Research Article

GC-MS Analysis of Ethanolic Bark Extract of *Alstonia scholaris* and Evaluation of its Pharmacological Studies

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ABSTRACT

The anti-arthritic effect of ethanolic extract of Bark of *Alstonia scholaris* Linn. (Apocynaceae) has been studied previously. The main purpose of this research work was to explore its chemical constituents which are responsible for the activity by GC-MS analysis and also to verify anti-arthritic effect of the ethanol extract of *Alstonia scholaris* bark. Since ethanol extract showed better activity than other successive extracts GC-MS analysis was carried out to identify the constituents responsible for the activity. The ethanolic extract of Bark of *Alstonia scholaris* Linn. were subjected to *in vitro* anti-arthritic activity by Inhibition of protein denaturation method. The GC-MS analysis of *Alstonia scholaris* bark extract within absolute alcohol was achieved using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% phenyl 95 % dimethyl polysiloxane) (30 nm × 0.25 mm ID × 0.25 µm df). The highest percentage inhibition of ethanolic extract of *Alstonia scholaris* was about 88.95 ± 0.41 % at concentration of 750µg/ml in contrast to standard which was about 91.88 ± 0.10 % at same concentration. GC-MS chromatogram of the ethanolic bark extract of *Alstonia scholaris* illustrates peaks indicating the presence of a wide range of compounds. The results achieved indicate that ethanol extract possess good anti-arthritic activity and it may be due to components mainly oxygenated hydrocarbons and primarily phenolic hydrocarbons, Steroids which was Identified by GC-MS analysis.

Keywords: *Alstonia scholaris*, Anti arthritic activity, GC-MS analysis, Phytochemical.

INTRODUCTION

Alstonia scholaris (L.) R.Br. fit in to the family Apocynaceae, is a tropical tree found enormously in Indian sub-continent region and widely spread over South East Asia. It was recognized as satiyana. It comprise of hazy irregular bark and gloomy sap affluent in poisonous alkaloid. The alkaloid fraction of *Alstonia scholaris* (L.) exhibited anti-tussive and antiasthmatic activities *in vivo.*[1] The bark also called dita. Bark is by tradition used by several ethnic groups of North-East India and also other part of the world as a source of remedy against bacterial infection, malarial fever, toothache, rheumatism, snake bite, dysentery, bowl disorder asthma etc. And latex is used in treating coughs, through sores and fever.[2,3] In modern pharmaceutical, *Alstonia scholaris* is well intentional for enormous activities viz., antimicrobial, antiamoebic, antidiarrhoeal, antiplasmodial, hepatoprotective, immunomodulatory, anticancer, antiasthmatic, free radical scavenging, antioxidant, analgesic, anti-inflammatory, antiulcer, Antifertility and wound healing. An antimalarial Ayurvedic preparation, Ayush-64, containing *A. scholaris* is marketed.[4] Folklore use includes application of milky juice of leaves on wounds, etc. And the juice was also served as a remedy for ulcers and for rheumatic pain and in the mixed form with oil was used in earache.[2] Plenty of Phytoconstituents was isolated by various techniques, characterized and formulated as drug from the bark, leaf, etc. On the other side, concerning scientific standardization or exceptionally few reports are available.

The current research work was attempted to describe the phytochemical composition using different solvents of increasing polarity and to establish the physicochemical standard of the stem bark of *Alstonia scholaris.*

MATERIALS AND METHODS

Collection of Plant

The Bark of the plant were collected from Ramanathapuram district, Tamilnadu, India and it was examined, recognized and authenticated by Professor P. Jayaraman, Plant Anatomy Research Centre, Chennai. The bark was shade dried for 28 days and pulverised in plant mill and accumulated in air tight box for future use.

Preparation of Extract

The Bark powder was subjected to successive extraction by cold maceration for 72 hours, 48 hours and then for 24 hours at room temperature. The powdered bark was consecutively extracted with 200 ml of solvents (petroleum ether, benzene, chloroform, ethyl acetate and Ethanol). Solvent was distilled from the extract under reduced pressure.

Phytochemical screening of the bark extract

The primary and secondary metabolites were recognized by performing a variety of biochemical tests. The bark extracts was assessed for the existence of phenols, glycosides, quinones, terpenoids, alkaloids, saponins, flavonoids, triterpenoids, steroids and tannins.

In vitro anti-arthritic activity

Inhibition of protein denaturation method

Chemicals

Sodium chloride, Potassium chloride, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Bovine serum albumin and HCl acid was procured from Merck, Qualigens and Sigma Aldrich. Diclofenac sodium 50mg and Rumagesic Capsule 500mg was procured from the local market.

