

PRELIMINARY PHYTOCHEMICAL EVALUATION AND SPECTRAL CHARACTERIZATION OF ACTIVE CONSTITUENTS IN THE DRIED EXTRACTS OF THE WHOLE PLANT *ARGYREIA IMBRICATA* (ROX) SANT & PATEL

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ABSTRACT

Objective: The present study was designed with the aim of preliminary phytochemical evaluation and spectral characterization phytochemicals present in the extracts of whole plant *Argyrea imbricata* (Rox) Sant & Patel.

Methods: The whole plant *A. imbricata* was collected, authenticated, and dried in the shade for powdering in mechanical grinder. The powdered plant material was extracted with Soxhlet apparatus using different solvents, and the dried extract obtained was subjected to preliminary phytochemical evaluation, and then column chromatography separation and the separated fractions were subjected to thin-layer chromatography (TLC) separation. Based on the yield in TLC, the selected compounds were subjected to carbon-13 nuclear magnetic resonance, proton NMR (¹H NMR), and mass spectroscopic evaluation.

Results: In the preliminary phytochemical evaluation, the presence of alkaloids, glycosides, phenolic compounds, flavonoids, carbohydrates, saponins, sterols, and terpenoids was found in different extracts. In column chromatography separation, totally 650 fractions were collected, and four compounds were isolated with the help of TLC. Among the four, two compounds were selected for spectral evaluation based on the yield obtained. The selected compounds were identified and named as, 3-ethyl-5-(hydroxymethyl) phenol and methyl 4-hydroxybenzoate with the help of spectral evaluation.

Conclusion: The outcome of this study is beneficial, and further investigation in the future directed toward the detailed pharmacological screening of the extracts may give more valuable results.

Keywords: *Argyrea imbricata*, Preliminary phytochemical evaluation, Chromatographic separation, Spectral evaluation

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INTRODUCTION

It is well known that the therapeutic usage of plant sources is time immemorial. Medicinal plants are commonly distributed all over the world, but, notably in tropical countries. Since the past, medicinal plants are continuously explored for their therapeutic potentials. According to the World Health Organization, plants are one of the best sources for a variety of drugs, and approximately 25% of present-day medicines are derived directly or indirectly from higher plants [1-3]. Of course, traditional medicines occupy a prime position in the primary health care need of a significant proportion of world's population, particularly, in developing countries. Importantly, usage of medicinal plants in Asian countries indicates a longstanding tradition of human relationships with the environment. These medicinal plants contain a wide range of bioactive compounds that include alkaloids, flavonoids, tannins, and phenolic compounds which can be used to treat numerous chronic as well as infectious diseases [4-7].

In general, the rural population of developing countries has rich knowledge in the medicinal value of the plants of that region [4]. Increasing demand for medicines from the plant sources, therefore, needs a systematic evaluation of plants used in traditional medicines [5]. With this view, the plant *Argyrea imbricata* was selected for the present study.

The genus *Argyrea* belongs to Convolvulaceae family consist around 220 species which are widely distributed all over India. The *A. imbricata* is one among them, commonly found in South India at an altitude up to 300m mean sea level. In Tamil, it is known as "Vellai unnankodi." It

is a dicotyledonous plant; grow well in rainy season has flowering and fruiting season in August–December period. It is a large climber, have white-woolly stem; leaves are 8–12 cm, ovate, obtusely acute, rounded or subcordate at base, strigose above, white-tomentose beneath, nerves impressed above; Petiole 3 cm long; cymes terminal; peduncles 5cm long; bracts and bracteoles small; flowers few, shortly pedicelled, calyx lobe orbicular; 10×7 mm, densely hairy outside, reddish inside, corolla 2 cm long, pink. Berry 5×5 mm, reddish, densely, and hairy outside. The present study focused on the preliminary phytochemical screening of the plant *A. imbricata* and the characterization of active constituents by different spectral analysis. The outcome of this study would provide a good platform for further researches.

METHODS

Plant collection and identification

The whole plant of *A. imbricata* was collected from Mekkarai, the rural area located near the foothills of Western Ghats, Tirunelveli District, Tamil Nadu, India. Identification and authentication of the collected plants were done by Dr. Chelladurai, Research Officer-Botany, Central Council for Research in Siddha, Tirunelveli, Tamil Nadu, India.

