SCREENING CHEMICAL COMPOSITION OF THE LEAF AND BARK EXTRACTS OF THE PLANTS ARTABOTRYS ZEYLANICUS AND ARTABOTRYS SAHYADRICUS FROM SOUTHERN WESTERN GHATS.

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ABSTRACT

Nowadays, we are looking for a new therapeutically potential drug to heal and resist various infectious diseases without any consequences. The leaves and stem bark part of *Artabotrys zeylanicus* and *Artabotrys sahyadricus* were extracted with aqueous and different solvents like petroleum ether, chloroform , ethyl acetate and ethanol. All extracts are subjected to phytochemical screening and followed by quantitative tests. Phytoconstituents like alkaloids, flavonoids, phenols, tannins, glycosides, steroids, terpanoids and cardiac glycosides were demonstrated in preliminary phytochemical screening. Alkaloids, flavonoids, steroids, terpanoids and cardiac glycosides were highly indicate. Steroids , terpanoids and cardiac glycosides were indicate highly in Stem bark part. *A. zeylanicus* permormed better activity than *Artabotrys sahyadricus* in qualitative and quantitative analysis. Results subjected that both plant extracts can give out a remarkable reference for future studies in biological activities.

Keywords: Qualitative and quantitative analysis, Phytoconstituents, *Artabotrys zeylanicus* and *Artabotrys sahyadricus, Annonaceae.*

1. INTRODUCTION

One of the largest genus *Artabotrys* belong about 100 species, all members are Climbing shrubs or woody lianas in custard apple family annonaceae (Li and Michael., 2011). *Artabotrys* genus mainly distributed in tropical and subtropical regions of the world (Kok and Christophe,2014). Remarkable medicinal pottential reported in numerous *Artabotrys* genus, Phytoconstituents like alkaloids, Flavonoids, Phenols, Tannins, Glycosides, Steroids, Terpanoids and Cardiac glycosides were already reported in *A. crassifolius (*Kok *et al.*, 2013*), A. hexapetalus (*Florence et al., 2014 ; Jamnian et al., 2012 and Tong *et al.*, 1997*), A. uncinatus (*Tian *et*

al., 2001), *A. oliganthus* (Zana *et al.,* 2016), *A. velutinus(* Mahudro *et al.,* 2016)and *A. brachypetalus* (Anne *et al.,* 2003). All records reflect the great potential of different plant parts of *Artabotrys* species hence all these plants can be used to find out new drugs for various ailments. In the present study, we report on the chemical composition of the leaf and stem bark of *A. sahyadricus* (Prabhukumar *et al.,* 2017) *and A. zeylanicus* (Jose *et al.,* 2013)through qualitative and quantitative phytochemical analysis.

2. MATERIALS AND METHODS

Plant Identification

Leaf and Stem part of *Artabotrys zeylanicus* Hook.f. & Thomson were collected from Muthikulam, Palakkadu district, Kerala, India and *Artabotrys sahyadricus* Robi, KMP Kumar & Hareesh were collected from Kuttampuzha Forest Range, Ernakulam district, India. They were identified and authenticated by Dr. Prabhukumar KM, Senior Scientist & Head, Plant Systematics & Genetic Resources Division & 'CMPR' Herbarium, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Malappuram - 676 503, Kerala, India (*A. zeylanicus* 8680 and *A. sahyadricus* 8693) and voucher specimen has been deposited in 'CMPR' Herbarium, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Malappuram - 676 503, Kerala, India.

Preparation of Plant Extract

The fresh leaf and stem bark parts of *A. zeylanicus and A.sahyadricus* were washed with tap water and shade dried for two month and powdered coarsely. Then they were finely powdered mechanically using pulverizer and passed through 40 mesh sieve and stored in airtight containers. About 250g of powdered aerial and root were extracted in soxhlet apparatus with petroleum ether, chloroform, ethyl acetate and ethanol. The extract was dried under reduced pressure at low temperature (40-50°C). The last traces of the solvent were removed under vacuum drier and the solid mass obtained was stored at 4°C until further use.

Quanlitative Analysis.

