



Preliminary Qualitative and Quantitative Phytochemical Profiling of *Aristolochia Tagala* Cham. A Rare Medicinal Plant

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Abstract: *Aristolochia tagala* Cham. is a rare medicinal plant found in certain tropical regions of the world. During the present investigation, a detailed preliminary qualitative and quantitative analysis were carried out to know the phytochemical profile of the leaves of *A. tagala*. The leaf powder was extracted with three solvents having different polarities and subjected for analysis. The extracts showed the presence of carbohydrates, proteins, alkaloids, phenolics, flavonoids, tannins, terpenoids, steroids, glycosides and volatile oils whereas saponins, non-volatile oils, gums and mucilage were completely absent. In quantification experiments, chloroform leaf extract showed high concentrations of phenolics, flavonoids and flavonols while methanolic leaf extract showed abundant alkaloids. This preliminary phytochemical profile can be used as a data source for the isolation of phenolic and alkaloid compounds. The isolated compounds can be used for their specific biological activity and based on their curative properties they may be used for the formulation of drugs.

Keywords: Preliminary phytochemical profile, alkaloids, phenolic, *Aristolochia. tagala* leaf extract

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I. INTRODUCTION

The genus *Aristolochia* consists of around 800 species of which some are found in temperate and others in tropical regions of the world. Many members of this genus are used in the traditional medicines all over the world as an analgesic, anti-inflammatory, antitussive, to cure obesity and even tumours. *Aristolochia* members are known to contain many active alkaloid and flavonoid components however the quantity varies in different species.¹ *Aristolochia tagala* Cham. is one such highly valued rare medicinal plant belonging to this genus found to occur in the tropical regions of Asia and Australia. The roots and leaves of this plant are used by tribes of India and Bangladesh to treat snake bite, tumour, bone fracture, malaria, to cure diarrhoea, vomiting indigestion and rheumatism.²⁻⁷ Kani tribes of Thirunelveli hills use the leaves of this plant to prepare medicated oil to cure venereal diseases and similar tribes of Agasthiaya malai biosphere reserve consume decoction made from flower to regulate menstrual disorder.^{8,9} Kurichyas tribal community of Kannur district of Kerala employ grounded paste of fresh leaves to reduce stomach ache.¹⁰ In Indonesia, the leaves are used to treat bilious disorders, in Malaysia fever is treated with the pounded leaves of this plant and in Philippines root powder is used as an emmenagogue.¹¹ Recent studies have shown that the leaf extracts are known to have anticancer, antimicrobial, antioxidant, and analgesic activities.¹²⁻¹⁹ These scientific studies tally with the above mentioned ethnomedicinal uses of the plant. Such immense medicinal properties are attributed to the phytochemicals present in them. Hence, in the present study, a detailed preliminary qualitative and quantitative tests were conducted to analyze the phytochemical profile of the leaves of *A. tagala*.

2. MATERIALS AND METHODS

2.1 Collection of *Aristolochia tagala*

Field survey was undertaken in the month of July at the Bisle ghat forest area (12°71'N, 75°69'E, at 803 m altitude,) of Sakleshpur taluk (Hassan district, Karnataka, India) to collect *A. tagala* for the present study. A herbarium was prepared from the collection and submitted to the BSI-Pune for authentication (Accession No. 136269). Healthy leaves were segregated, washed, dried (at room temperature) and powdered.

2.2 Extraction

The leaf powder was extracted with three solvents of different polarities separately. The solvents used were methanol, chloroform and petroleum ether. The leaf powder was treated with solvent at the ratio 1:5 in a tightly closed conical flask at 50°C in hot water bath for four hours after which the extract was filtered (with Whatman No. 1 filter paper) and collected for further evaporation of the solvent (at 50°C in Hot air oven). The crude extracts in the form of paste were collected and stored in eppendorf tubes (sealed with paraffin tape and stored at 4°C in the refrigerator) until further use.

2.3. Phytochemical Profiling

All three solvent leaf extracts viz., methanolic (AT-M), chloroform (AT-C) and petroleum ether leaf extract (AT-PE)

were subjected to the following preliminary qualitative and quantitative tests to obtain a phytochemical profile of the leaves.

