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Estimation Of Secondary Metabolites From The Root Bark Extracts Of *Artocarpus Heterophyllus* Lams.

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ABSTRACT

Artocarpus heterophyllus Lam. is an evergreen tree that widely grows in tropical regions. Each part of the tree is useful. The plant is also used in traditional medicines. The current study is concerned with the estimation of total phenolic and flavonoid contents in the root bark extract of Artocarpus heterophyllus Lam. The estimation of total phenolic and flavonoid contents were carried out on chloroform and ethyl acetate fractions. Total phenolic content was estimated by Folin Cio-calteau method and the total flavonoid content was estimated using Aluminium Chloride Colorimetric method.The study showed the chloroform fraction had more phenolic (27.27µg/mL) and flavonoid (651 µg/mL)content than ethyl acetate fraction.

Keywords: Artocarpus heterophyllus, Folin Cio-calteau, phenolic and flavonoids.

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INTRODUCTION

Green plants synthesise and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. In some cases, the crude extract of medicinal plants may be used as medicaments. The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance(1). A few of these genera viz. Morus, Ficus, and Artocarpus are economic sources of food and widely used in traditional medicine, agriculture and industry. These genera received a great level of scientific interest as they contain medicinally important secondary metabolites possessing useful biological activities(2).

Moraceae is large family comprising sixty genera and nearly 1400 species, including important group such as Artocarpus, Morus, and Ficus. Artocarpus heterophyllus or Jackfruit (family of Moraceae) is a monoecious evergreen tree that is grown in several tropical countries. A.heterophyllus is widely distributed in tropical region and has been used as traditional folk medicine against inflammation, malarial fever and so on. Moraceae plants including A. heterophyllus are rich sources of the isoprenylated phenolic compounds, including flavonoids (3). From the reviews, we found that, all the parts of the plant A.heterophyllus had medicinal importance. In the present study we selected the root bark of the plant A. heterophyllus belonging to the family Moraceae. The aim of our study is to establish the total phenolic and total flavonoid content in selected the root bark of the plant A.heterophyllus.

MATERIALS AND METHODS

Source of Material

Fresh roots of A.heterophyllus used for study was collected from the outskirts of Kaduthuruthy village, Kottayam district, Kerala during January 2017. The sample drug has been identified and authenticated by the botanist, Mr. Rogimon P Thomas, Assistant Professor, Department of botany, CMS College Kottayam. A voucher specimen is preserved at CMS College Kottayam.

Preparation of extract

The root barks of A. heterophyllus were peeled off, shade dried and powdered. The powdered drug was soaked in ethanol in a round bottom flask. After soaking it for overnight, it was refluxed with ethanol 95% for 3 hours and the solution was decanted off. The extraction was repeated thrice (till colour less extract is obtained). All the extracts were combined and concentrated to a semisolid consistency. Thus total ethanolic extract was obtained. The fractionation of the ethanolic extract was carried out using solvents in the increasing order of polarity i.e. petroleum ether, chloroform and ethyl acetate. Each fraction were concentrated, weighed and stored for further studies (4).

Estimation of total Phenolic content

Method: Folin Cio-calteau method

Preparation of reagents:

- **A.** Folin Cio-Calteau reagent (2 N): - It was diluted to 1:10 ratio with distilled water.
- **B.** Sodium carbonate solution: -7.5 % solution of sodium carbonate (anhydrous) was made with distilled water

Preparation of standard graph of Gallic acid:

Gallic acid (10 mg) was dissolved in a small quantity of methanol in a 10 mL volumetric flask and made up to the mark with the solvent. The above solution (1mL), containing 1mg/mL was pipetted out and made up to 10 mL with methanol to get 100 µg/mL gallic acid standard solution (stock solution). From the stock 0.1, 0.2, 0.4, 0.8 and 1.0 mL were pipetted out successively and treated with Folin Cio-calteau reagent (5 mL). 7.5% sodium carbonate (4mL) solution was added to it after 5 minutes. It was stirred and incubated at room temperature for 2 hours. After 2 hours, absorbance of the solutions was measured at 750 nm using UV-VISIBLE

spectrophotometer (Agilent, Cary 60). The absorbance values were plotted against different concentration and the standard graph was plotted (5)

Preparation of sample solution

Each extract (10 mg) was dissolved in small quantity of methanol and made upto 10 mL with solvent. 1mL was pipetted out from each extract solution and 5 mL of Folin Cio-calteau reagent was added. After 5 minutes, 4 mL of Sodium carbonate solution was added and incubated at room temperature for 2 hours. The absorbance was measured at 750 nm and the values obtained were interpreted from the standard graph of gallic acid to get the milligram equivalents of gallic acid.