The qualitative tests were done to find out the presence of the active phytochemical constituents in the defatted extracts (Harborne**,** 1984 and Wagner *et al.,* 1984)

- **Phytochemical Screening.**

Alkaloids (Mayer's test)

To the extract added 1% HCl and 6 drops of Mayer's reagent were added. An organic yellow precipitate indicated the presence of alkaloids in the sample.

Flavonoids (Lead acetate test)

The aqueous extract was treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Terpenoids (Salkowski test)

10mg of the extract was dissolved in 1ml of chloroform, 1ml of acetic anhydride was added following the addition of 2ml of conc. H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoides.

Cardiac glycosides (Keller-Killiani test)

0.5g of extract diluted to 5ml of water then added 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Phenols (Ferric chloride test**)**

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Sterols (Liberman-Burchard's test)

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Saponins (Froth Test)

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

Tannins (Lead acetate test)

In a test tube containing about 5ml of an aqueous extract a few drops of % solution of lead acetate was added. A yellow or red precipitate was formed indicating the presence of tannin.

Resins

To 2ml of chloroform extract 5-10ml of acetic anhydride was added, dissolved by gently heating coding and then 0.5ml of sulphuric acid was added. Bright purple colour was produced. It indicates the presence of resins.

Glycosides

A small amount of alcohol extract samples was dissolved in 1ml water and then aqueous sodium hydroxide solution was added. Formation of a yellow colour indicators the presence of glycosides.

Triterpenoids

10mg of the extract was dissolved in 1ml of chloroform, 1ml of acetic anhydride was added following the addition of 2ml of conc. H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoid.

Reducing sugar

The crude extract of each plant was shaken with 5ml of distilled water and filtered. The filtrate was boiled with drops Fehling's solution A & B for 2 minutes. An orange red precipitate indicates the presence of reducing sugar.

2.4 Quantitative Analysis.

- **Determination of Flavonoids** (Zhishen *et al.*, 1999).

Total flavonoid content was estimated by the aluminium chloride colorimetric assay. An aliquot (1ml) of extract and standard solution of Catechin (100mg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To this 0.3ml of 5% $NaNO_3$ was added. After 5 min, 0.3ml of 10% $AlCl_3$ was added. After 1 min, 2ml of 1M NaOH was added and the total volume was made up to 10ml with distilled water. The solution was mixed well and the absorbance was measured against reagent blank at 510nm. The value of optical density was used to calculate the total flavonoid content present in the sample. The mean of the three values were expressed as milligrams of Rutin equivalents (mg RE)/ g extract on a dry weight basis.

- **Determination of Total phenols** (Singleton *et al.*, 1999).

Total phenolics were quantified and expressed as Gallic acid equivalents. About 3.9ml of distilled water and 0.5ml of Folin-ciocalteau reagent were added to 0.1ml of extract in a tube and incubated at room temperature for 3min after which 2ml of 20 % sodium carbonate was added and kept in a boiling water bath for 1min. Phenols react with phosphomolybdic acid in the Folin-ciocalteau reagent in alkaline medium and produce a blue coloured complex (molybdenum blue) that can be estimated colorimetrically at 650nm. The total phenol content of the extract was calculated and expressed as Gallic acid equivalent (GAE) mg/ g extract.

- **Determination of Alkaloids** (Harborne, 1973).

5g of the sample was weighed in a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. This solution was allowed to settle and the precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried, weighed and expressed as g/gm extract.

- **Determination of total terpenoids** (Ferguson, 1956).

About 2g of plant powder was weighed and soaked in 50ml 95% ethanol for 24 hrs. The extract was filtered and the filtrate extracted with petroleum ether (60 to 80^0C) and concentrated to dryness. The dried ether extract was treated as total terpenoids.

3. RESULTS AND DISCUSSION

In the present study the qualitative and quantitative analysis of *A. zeylanicus* and *A. sahyadricus* leaves and stem bark extracts were carried out for dried plant samples. Chemical test were carried out in aqueous and different solvents like petroleum ether, chloroform , ethyl acetate and ethanol. Preliminary screening for phytochemicals in the plant extracts revealed presence of various secondary metabolites in both *A. zeylanicus* and *A. sahyadricus* such as alkaloids, flavonoids, phenols, tannins, glycosides, steroids, terpanoids and cardiac glycosides. Alkaloids and flavonoids were highly present in both plant leaves and stem bark extracts tested (Table 1 and 2). Ethyl acetate extract of leaves and stem bark of *A. zeylanicus* and *A. sahyadricus* had the most phytochemicals. Steroids , terpenoids, phenols and cardiac glycosides were present in almost leaves and stem bark part of plants tested, but were most abundant in stem part of both plants and flavonoides were highly present in leaves extract than stem bark extracts. *A. zeylanicus* permormed better activity than *A. sahyadricus* in qualitative and quantitative analysis (Table 3 and 4).