Preparation of reagents

5% Bovine serum albumin (BSA) **-** Dissolved 5g of BSA in 100ml of water.

Phosphate buffer saline pH 6.3 **-** Dissolved 8g of sodium chloride (NaCl), 0.2g of potassium chloride (KCl), 1.44g of disodium hydrogen phosphate (Na_2HPO_4) and 0.24g of potassium dihydrogen phosphate (KH_2PO_4) in 800ml distilled water. The pH was adjusted to 6.3 using 1N HCl and make up the volume to 1000ml with distilled water.

- Test **so**lution-0.45ml of Bovine serum albumin and 0.05ml of test solution of various concentrations
- Test control solution- 0.45ml of bovine serum albumin and 0.05ml of distilled water.
- Product control solution-0.45ml of distilled water and 0.05 ml of test solution
- Standard solution- 0.45ml of Bovine serum albumin and 0.05ml of Diclofenac sodium of various concentrations.

Procedure

0.5ml of Test solution, Test control solution, Product control solution, Standard solution was prepared. Various concentrations (100, 250, 500 and 750µg/ml) of test dugs and standard drug Diclofenac sodium (100, 250, 500 and 750µg/ml) were prepared. 1N HCl was used to adjust the pH to 6.3 for all the above solutions. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer was added to the above solutions. The absorbance was measured at 416nm.[5,6] The control represents 100% protein denaturation. The percentage inhibition of protein denaturation can be calculated as

Percentage Inhibition =

100 – [{(optical density of test control – optical Density of product control)/optical density of test solution} × 100

The control represents 100% protein denaturation

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The additional defined information in qualitative examination can be attained by Gas-chromatography together with mass spectrometry (GC-MC).[7] The GC-MS analysis of *Alstonia scholaris* bark extract within absolute alcohol was achieved using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% phenyl 95 % dimethyl polysiloxane) (30 nm × 0.25 mm ID × 0.25 µm df). Mass detector Turbomass gold of the company which was functioning in EI mode. Helium was the carrier gas at a run time of 1 ml/min. The injector was controlled at 250°C and the oven temperature was automated as follows; 50°C at 8°C/min to 200°C(5 min) at 7°C/min to 290°C(10min) at 110°C (2min) to 200°C at 10°C/min to 280°C (9 min) at 5°C/min. Total operation time is 36 minutes. GC-MS analysis of some of the effective volatile constituents present in the extract was achieved and the interpretation on mass spectrum of GC-MS was concluded using the Database of Indian Institute of Crop Processing Technology (IICPT) having more than 75,000 patterns. The plant extract was liquefied in ethanol and pass through a filter with polymeric solid phase extraction (SPE) column and investigated in GC-MS for different components.

RESULTS AND DISCUSSION

Preliminary phytochemical studies

Preliminary phytochemical learning of the successive extracts of the bark of *Alstonia scholaris* was made. The extracts exposed the occurrence of metabolites like flavonoids, steroids, saponins, terpenoids, triterpenoids, glycosides, alkaloids, phenols and tannins, which is specified in Table 1.

Table 1: Preliminary phytochemical analysis of *Alstonia scholaris*

Name of test	Pet ether	Benzene	Chloroform	Ethyl acetate	Ethanol
Quinones	-	-	+	-	-
Glycosides	-	-	-	+	+
Terpenoids	-	+	-	+	+
Alkaloids	-	-	+	-	-
Saponins	-	-	-	-	+
Flavonoids	-	-	-	+	+
Triterpenoids	-	+	-	+	-
Steroids	+	+	-	-	-
Tannins	-	-	-	-	+
Phenols	-	-	-	-	+

Anti-Arthritic Activity

Inhibition of Protein Denaturation Method

Protease inhibitor activity of ethanolic bark extract of *Alstonia scholaris* was estimated as described in the literature. Diclofenac sodium was included as standard. *In vitro* Anti-Arthritic assay was done by inhibition of protein denaturation method. The highest percentage inhibition of ethanolic extract of *Alstonia scholaris* was about 88.95 ± 0.41 % at concentration of 750µg/ml in contrast to standard which was about 91.88 ± 0.10 % at same

concentration. The results were exposed in the Table 2 and graphically in Figure 1.