Estimation of Total Flavonoid content

Method: Aluminium Chloride Colorimetric method

Preparation of standard

Quercetin (10 mg) was dissolved in small quantity of methanol in a 10 mL volumetric flask and made upto the mark with the solvent. The above solution (1mL), containing 1mg/mL was pipetted out and made up to 10 mL with methanol to get 100 µg/mL of quercetin standard solution (stock solution). From the stock solution, 0.1, 0.2, 0.4, 0.6, 0.8 and 1mL were pipetted out successively for the experiment. To each of these, 1.5 ml methanol, 0.1 mL aluminium chloride, 0.1 mL potassium acetate solution and 2.8 mL distilled water were added and mixed well. Then total volume was made up to 10 mL with distilled water. A blank was prepared without the addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured at 415 nm using UV-VIS spectrophotometer. A standard graph was plotted using concentration and absorbance (6) (7).

Stock Solution of Extracts:

10 mg of each extract was accurately weighed and made upto 1mL with DMSO

Preparation of sample solution

0.5 mL of each extract stock solution, 1.5 mL methanol, 0.1 mL aluminium chloride, 0.1 mL potassium acetate solution and 2.8 mL distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all extracts were prepared and their absorbance was measured at 415 nm. All prepared solutions were filtered through Whatmann filter paper before measuring.

RESULTS

The dried root bark of A. heterophyllus was extracted with ethanol and fractionated with different solvents of increasing polarity. The percentage yield of total ethanolic extract was 8.5% w/w. The percentage yield of petroleum ether, chloroform, ethyl acetate and remaining alcoholic extracts were 0.54%w/w, 47.81% w/w, 17.29% w/w and 34% w/w respectively. Preliminary phytochemical screening on total ethanolic extract showed the presence of alkaloids, phenolics, flavonoids. Estimation of total phenolic and flavonoid content indicated that A. heterophyllus is rich in phenolic and flavonoid contents. The total phenolic content of chloroform and ethyl acetate fractions were estimated by Folin cio-calteau method. The phenolic content of the extracts were expressed as gallic acid equivalents. Chloroform fraction (CE) showed higher amount of phenolic content (27.27µg/mL) than ethyl acetate fraction (EAE) (21.75 µg/mL). The total flavonoid content of chloroform and ethyl acetate fractions were estimated by aluminium chloride colorimetric method. The flavonoid content of the extracts were expressed as quercetin equivalents. Chloroform fraction showed higher amount of flavonoid content (651 µg/mL) than ethyl acetate fraction (418 µg/mL).

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Estimation of Total Phenolic content

The total phenolic content in the chloroform fraction and ethyl acetate fraction was found out by Folin cio-calteau method. The absorbance values obtained for different concentrations of standard gallic acid is tabulated in table no: 1and the standard graph is shown in fig: 1.

Table 1: Estimation of gallic acid

SL no:	Concentration of gallic acid (µg/mL)	Absorbance
1.	10	0.180 ± 0.0002
2.	20	0.271 ± 0.0003
3.	40	0.343 ± 0.0119
4.	80	0.680 ± 0.0001
5.	100	0.850 ± 0.0001

Values are means ± standard deviations of triplicate determinations.