Preparation of powdered material and extraction

The collected whole plant was dried in the shade for about 10 days, powdered with mechanical grinder and stored in the airtight container for further study. The powdered plant material was extracted in Soxhlet apparatus assembly successively with solvents of increasing polarity,

namely, petroleum ether, chloroform, ethyl acetate, and methanol. For that, about 25 g of dried coarse powder was weighed, moistened with the respective solvents, packed in the Soxhlet extractor and extracted individually with 500 ml of each solvent. After each extraction, the same dried marc was used for the subsequent extraction. Each extract was filtered, distilled off the solvent to obtain the dried extract. The percentage yield of each dried extract was calculated.

Preliminary phytochemical screening

Chemical test for alkaloids

Little quantity of dried extract with alcohol was shaken with dilute hydrochloric acid and filtered. The acidified filtrate was used to detect the presence of alkaloids by the following tests.

Mayer's test

The acidified filtrate (2 ml) was treated with Mayer's reagent (1 ml), shaken well and observed for the presence of creamy precipitate.

Wagner's test

The acidified filtrate (2 ml) was treated with Wagner's reagent (1 ml) and observed for the presence of reddish-brown precipitate.

Hager's test

The acidified filtrate (2 ml) was treated with Hager's reagent (1 ml) and observed for the presence of yellow precipitate.

Dragendorff's test

The acidified filtrate (2 ml) was treated with Dragendorff's reagent (2 ml) and observed for the presence of orange-red precipitate.

Chemical tests for glycosides

Little quantity of dried extract was hydrolyzed with dilute hydrochloric acid on a water bath for a few hours, and the hydrolysate obtained was used to detect the presence of glycosides by following tests.

Legal test

The hydrolysate (2 ml) was dissolved in pyridine (2 ml). Freshly prepared sodium nitroprusside solution (2 ml) was added to it. Made the mixture alkaline with sodium hydroxide solution and observed for the formation of a pink color.

Baljet test

The hydrolysate (2 ml) was treated with sodium picrate solution (1 ml) and observed for the formation of a yellow to orange color.

Borntrager's test

A little quantity of the residue obtained from the evaporation of hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated and equal quantity of dilute ammonia solution was added to it and shaken well and observed for the formation of pink color in the ammoniacal layer.

Modified Borntrager's test

A little quantity of the residue obtained from the evaporation of hydrolysate was treated with ferric chloride and dilute hydrochloric acid. Then, it was extracted with chloroform. The chloroform layer was separated, and an equal quantity of dilute ammonia solution was added to it and shaken well and observed for the formation of pink color.

Chemical tests for phenolic compounds and tannins

Ferric chloride test

A small quantity of the dried extract was mixed with water and treated with dilute ferric chloride solution (5%) and observed for the presence of a blue color.

Gelatin test

The dried extract dissolved in the water was filtered. To the filtrate, a 2% solution of gelatin containing 10% sodium chloride was added and observed for the presence of milky white precipitate.

Lead acetate test

The dried extract dissolved in the water was treated with a 10% lead acetate solution and observed for the presence of bulky white precipitate.

Decolorization test

The dried extract dissolved in water was treated with dilute potassium permanganate solution and observed for the decolorization of potassium permanganate.

Chemical tests for flavanones and flavonoids

Aqueous sodium hydroxide test

Aqueous sodium hydroxide solution was added to the little quantity of dried extract and observed for the yellow coloration of the solution.

Ammonia test

The filter paper wetted with a small quantity of an alcoholic solution of the dried extract was exposed to ammonia vapor and observed for the formation of yellow color.

Shinoda test

The dried extract mixed with alcohol was treated with magnesium or zinc and dilute hydrochloric acid and observed for the formation of orange-red or violet color.

Chemical tests for carbohydrates

A small quantity of ethanolic extract was mixed with water or alcohol and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

Molisch's test

The filtrate (2 ml) was treated with a few drops of Molisch's reagent and concentrated sulfuric acid (2 ml) was added through the side of the test tube without shaking and observed for the presence of violet ring at the junction of two solutions.

Fehling's test

The filtrate (1 ml) was treated with 1 ml each of Fehling's solution A and B and boiled in a water bath and observed for the formation of a reddish precipitate.

Benedict's test

The filtrate (2 ml) was treated with Benedict's reagent (2 ml). Then, the mixture was heated in a boiling water bath and observed for the presence of reddish precipitate.