2.3.1 Preliminary Qualitative Tests

All the three extracts were tested for the presence of carbohydrates, proteins, alkaloids, phenolics, flavonoids, tannins, terpenoids, steroids, glycosides, fixed & volatile oils, gums and mucilage. These preliminary qualitative tests were conducted according to the standard methods as mentioned by Raman (2006), Khandelwal (2008) and Kumar (2016).^{20-22,14}

2.3.2. Preliminary Quantitative Tests

2.3.2.1. Determination of Total Alkaloids

In present study for the determination of total alkaloids, the experimental procedure as mentioned by Ajanal *et al* (2012) with suitable modifications were followed.²³ Stock solutions of the extracts were prepared by vortexing 10 mg of extract in 1 ml of 2 N HCl for about 10 minutes. From these stocks 1 mg / ml sample solutions of all the three extracts were prepared. The samples were neutralized to pH 7 using 0.1 N NaOH. To 1 ml of such neutralized sample 5 ml of 0.1mM bromocresol green solution and 5 ml of phosphate buffer (4.7 pH) were added and mixed well. This mixture was shaken vigorously with 1, 2, 3 and 4 ml chloroform (in consecutive steps). The lower layer was collected and absorbance was read at 470 nm using a spectrophotometer. Simultaneously the same experiment was conducted on various concentrations of Atropine solution to build a standard curve. Total alkaloids present in the extracts were calculated based on atropine standard curve and expressed as mg of Atropine Equivalents per gram weight of extract (mg AE / g)

2.3.2.2. Determination of Total Phenolics

For the current research work, the procedure for the estimation of total phenolics and total flavonols were followed as indicated by Iqbal *et al* (2015) but with slight modifications.²⁴ Using the crude extracts, sample solutions having concentration of 1 mg /ml were prepared using methanol. 200 l of sample was mixed well with 200 l of 15 fold diluted FCP reagent and incubated at room temperature in dark for 5 minutes. Later 200 l of 7.5 % sodium carbonate solution was added and made upto 2 ml with distilled water. The mixture was vortex well and absorbance was read at 765 nm. Parallely the same reaction was carried out with different concentrations of gallic acid solutions to construct a standard curve. Total phenolic contents present in the extracts were calculated based on gallic acid standard curve and expressed as mg of Gallic Acid Equivalents per gram weight of extract (mg GAE / g)

2.3.2.3. Determination of Total Flavonols

1 ml of sample solution having a concentration of 100 g / ml (in methanol) were mixed well with 4 ml of distilled water, 500 l of 1 % aluminium chloride and 500 l of 5 % sodium acetate solutions. The mixture was incubated for 30 minutes at room temperature and later absorbance was noted at 440 nm. Similar tests were conducted on various concentrations of quercetin and standard curves were built.

Total flavonoids present in the extracts were evaluated based on quercetin standard curve and expressed as mg of Quercetin Equivalents per gram weight of extract (mg QE/g)

2.3.2.4. Determination of Total Flavonoids

For this experiment the protocol as cited by Baba & Malik (2015) was followed with certain changes.²⁵ To 1 ml of sample solution having a concentration of 100 g / ml (in methanol), 4 ml of distilled water, 300 l of 5 % NaOH and 300 l of 10 % AlCl₃ solutions were added, mixed well and incubated for 10 minutes at room temperature. Later, 2 ml of 1 M NaOH and 2.4 ml of distilled water were added and further incubated for another 15 minutes at room temperature. Finally the contents were mixed well and absorbance was read at 510 nm. Parallel experiment was carried out with various concentrations of Quercetin for comparison and calculations. Total flavonoids were expressed as mg QE / g.