Table 2: Anti-arthritic activity of ethanol Bark extract of Alstonia *scholaris* by protein denaturation

Concentration(µg/ml)	% Inhibition	
	Diclofenac sodium	*Alstonia scholaris*
100	58.45 ± 0.30	46.13 ± 0.40
250	79.05 ± 0.90	63.7 ± 0.6245
500	86.90 ± 0.75	79.20 ± 0.90
750	91.88 ± 0.10	88.95 ± 0.41

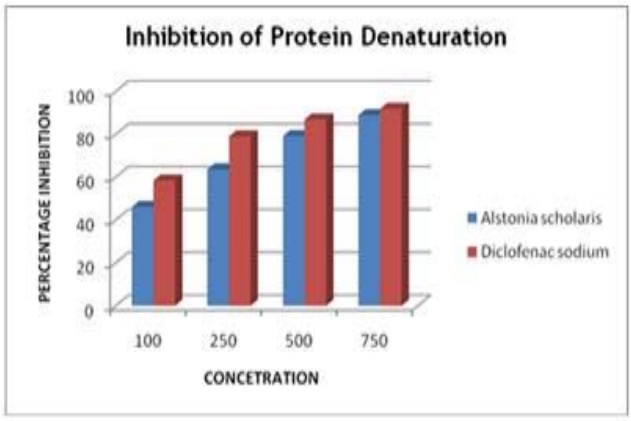

Figure 1: Inhibition of Protein Denaturation

Table 3: Phytocomponents Identified in the *Alstonia scholaris* ethanolic Bark extract by GC-MS

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	8.28	Pentanoic acid	$C_5H_{10}O_2$	102	1.45
2.	12.70	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	7.25
3.	12.78	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.72
4.	26.58	3,6-Bis[2-methylphenyl]-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	$C_{20}H_{16}N_2O_2$	316	1.81
5.	30.37	Stigmasterol	$C_{29}H_{48}O$	412	3.62
6.	31.15	D-Friedoolean-14-en-3-one	$C_{30}H_{48}O$	424	1.81
7.	31.82	Guaia-3,9-diene	$C_{15}H_{24}$	204	38.04
8.	32.19	2H-1-Benzopyran-2-one,7-acetyl-8-[acetyloxy]-4-methyl-	$C_{14}H_{12}O_5$	260	21.74
9.	33.24	6βBicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α,5α-dimethyl-	$C_{15}H_{25}I$	332	18012
10.	35.37	B – Amyrin	$C_{30}H_{50}O$	426	5.43

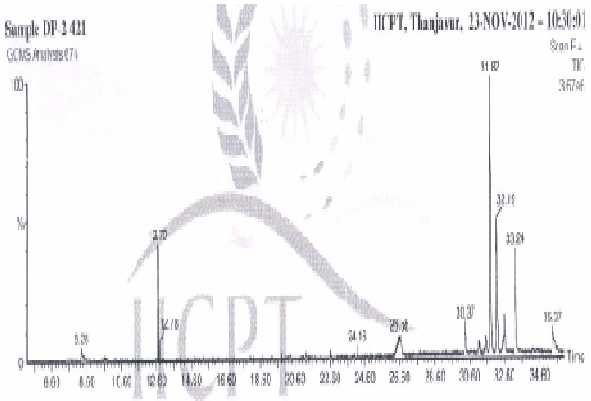

Figure 2: GC/MS spectrogram for the *Alstonia scholaris* ethanolic Bark extract

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

GC-MS chromatograms of the ethanolic bark extract of Alstonia scholaris (Figure 2) illustrate peaks indicating the presence of a wide range of compounds. It was shown in Table no: 3. The compound recognized in GC-MS analysis was Pentanoic acid (1.45%), Dibutyl phthalate (7.25%), n-Hexadecanoic acid (0.72%). 3,6-Bis (2-methylphenyl)-2,5-dihydropyrrolo [3,4-C]pyrrole-1,4-dione (1.81%), Stigmasterol (3.62%), D-Friedoolean-1,4-en-3-one (1.81%), Guaia-3,9-diene (38.04%), 2H-1-Benzopyran-2-one, 7-acetyl-8-(acetyloxy)-4-methyl- (21.74%), 6βBicyclo[4.3.0]nonane, 5β-iodomethyl-1β-isopropenyl-4α, 5α-dimethyl-(18.12%), β-Amyrin (5.43%). Most of them are oxygenated hydrocarbons and primarily phenolic hydrocarbons, Steroids. These phytochemicals may be accountable for various pharmacological behaviour like anti-oxidant and anti-arthritic activity, etc.

CONCLUSION

In view of preliminary phytochemical studies and anti-arthritic study, ethanolic extract showed more appreciable results, so it was subjected to GC-MS analysis to identify the components responsible for the potency. The present study in *Alstonia scholaris* suggests that the contribution of these components on the pharmacological activity should be evaluated future by *in vivo* studies.

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