From previous studies according to *A.hexapetalus l*eaf extracts of contain alkaloids, flavonoids, glycosides, phenols, steroids and terpenoides (Florence *et al.,* 2014; Savithramma *et al., 2011*). Hydroalcoholic leaves extract of *A. hexapetalus* also resulted the presence of alkaloids, flavonoids, steroids and terpenoides (Karthik *et al.,* 2012). These reports are stoutly supported the present study. All resulted Phytochemical constituents are responsible for the medicinal properties of the medicinal plants which produce a definite and specific action on the human body.

4. CONCLUSION

The study was performed to reveal the phytochemical constituents from the plants . *A. zeylanicus* and *A. sahyadricus*. The results obtained from the qualitative and quantitative phytochemical studies can be used as a investigative tool for future biological studies.

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TABLE

Table 1: Preliminary phytochemical analysis in different solvent extract of *A. zeylanicus.*

Secondary Metabolites	Petroleum ether		Chloroform		Ethyl acetate		Ethanol		Aqueous	
	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem
Alkaloids	+++	+++	+++	+++	+++	+++	+	+	+	+
Flavonoids	+++	++	+	+	+++	++	+	+	+	-
Phenols	+	+	+	+	++	+++	-	+	-	-
Tannins	+	-	+++	-	++	-	-	-	-	-
Glycosides	+	+	+	-	++	++	+	+	-	-
Saponins	-	-	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	-	-	-	-
Steroids	++	+++	+	+++	+++	+++	-	-	+	-
Terpanoids	++	+++	+	+++	+	+++	+	++	+	-
Triterpanoids	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	+	+++	+	+++	+++	+	+	-	-

Table 2: Preliminary phytochemical analysis in different solvent extract of *A. sahyadricus.*

Secondary Metabolites	Petroleum ether		Chloroform		Ethyl acetate		Ethanol		Aqueous	
	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem
Alkaloids	+++	+++	+++	+++	+++	+++	+	+	-	-
Flavonoids	+	+	++	+	+++	+++	+	+	-	+
Phenols	+++	+++	++	++	+++	+++	+	-	-	-
Tannins	+	+++	++	++	+++	+++	+	-	-	-
Glycosides	+	-	-	+	++	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-
Resins	-	+	-	+	-	++	-	+	-	+
Steroids	+	++	++	+	++	+	++	++	-	-
Terpanoids	-	+	-	+	+	++	+	+	-	+
Triterpanoids	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+++	+++	+++	++	+++	+++	+	+	-	+

Table 3: Quantitative determination of total flavonoid and total phenol content of leaf and stem parts of

Name of plants	Plant parts used	Total flavonoid gm of RE/gm extract	Total phenol (gm of GAE/gm extract)
A. zeylanicus.	Leaf	63.43±0.459	1.21±0.14
	Stem	54.14±0.069	3.65±0.03
A sahyadricus.	Leaf	54.63±0.105	0.15±0.00
	Stem	36.23±0.121	0.41±0.01

RE-Rutin; Values are presented as the mean ± standard deviation. GAE-Gallic acid Values are presented as the mean ± standard deviation. Values are performed in triplicates and represented as mean ± SD (standard deviation). Mean values followed by different superscripts in a column are significantly different (p<0.05).

Table 4: Quantitative determination of total flavonoid and total phenol content of leaf and stem parts of

Phytochemical	*A. zeylanicus.*		*A. sahyadricus.*	
	Leaf	Stem	Leaf	Stem
Total Alkaloids (mg/g extract)	0.235	0.2396	0.3795	0.281
Total Terpanoids(mg/g extract)	0.1368	0.1706	0.1112	0.1424