2.3.2.5. Determination of Total Tannins

The experimental procedure was carried out as mentioned by Muthukumaran et al (2016) with slight modifications.²⁶ 1 ml of sample with a concentration of 100 g / ml (in respective solvents) was mixed well with 4 ml of distilled water, 500 l of 8mM potassium ferricyanide and 500 l of 0.1 M ferric chloride solution in 1 N HCl. To this mixture, another 14 ml of distilled water was added and mixed well. Immediately the absorbance was read at 750 nm. The standard curve was built by conducting the same experiment on various concentrations of tannic acid. Total tannins in the extracts were estimated based on the tannic acid standard curve and expressed as mg of Tannic Acid Equivalent/g weight of sample (mg TO / g).

3. STATISTICAL ANALYSIS

All the tests were conducted in triplicates. For qualitative experiments, construction of the standard curve and all other calculations were carried out on MS-Excel spread sheet, values were presented as Mean \pm SE.

4. RESULTS AND DISCUSSION

In the present study preliminary qualitative and quantitative phytochemical analysis were done to obtain a phytochemical profile of the leaves of *Aristolochia tagala*. The results of qualitative phytochemical tests (Table 1) revealed that AT-M and AT-C contained moderate amounts of carbohydrates, phenolics and flavonoids and traces of proteins and tannins. AT-M exhibited moderate results in the tests for the presence of alkaloids while AT-C and AT-PE had traces of terpenoids. All the extracts showed high quantities of glycosides and moderate amounts of steroids. Saponins, fixed oils, gums and mucilage are found to be absent in all the tested extracts. The current experiments on the quantification of phytochemicals present in the different solvent extracts of leaves of *A. tagala* show that methanolic extract has incredibly high quantity of alkaloids (752.136 mg AE / g), moderate amount of total phenolics, total flavonoids and total flavonols (Table 2, Figure 1 & 2). However chloroform leaf extract showed a high quantity of total phenolics, flavonoids and flavonols (87.928 mg GAE / g, 28.909 mg QE / g and 8.368 mg QE / g respectively). Tannins were found to be present in trace amounts (2.559 mg TAE / g) only in methanolic leaf extract.

Table 1: Preliminary qualitative phytochemical screening of *A. tagala* leaf extracts

Tests Conducted	Samples		
	AT-M	AT-C	AT-PE
Tests for Carbohydrates			
Molisch Test	+	+	+
Benedict's Test	+	++	-
Fehling's Test	++	++	+
Test for Proteins			
Ninhydrin Test	-	-	-
Biuret test	+	+	-
Precipitation test	+	+	-
Test for Glycosides			
Kellerkillane's test	++	+	+
Modified Borntrager's test	+++	+++	+++
NaOH test	+++	++	++
Test for saponins			
Foam test	-	-	-
Test for alkaloids			
Mayer's test	++	-	+
Hager's test	++	+	++
Dragendorff's test	++	+	-
Test for Phenolics			
FeCl ₃ test	++	+++	+
Libermann's test	++	+	+
Bromine Water test	+	++	+
Test for flavonoid			
Lead acetate test	++	+	+

Alkaline reagent test	++	++	+
Ammonium test	++	+	+
Test for terpenoids			
Salkowski's test	-	+	+
Test for tannins			
Gelatin test	+	+	-
Lead acetate test	+	+	-
Test for steroids			
Liebermann Burchard's test	++	+	++
Test for oils			
Test for non-volatile oils		-	
Test for volatile oils	+	++	+
Test for gums & mucilage			
Absolute alcohol test	-	-	-
KOH test		-	

AT-M = methanolic leaf extract, AT-C = chloroform leaf extract, AT-PE = petroleum ether leaf extract,
+++ = high, ++ = moderate, + = traces, - = absent

Table 2 :Preliminary quantitative phytochemical screening of *A. tagala* leaf extracts

Sample	Total Phenolics as GAE mg/g (Mean \pm SE)	Total Flavonoids as QE mg/g (Mean \pm SE)	Total Flavonols as QE mg/g (Mean \pm SE)	Total Tannins as TAE mg/g (Mean \pm SE)	Total Alkaloids as AE mg/g (Mean \pm SE)
AT-M	10.07142857 10.230 \pm 0.158	4.363 \pm 0.349	6.719 \pm 0.015	2.559 \pm 0.008	752.136 \pm 2.992
AT-C	87.928 \pm 0.238	28.909 \pm 0.174	8.368 \pm 0.015	NA	5.337 \pm 0.036
AT-PE	NA	5.474 \pm 0.101	5.478 \pm 0.015	NA	4.286 \pm 0.045