Chemical tests for proteins and amino acids

Millon's test

Little quantity of dried extract was treated with of Millon's reagent (2 ml) and observed for the formation of white precipitate, which on warming turn into a red colored solution.

Biuret test

Little quantity of dried extract was treated with a few drops of 2% copper sulfate solution. To this excess of potassium hydroxide solution was added and observed for the formation of violet colored solution.

Ninhydrin test

Little quantity of dried extract was treated with few drops of ninhydrin solution and heated on a water bath and observed for the presence of a violet color.

Chemical test for terpenoids

Salkowski test

Little quantity of dried extract was dissolved in chloroform. An equal volume of concentrated sulfuric acid was added to it and observed for the appearance of red color in the chloroform layer and greenish-yellow fluorescence in the acid layer.

Chemical tests for sterols

A little quantity of the alcoholic extract was refluxed with alcoholic potassium hydroxide solution until the saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated, and the residue obtained was used in the tests for sterols.

Liebermann–Burchard test

The residue was taken with dry chloroform (1 ml), and then it was mixed with 2 ml of specially distilled acetic anhydride followed by a few drops of concentrated sulfuric acid through the sides of the test tube and observed for the formation of green color in the upper portion which changes to bluish violet.

Salkowski test

The residue was dissolved in chloroform, and an equal volume of concentrated sulfuric acid was added to it and observed for the red color in the lower layer.

Chemical tests for saponins

Foam (froth) test

A small quantity of dried extract was diluted with distilled water (20 ml) in a graduated cylinder. The suspension was shaken for 15 min and observed for the formation of froth.

Hemolysis test

A drop of blood was placed in a slide and mixed with a small quantity of dried extract and observed for hemolysis.

Chemical tests for gum and mucilage

Absolute alcohol (25 ml) was added with an aqueous extract (10 ml) with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

Chemical test for volatile oil

Powdered material (50 g) was subjected to hydro-distillation in volatile oil estimation apparatus (Clevenger apparatus). Collect the distillate and observed for the presence of volatile oil layer.

Isolation by chromatography

Fractionation of the crude extract by column chromatography

Dried extract (10 g) was mixed with Silica gel (60–120 mesh; 20 g) and hexane and the admixture was packed in the column (2.4 dia.) with silica gel and eluted with solvents in the order of increasing polarity from hexane to ethyl acetate in the ratio from 95:5 to 0:100. The collected fractions were subjected to thin-layer chromatography (TLC) study. The fractions showed that the same Rf value was combined and concentrated using the rotary evaporator.

TLC

An aliquot of all the concentrated fractions was loaded on the activated silica gel TLC plates (20×20 cm) and eluted with different ratio of



Fig. 1: (a and b) *Argyreia imbricata* plant

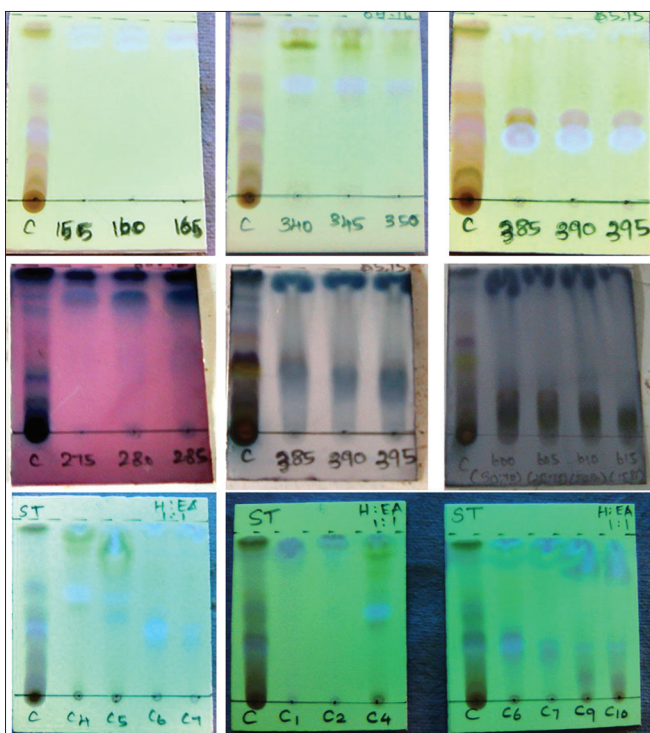


Fig. 2: Thin-layer chromatography plates of some isolated fractions

Table 1: Preliminary phytochemical evaluation of the dried extract of whole plant *Argyreia imbricata*

