5186.82970.
7. Baliga MS. Review of the phytochemical, pharmacological and toxicological properties of *Alstonia scholaris* Linn. R. Br (Saptaparna). Chin J Integr Med. 2012:1-14. doi: 10.1007/s11655-011-0947-0.
8. Khyade MS, Kasote DM, Vaikos NP. *Alstonia scholaris* (L.) R. Br. and *Alstonia macrophylla* Wall. ex G. Don: A comparative review on traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 2014;153(1):1-18. doi:10.1016/j.jep.2014.01.025.
9. Kanase V, Mane DJ. A pharmacognostic and pharmacological review on *Alstonia scholaris*. Asian J Pharm Clin Res. 2018;11(12):22-6. doi: 10.22159/ajpcr.2018.v11i12.28124.
10. Pandey K, Shevkar C, Bairwa K, Abhijeet S, Kate AS. Pharmaceutical perspective on bioactives from *Alstonia scholaris*: ethnomedicinal knowledge, phytochemistry, clinical status, patent space, and future directions. Phytochem rev. 2020;19(1):191-233. doi: 10.1007/s11101-020-09662-z.
11. Arulmozhi S, Rasal VP, Sathiyanarayanan L, Ashok P. Screening of *Alstonia scholaris* Linn. R. Br., for wound healing activity. Orient Pharm Exp Med. 2007;7(3):254-60. doi: 10.3742/OPEM.2007.7.3.254.
12. Cai X, Shang J, Feng T, Luo X. Novel Alkaloids from *Alstonia scholaris*. Zeitschrift für Naturforschung B. 2010;65(9):1164-8. doi: 10.1515/znb-2010-0918.
13. 13.Subraya CK, Harikiran, Gupta D. Antioxidant and Anti-inflammatory Activity of *Alstonia scholaris* R.Br. Stem Bark Extract. Free Rad Antiox. 2012;2(2):55-7. doi: 10.5530/AX.2012.2.2.9.
14. Feng L, Chen Y, Yuan L, Liu X, Gu JF, Zhang MH, *et al*. A combination of alkaloids and triterpenes of *Alstonia scholaris* (Linn.) R. Br. leaves enhances immunomodulatory

activity in C57BL/6 mice and induces apoptosis in the A549 cell line. Molecules. 2013;18(11):13920-39. doi: 10.3390/molecules181113920, PMID 24225772.

15. Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed. 2012;2:S178-S180. doi:10.1016/S2221-1691(12)60154-3.
16. Banerjee S, Chanda A, Adhikari A, Das A, and Biswas S. Evaluation of Phytochemical Screening and Anti-Inflammatory Activity of Leaves and Stem of *Mikania scandens* (L.) Wild. Ann Med Health Sci Res. 2014;4(4):532-6. doi:10.4103/2141-9248.139302.
17. Mohamed Saleem TK, Azeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extacts of *Gendarussa vulgaris* Nees. Asian Pac J Trop Biomed. 2011;1(2):147-9. doi:10.1016/S2221-1691(11)60014-2, PMID 23569746.
18. Parvin MS, Das N, Jahan N. *et al*. Evaluation of *in vitro* anti-inflammatory and antibacterial potential of *Crescentia cujete* leaves and stem bark. BMC Res Notes. 2015;8:412. doi:10.1186/s13104-015-1384-5,
19. Das SN, Chatterjee S. Long term toxicity study of ART-400. Ind Indg Med. 1995;16(2):117-23.
20. Gandhidasan R, Thamaraichelvan A, Baburaj S. Anti-inflammatory action of *Lanneacoro mandelica* by HRBC membrane stabilization. Fitoterapia. 1991;62(1):81-3.
21. Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. Evaluation of anti-inflammatory activity of *Tephrosia purpurea* in rats. Asian Pac J Trop Med. 2010;3(3):193-5. doi: 10.1016/S1995-7645(10)60007-7.
22. Vadivu R, Lakshmi KS. *In vitro* and *in vivo* anti-inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour.) Moore ssp. laurina. Bangladesh J Pharmacol. 2008;3(2):121-4.
23. Perumal SS, Ekambaram SP, Dhanam T. *In vivo* antiarthritic activity of the ethanol extracts of stem bark and seeds of *Calophyllum inophyllum* in Freund's complete adjuvant induced arthritis. Pharm Biol. 2017;55(1):1330-6. doi: 10.1080/13880209.2016.1226346, PMID 27593261.
24. Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. J Exp Med. 1962;115(3):597-608. doi: 10.1084/jem.115.3.597, PMID 14482110.

Cite this article: Banik B, Das MK. Therapeutic potential of *Alstonia scholaris* Latex in the Management of Inflammatory Diseases: An *in vitro* Approach. Pharmacog Res. 2023;15(3):478-83.