Values are expressed as Mean \pm SE (n=3); AT-M = methanolic leaf extract, AT-C = chloroform leaf extract, AT-PE = petroleum ether leaf extract, GAE = Gallic Acid Equivalent, QE = Quercetin Equivalent, TAE = Tannic Acid Equivalent, AE = Atropine Equivalent

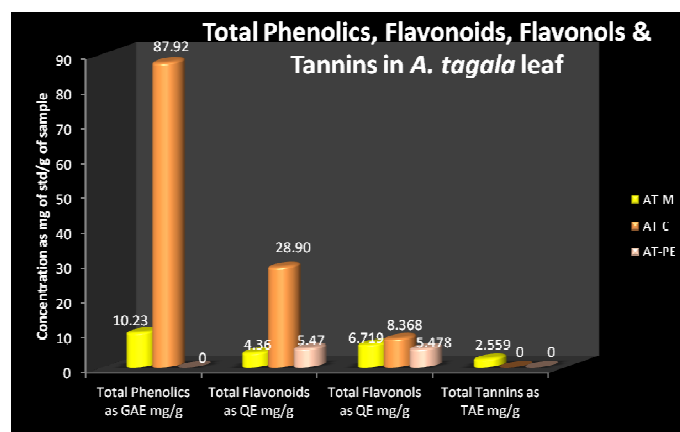


Fig 1: Comparative account of total phenolics, flavonoids, flavonols and tannins in three different solvent extracts of *A. tagala* leaves

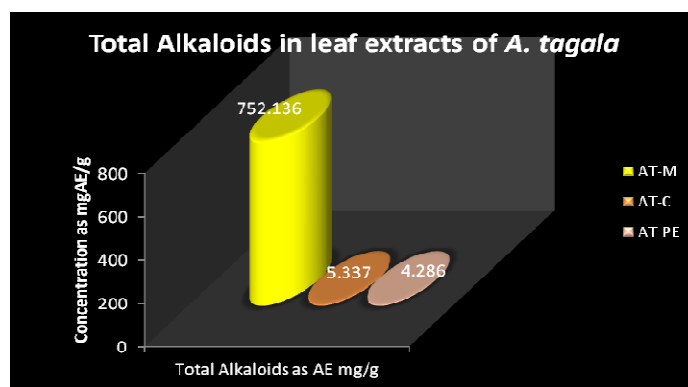


Fig 2:Comparative account of total alkaloids in three different solvent extracts of *A. tagala* leaves