S. No.	Chemical test	I	II	III	IV
1.	Alkaloids	-	++	+	+
1a.	Mayer's test	-	++	+	+
1b.	Wagner's test	-	++	+	+
1c.	Hager's test	-	++	+	+
1d.	Dragendorff's test	-	++	+	+
2.	Glycosides				
2a.	Legal test	+	-	++	++
2b.	Baljet test	+	-	++	++
2c.	Borntrager's test	-	-	-	-
2d.	Modified Borntrager's test	+	-	+	+
3.	Phenolic compounds				
3a.	Ferric chloride test	-	-	+	+
3b.	Lead acetate test	-	-	+	+
3c.	Gelatin test	-	-	+	+
4.	Flavanones and flavonoids				
4a.	Aqueous NaOH test	-	-	+	+
4b.	Ammonia test	-	-	+	+
4c.	Shinoda test	-	-	+	+
5.	Carbohydrates				
5a.	Molisch's test	-	-	+	+
5b.	Fehling's test	-	-	+	+
5c.	Benedict's test	-	-	+	+
6.	Proteins and amino acids				
6a.	Millon's test	-	-	-	-
6b.	Biuret test	-	-	-	-
6c.	Ninhydrin test	-	-	-	-
7.	Terpenoids				
7a.	Salkowski test	-	-	+	+
8.	Sterols				
8a.	Liebermann–Burchard test	+	-	++	++
8b.	Salkowski test	+	-	++	++
9.	Saponins				
9a.	Foams test/froth test	-	-	-	+
9b.	Hemolysis test	-	-	-	+
10.	Gum and mucilage	-	-	-	-
11.	Volatile oil	-	-	-	-

I: Petroleum ether extract, II: Chloroform extract, III: Ethyl acetate extract, IV: Methanol extract, +: Presence of active constituents, ++: Significant presence of active constituents, -: Absence of active constituents

hexane:ethyl acetate such as 90:10, 75:25, 50:50, and 25:75. Spots were located by exposing the plate to vanillin in sulfuric acid reagent. The compounds isolated were characterized by spectral evaluation.

Characterization of the isolated compounds by spectral study

Carbon-13 nuclear magnetic resonance (13C NMR)

¹³C NMR spectral evaluation of the isolated compounds was done using the Bruker Avance 100 MHz NMR instrument. About 30–40 mg of

isolated compound was dissolved in CDCl₃: DMSO-d₆, 90:10 in an NMR tube. All the spectra obtained were corrected with equivalent to the solvent signal.

Proton NMR (¹H NMR) spectrum

¹H NMR (300 MHz) spectra of the isolated compounds were recorded in CDCl₃. Chemical shifts were recorded in parts per million downfield with reference to internal standard tetramethylsilane on Bruker Ultrashield model 400.

Mass spectra

Mass spectra of the isolated compounds were recorded by TOF MS ES Mass Spectroscopy using an electron impact process.

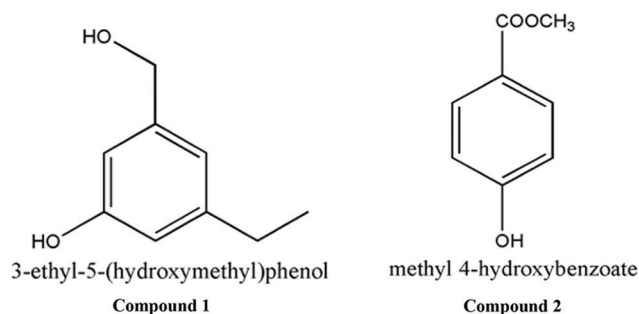


Fig. 3: Structure of the isolated compounds

RESULTS AND DISCUSSION

In the present study, the whole plant *A. imbricata* was collected (Fig. 1) and made into coarse powder after proper drying and extracted with different solvents such as petroleum ether, chloroform, ethyl acetate, and methanol. Regarding the percentage yield of dried extracts, the petroleum ether extract has given 4.5 g of dried extract. From the chloroform extract, 2.33 g of dried extract was obtained. In case of ethyl acetate and methanol extract, 3.16 and 10.1 g of dried extract were obtained, respectively.