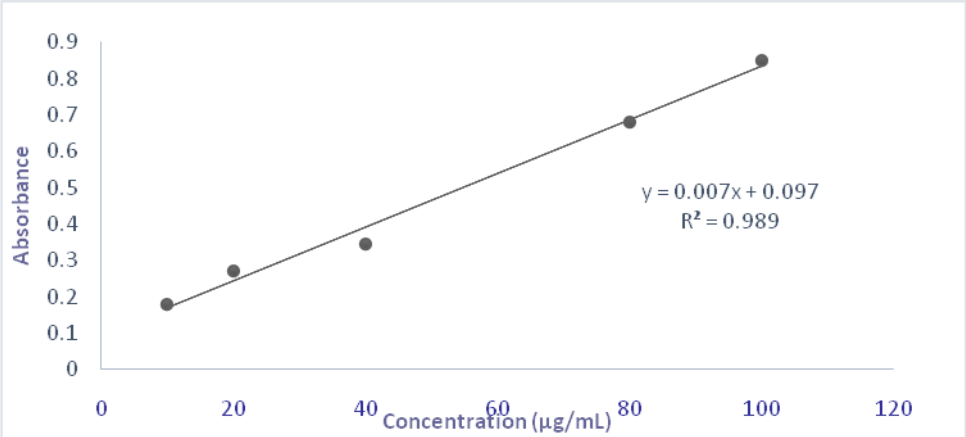

Fig 1: Standard graph of gallic acid

The absorbance values for both fractions are recorded and the total phenolic content in gallic acid equivalent is given in table: 2 and fig: 2.

Table 2: Estimation of total phenolic content of A. heterophyllus Lam.

SL no:	Extract	Absorbance	Gram equivalent of gallic acid per 100g
1.	CE	0.298 ± 0.0006	27.27
2.	EAE	0.258 ± 0.0008	21.75

Values are means ± standard deviations of triplicate determinations.

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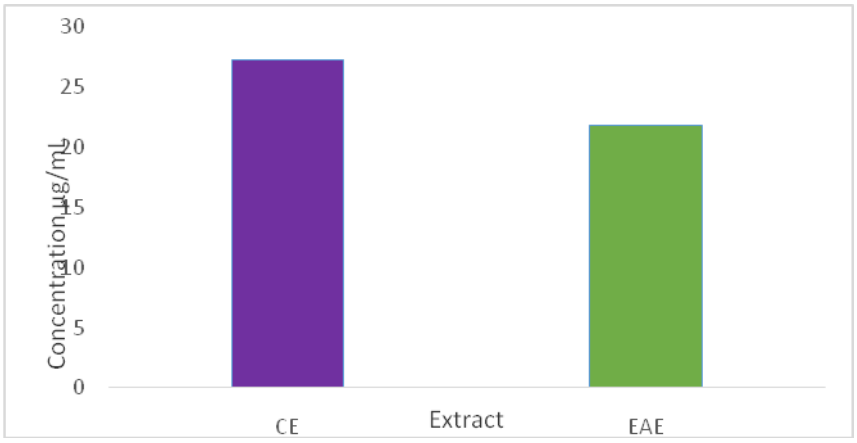

Fig 2: Evaluation of total phenolic content of CE and EAE fractions of A. heterophyllus Lam.

The result from the above table shows that the chloroform fraction (CE) has the highest (27.27) gram equivalence of gallic acid per 100g. Thus the total phenolic content was found high for the chloroform fraction (CE) compared to ethyl acetate fraction (21.75).

ESTIMATION OF TOTAL FLAVONOID CONTENT

Estimation of total flavonoids in both the fractions were carried out by using aluminium chloride colorimetric method. The absorbance values obtained for different concentrations of standard quercetin is tabulated in table:3 and the standard graph is given in fig: 3.

Table 3: Estimation of Quercetin

SL no:	Concentration of Quercetin (µg/mL)	Absorbance
1.	10	0.0274 ± 0.0001
2.	20	0.0301 ± 0.0001
3.	40	0.0716 ± 0.0003
4.	80	0.116 ± 0.001
5.	100	0.142 ± 0.001

Values are means ± standard deviations of triplicate determinations.