Kalaierasi et al (2014) have tested for the presence of various phytochemicals in the whole plant powder of *A. tagala* by extracting it with six different solvents at room temperature for 72 hours. In those extracts they were able to identify only phenolic groups in acetone, ethanol and aqueous extracts, flavonoids, in ethanol and aqueous extracts, and steroids in chloroform, acetone and ethanol extracts. They have shown the complete absence of alkaloids in all these extracts.¹⁶ Contrastingly in the present study high concentrations of alkaloids were found to be present in the methanol extract. These differences may be due to the method of extraction followed. As principle mentioned for hot maceration in the current experiment, the heat of 50°C provided for short duration of 4 hours during extraction process might have helped in the softening and breaking of the cell walls to release the soluble phytochemicals without affecting the integrity of the heat-labile compounds.¹³ The phenolic compounds are the compounds that have an aromatic ring with a hydroxyl (-OH) group. Vermerris and Nicholson (2007) have classified the plant phenolic compounds into 20 groups based on the number of carbons in the molecule. They include compounds like coumarins, flavonoids, flavonols, tannins etc.²⁸ The flavonoids can be further divided into six main categories - flavanols, flavanones, flavonols, isoflavones, flavones and anthocyanins.²⁹ The polarity of different phenolic substances vary widely and their solubility in different solvents also depend on the interaction with other phytochemicals which may lead to the formation of complexes with different polarity.³⁰ Hence in the present study it was observed that the high quantity of phenolics were present in chloroform - a mid polar solvent and tannins which are highly polar in nature, were present only in trace amount in the methanolic leaf extract and completely absent in the mid polar (chloroform) and non-polar solvent (petroleum ether) leaf extracts. The current research work also shows that *A. tagala* leaves have large quantities of phenolic compounds of intermediate polarity which were obtained by extracting with chloroform solvent and a small amount of phenolics with comparatively high polarity. Among the different groups of phenolic compounds, phenolic acids, flavonoids and tannins are regarded as the chief dietary phenolic compounds. Phenolic compounds in general are known to possess antioxidant activity by which it decreases lipid oxidation and oxidative stress, thereby reducing the risk of developing certain diseases like arteriosclerosis, diabetes, cataract, cardiological diseases and even cancer. Phenolic compounds also have anti-inflammatory property.³¹ Alkaloids are nitrogen containing organic compounds. They can be extracted with highly polar solvents or solvents with medium polarity which is reflected in the present study.³² Alkaloids occur naturally in a wide range of organisms, like marine sponges, arthropods, amphibians, birds, bacteria, fungi and plants. In plants they are part of defence mechanisms against pathogens, herbivores etc. They are known for performing a wide range of biological activities such as neurotransmitters, growth stimulators, inhibitors and even destroyers. Many of the alkaloids are used as antirheumatic, anti inflammatory, anti malarial, and antitumour drugs.^{33, 34} Hadem and Sen (2020) have identified Aristolochic acid I - an alkaloid as the most active compound present in the methanolic root extracts of *A. tagala* which is responsible for the anticancerous efficacy.¹³ In our previous study we have shown that methanolic leaf extracts of the *A. tagala* can efficiently induce apoptosis in HepG2 cell line.¹² In the present study it was found that

methanolic leaf extract has high quantity of alkaloids and hence tally with above mentioned literature. Phenolic compounds found in *A. longa* are known to have antimicrobial activity.³⁵ Polyphenolic compounds of *Aristolochia* species are known to inhibit action of phospholipase A2 enzyme a major constituent of venoms of snakes and scorpions and thereby reduce the inflammation caused by the venom.³⁶ Aristolochic acid an alkaloid present in most of the *Aristolochia* members is known to inhibit hyaluronidase enzyme action of the snake venom.³⁷ Alkaloids like aristolactam, aristolochic acids, aristolochene, cepharanones and magnoflorine from various *Aristolochia* species are known to possess antitussive, analgesic, anti-inflammatory, anti-malarial, anti-rheumatic, diuretic and anti-cancer properties.^{38, 39} Present study on *A. tagala* in relation with all the above mentioned studies shows that methanolic leaf extract is a rich resource of alkaloids and chloroform leaf extracts have high quantities of phenolic compounds which may have similar effects as that of other *Aristolochia* species. Presence of these phytochemicals in the plant also partially validates its ethnobotanical usage to cure various ailments including cancer.

5. CONCLUSION

It is known that roots of *Aristolochia tagala* has high medicinal properties. But uprooting the plant to collect the roots can affect the plant's growth and even kill the plant. Therefore an alternative source is the leaves which are abundantly available. Hence the current work aimed at obtaining the phytochemical profile of the leaves. The present preliminary qualitative and quantitative phytochemical analysis has resulted in obtaining a brief phytochemical profile of *Aristolochia tagala* leaves, which shows the presence of very large quantities of alkaloids, moderately high quantities of phenolic compounds including flavonoids and flavonols. The current study also revealed suitable solvent needed for the extraction of above compounds at low cost and less time consuming methods. These results can serve as a ground work for the further analysis and isolation of bioactive compounds from the leaves of *A. tagala*. The isolated compounds can be used for their specific biological activity and based on their curative properties they may be used for the formulation of drugs.

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7. AUTHORS CONTRIBUTION STATEMENT

Prof. L. Rajanna planned the objectives and also supervised the entire research work. Ms. G. S. Shailaja Sharma collected the literature data and conducted the experiment. Both the authors discussed the results and contributed equally to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none

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