A COMPARISON ON PHYTOCHEMICAL AND ANTIOXIDANT PROFILE OF ARTOCARPUS HETEROPHYLLUS SEEDS

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ABSTRACT
The present study was carried out with the objective to study the variation in phyto constituents and anti-oxidant property among the various extracts of Artocarpus heterophyllus seeds. Phytochemical screening revealed the presence of phenols, flavonoids, saponins, tannins, steroids and alkaloids. The antioxidant property was evaluated using the DPPH free radical scavenging assay using quercetin as the standard. The antioxidant activity of ethanolic extract was found to be the strongest, followed in descending order by dichloromethane: methanol and acetone extract. The IC\(_{50}\) values of the ethanol, dichloromethane: methanol and acetone extracts were found to be 320,470, 362 µg/ml respectively.

KEYWORDS: DPPH, antioxidant, phytochemical, flavonoid.

INTRODUCTION
Oxidative process is one of the most important routes for producing free radicals in living systems. The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Antioxidant compounds like polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide, lipid peroxyl and inhibit the oxidative mechanisms that lead to degenerative diseases.\(^1\)

Artocarpus heterophyllus belonging to the family moraceae is a monoecious evergreen tree grown in several tropical and subtropical regions. In the present investigation, the
phytochemical evaluation and antioxidant property of *Artocarpus heterophyllus* seed was evaluated.

**MATERIALS AND METHODS**

**Collection of plant material**
The fresh seeds of *Artocarpus heterophyllus* were collected from the local farms of Nagercoil, Kanyakumari district in the month of August. The seeds were authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, West Tambaram, Chennai-45.

**Preparation of the extract**
The freshly collected seeds of *Artocarpus heterophyllus* were chopped and shade dried at room temperature. The seeds were subjected to size reduction to a coarse powder by using electric blender. 100g of powdered seeds were extracted with ethanol, dichloromethane: methanol and acetone using soxhlet extractor. The yield obtained was weighed and expressed as % yield.

**Phytochemical analysis**
Phytochemicals are secondary plant metabolites produced by the plants which are generally useful medicinally. The phytochemical analysis was carried out using the following standard procedures.[2-3]

**Test for tannins**
- To 1 ml of the extract, few drops of 0.1 % ferric chloride was added and observed for the development of brownish green or blue black coloration.
- To the extract, 1 % gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

**Test for saponins**
- To 1 ml of extract, 2 ml of water was added. The suspension was shaken in a graduated cylinder for 15 minutes. A formation of a layer of foam indicates the presence of saponin.
Test for flavonoids

- To 1 ml of extract, few drops of sodium hydroxide solution was added. Formation of intense yellow color, which becomes colorless on further addition of diluted hydrochloric acid, indicates the presence of flavonoid.
- To 1 ml of extract, few drops of lead acetate solution was added. Formation of yellow precipitate indicates the presence of flavonoids.

Test for alkaloids

The extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was further tested with following reagents for the presence of alkaloids.

- The filtrate was treated with Dragendroff’s reagent. Formation of orange red precipitate indicates the presence of alkaloids.
- The filtrate was treated with Mayer’s reagent. Formation of a whitish yellow or cream colored precipitate indicates the presence of alkaloids.

Test for protein

- To 1 ml of extract, a few drops of Millon’s reagent was added. Formation of a white precipitate indicates the presence of protein.
- To the 1 ml extract in methanol, 4 % sodium hydroxide and 1 % copper sulphate solution were added. Formation of violet or pink color indicates the presence of proteins.

Test for steroids

- A small amount of extract was dissolved in 5 ml of chloroform, to that a few drops of concentrated sulphuric acid was added and allowed to stand. The appearance of brown ring indicates the presence of steroids.
- To 1 ml of extract in chloroform, few drops of acetic anhydride was added. It was boiled and cooled. To this, 1 ml of concentrated sulphuric acid was added along the sides of the tube. Formation of bluish green color confirms the presence of steroids.

Test for anthraquinones

- To 1 ml of extract, aqueous ammonia was added and observed for change in color. Formation of a pink color indicates the presence of anthraquinones.
Test for phenol

- To 1 ml of extract, 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.
- To 1 ml of extract, 1 ml of 5% ferric chloride solution was added. A bluish black color indicates the presence of phenols.