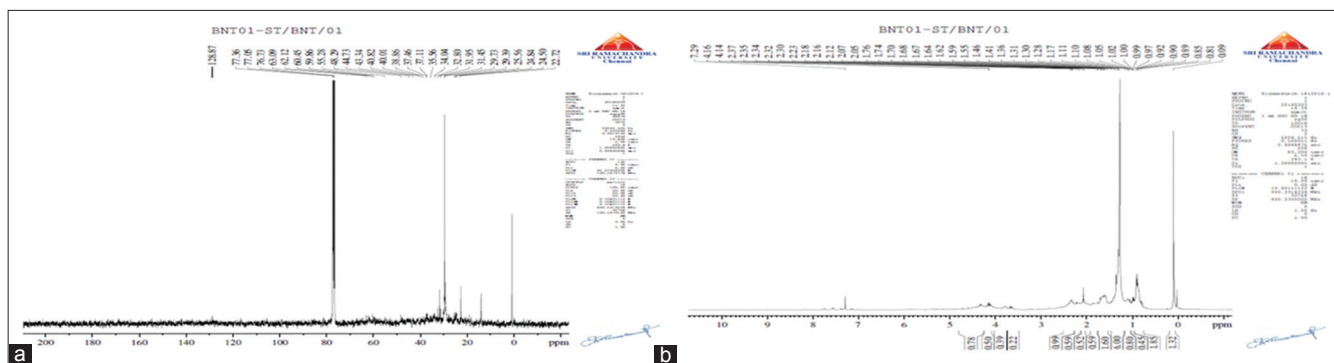


Fig. 4: Carbon-13 nuclear magnetic resonance (a) and proton nuclear magnetic resonance (b) reports of compound 1

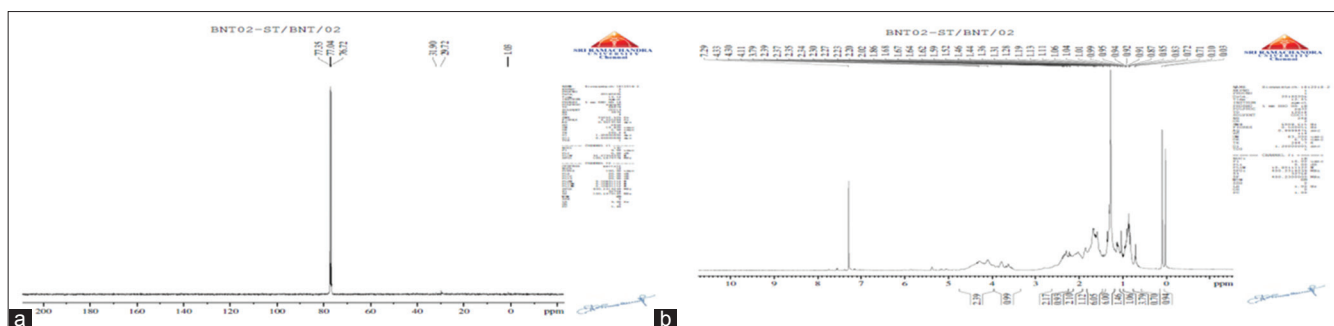


Fig. 5: Carbon-13 nuclear magnetic resonance (a) and proton nuclear magnetic resonance (b) reports of compound 2

Table 2: TLC study of isolated fractions

S. No.	Number of fractions	% of solvent	Volume of solvent used in column fractionation (ml)	TLC spot
1.	1–70	100% Hexane	500	
2.	71–110	95% Hexane: 5% Ethyl acetate	800	Compound 1
3.	110–180	90% Hexane: 10% Ethyl acetate	800	Compound 2
4.	181–240	85% Hexane: 15% Ethyl acetate	900	
5.	241–300	80% Hexane: 20% Ethyl acetate	500	
6.	301–370	75% Hexane: 25% Ethyl acetate	800	Compound 3
7.	371–430	70% Hexane: 30% Ethyl acetate	900	Compound 4
8.	431–600	50% Hexane: 50% Ethyl acetate	500	
9.	601–650	100% Ethyl acetate	200	