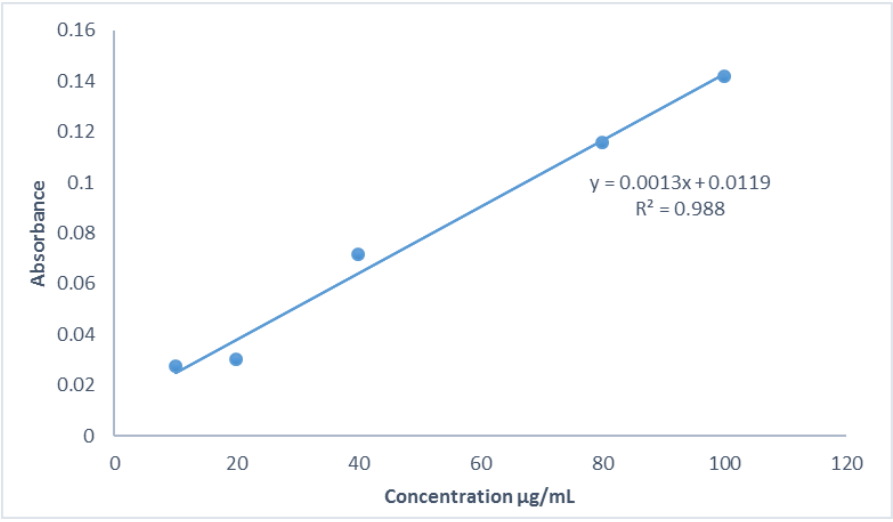

Fig 3: Standard graph of Quercetin

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The absorbance values for both fractions are recorded and the total flavonoid content as Quercetin equivalent per 100g is given in table: 4 and fig: 4.

Table 4: Estimation of total flavonoid content of A. heterophyllus Lam.

SL no:	Extract	Absorbance	Gram equivalent of quercetin per 100g
1.	CE	0.859 ± 0.0003	651
2.	EAE	0.556 ± 0.0005	418

Values are means ± standard deviations of triplicate determinations.

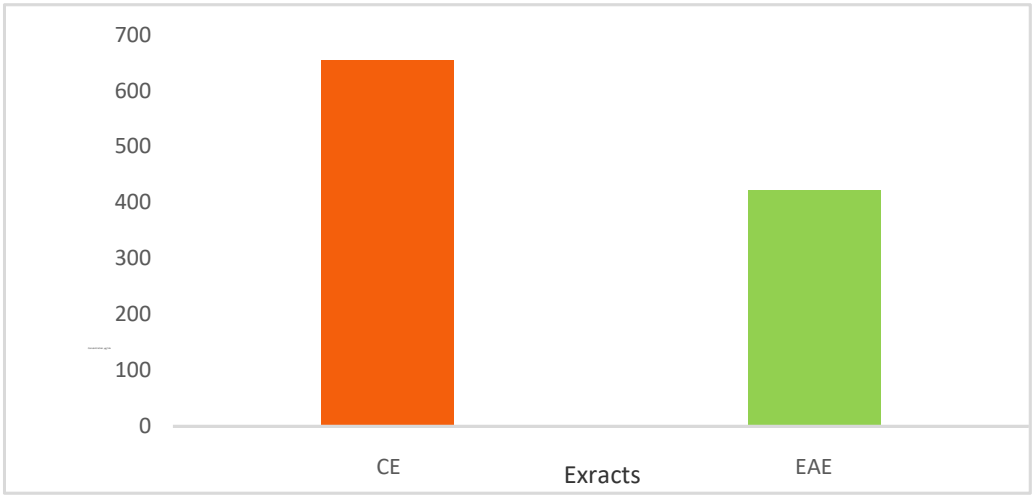

Fig 4: Evaluation of total flavonoid content of CE and EAE fractions of A. heterophyllus Lam.

The result from the above table shows that the chloroform fraction (CE) has the highest gram equivalent of Quercetin per 100g. Thus the total flavonoid content was found high in the chloroform fraction (CE) when compared to ethyl acetate fraction (EAE).