TLC: Thin-layer chromatography

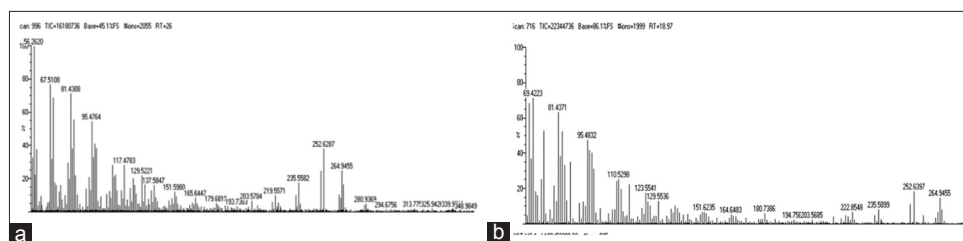


Fig. 6: Mass spectra of compound 1 (a) and compound 2 (b)

In the preliminary phytochemical evaluation, the tests for alkaloids revealed its presence in all the four extracts. The results indicated its significant presence in the chloroform extract comparing with other petroleum ether, ethyl acetate, and methanol extracts. In case of glycosides, it was present in all extracts except chloroform extract. The results indicated the significant presence of glycosides in both ethyl acetate and methanol extracts. In this category, Borntrager's test gave negative, but the modified Borntrager's gave the positive result, which indicated the presence of C-glycosyl compound in the extract. The phenolic compounds and tannins, flavanones and flavonoids, carbohydrates, terpenoids, and significant quantity of sterols were present in the ethyl acetate, and methanol extracts only. Saponins were present in the methanol extracts only. Proteins and amino acids, gum and mucilage, and volatile oil content were not found in the preliminary phytochemical evaluation (Table 1).

In column chromatography, 650 fractions were collected and the TLC was done for each collected fraction. Four pure compounds were isolated with the help of TLC (Table 2 and Fig. 2).

Two out of 4 compounds were selected for spectral evaluation based on the yield obtained. The two selected compounds were subjected to ^{13}C NMR, ^1H NMR, and Mass spectroscopic evaluation. From the evaluation, compound 1 was identified as 3-ethyl-5-(hydroxymethyl) phenol and compound 2 was identified as methyl 4-hydroxybenzoate (Fig. 3).

The ^{13}C NMR of (3-ethyl-5-(hydroxymethyl) phenol showed 24.50(methyl), 32.80–37.46(CH₂), 60.45–77.36, 100.45(C-OH), 128.87(aryl ring) and its ^1H NMR data showed 0.99–2.37(CH₃ and CH₂), 4.16(OH), 7.29(aryl proton). In the case of methyl 4-hydroxybenzoate, the ^{13}C NMR showed 31.9(CH₃), 145(double bonds), 162.5(ester), and ^1H NMR showed 0.9–2.39(methyl and CH₂), 3.79–5.23(OH), 7.2(aromatic protons). ^{13}C NMR and ^1H NMR reports of compounds 1 and 2 are shown in Figs. 4 and 5. The mass spectral reports of compounds 1 and 2 are shown in Fig. 6.

Previous reports by Haseebur *et al.* [2], Pushpa *et al.* [6], Kumar *et al.* [8], Jamuna *et al.* [9], Rahman *et al.* [10], and Gayathri *et al.* [11], Maruthamuthu *et al.* [12], and Harun *et al.* [13] declared the presence of various phytochemicals and these reports revealed diverse biological activities significantly. Obviously, it was able to identify the presence of different phytochemicals and, importantly, able to isolate and characterize two compounds in the present study. Of course, the outcome of this study would provide a base for further research, which may give more valuable results.

CONCLUSION

It was reported that various species of the genus "Argyrea" possess medicinal value. With this view, the plant *A. imbricata* was selected for the candidate for our research. Initially, preliminary phytochemical evaluation and characterization of chemical constituents were done successfully. Our further studies directed toward the detailed pharmacological screening of the extracts of the whole plant *A. imbricata* may give significant results.

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AUTHORS' CONTRIBUTIONS

V. Sebastin: Carried out the whole experiment with the assistance of Mr. K. I. Anoo Kumar, Dr. G. Gopalakrishnan: Design the whole research work, and Dr. M. Sreejith: Carried out the supervision of experiments.

CONFLICTS OF INTEREST

None.

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