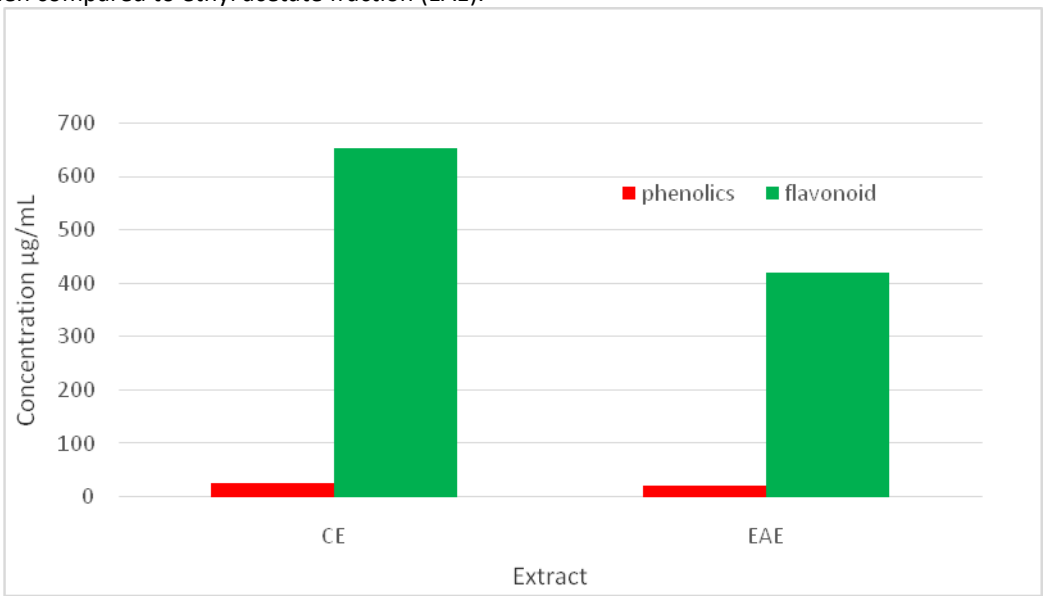

Fig 5: Comparison of total phenolic and flavonoid contents of CE and EAE fractions of A. Heterophyllus

From the estimation of total phenolic and total flavonoid content, it was found that the root bark of A. heterophyllus is rich in flavonoid and phenolic compounds. Among the extracts, chloroform extract has shown higher phenolic and flavonoid content than ethyl acetate extract.

DISCUSSION

The dried root bark of A. heterophyllus was extracted with ethanol and fractionated with different solvents of increasing polarity. The percentage yield of total ethanolic extract was 8.5% w/w. The percentage yield of petroleum ether, chloroform, ethyl acetate and remaining alcoholic extracts were 0.54% w/w, 47.81% w/w, 17.29% w/w and 34% w/w respectively. Preliminary phytochemical screening on total ethanolic extract showed the presence of alkaloids, phenolics, flavonoids, carbohydrates, steroids, terpenoids, proteins and amino acids. Estimation of total phenolic and flavonoid content indicated that A. heterophyllus is rich in phenolic and flavonoid contents. The total phenolic content of chloroform and ethyl acetate fractions were estimated by Folin cio-calteau method. The phenolic content of the extracts were expressed as gallic acid equivalents. Chloroform fraction showed higher amount of phenolic content (27.27 µg/mL) than ethyl acetate fraction (21.75 µg/mL). The total flavonoid content of chloroform and ethyl acetate fractions were estimated by aluminium chloride colorimetric method. The flavonoid content of the extracts were expressed as quercetin equivalents. Chloroform fraction showed higher amount of flavonoid content (651 µg/mL) than ethyl acetate fraction (418 µg/mL).

CONCLUSION

Dried root bark of A. heterophyllus Lam was selected for study. The present study was mainly focused on the extraction of dried root bark of A. heterophyllus Lam with ethanol. Then fractionating the ethanol extract with petroleum ether, chloroform and ethyl acetate. Preliminary phytochemical screening on total ethanolic extract showed the presence of phenolics, flavonoids. The phytochemical constituents phenolics and flavonoids of chloroform fraction and ethyl acetate fraction were quantitatively estimated. The chloroform fraction had more phenolic and flavonoid content than ethyl acetate fraction.

The secondary metabolites can be further isolated and the structure may be identified in the future.

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