Quantitative estimation of flavanoids\(^{[4-5]}\)

1 mg of each extract was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was shaken well and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 416 nm. 10 mg quercetin dissolved in 95% ethanol was used as standard. The total flavonoids content was obtained from a calibration curve, which was constructed by plotting the known concentrations of quercetin against the absorbance. All the readings were obtained in triplicate.

The total flavonoid content (TFC) was determined using the formula,

\[
TFC = \frac{\text{Concentration x Dilution factor x Volume of stock solution}}{\text{Weight of the extract used}} \times 100
\]

Quantitative estimation of saponin\(^{[6-7]}\)

Standard saponin solution was prepared by dissolving 10 mg of diosgenin in a mixture of 16 ml methanol and 4 ml distilled water. To the aliquots in each tube, 0.25 ml of 8% vanillin reagent was added and 2.5 ml of 72% v/v sulphuric acid was added slowly on the inner side of the wall. The solutions were mixed well and the tubes were transferred to a water bath maintained at 60°C. After 10 minutes, the tubes were cooled in ice cold water bath for 3 – 4 minutes. The absorbance was measured at 544 nm against the reagent blank. 1 mg of extract was dissolved in aqueous methanol (80%, 0.1 ml). 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard curve.

Quantitative estimation of alkaloids\(^{[8-9]}\)

1 mg of each extract was dissolved in 1 ml of methanol. To this 5 ml of phosphate buffer (pH 4.7) and 5 ml bromo cresol green (BCG) solution was added. The mixture was shaken well with 4 ml of chloroform and were collected in a 10 ml volumetric flask and then diluted to
the volume with chloroform. The absorbance was measured at 470 nm against blank. Atropine was used as standard and values are expressed as atropine equivalents.

**DPPH free radical scavenging assay**[^10-11]

The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay. DPPH (1,1-diphenyl -2-picryl hydrazyl) is considered as a stable radical because of the paramagnetism conferred on it by the presence of an odd electron (delocalization of the spare electron over the molecule as a whole). The effect of extract on DPPH radical was assayed using the method of Mensor *et al*. A methanolic solution of 0.5 ml of DPPH was added to 1 ml of the different concentrations of plant extract (200,400,600,800,1000 µg/ml) and allowed to react at room temperature for 30 minutes. Methanol served as the blank and quercetin, a well-known natural antioxidant, served as the positive control. After 30 minutes, the absorbance was measured at 517 nm using a UV visible spectrophotometer and converted into percentage radical scavenging activity as follows.

\[
\text{Scavenging activity (\%) = } \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100
\]

Where \(A_{\text{blank}}\) is the absorbance of DPPH radical + methanol; \(A_{\text{sample}}\) is the absorbance of extract. The inhibitory concentration for reduction of 50% absorbance (IC\(_{50}\)) was calculated using linear regression analysis. The study was carried out in triplicate and the average value was used for the calculation.

**RESULTS**

**Percentage yield**

*Table 1: Percentage yield of various extracts of Artocarpus heterophyllus seed.*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Solvent</th>
<th>Percentage yield (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetone</td>
<td>4.19</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>7.60</td>
</tr>
<tr>
<td>3</td>
<td>Dichloromethane : methanol(1:1)</td>
<td>10.12</td>
</tr>
</tbody>
</table>

Among the three extracts, dichloromethane + methanol extract showed a higher percentage yield of 10.12% when compared to the other two extracts.
Preliminary phytochemical screening

Table 2: Phytochemical analysis of various extracts of *Artocarpus heterophyllus* seed.

<table>
<thead>
<tr>
<th>PHYTOCHEMICAL</th>
<th>EXTRACT</th>
<th>Acetone</th>
<th>Dichloromethane : methanol (1:1)</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+ – Present (-) – Absent)

Preliminary qualitative phytochemical screening for the seeds of *Artocarpus heterophyllus* revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins in all the three extracts.

Quantitative estimation of flavonoids & saponins

Table 3: Quantitative estimation of flavonoids and saponins.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extracts</th>
<th>Flavonoids (mg of QE/g of extract)</th>
<th>Saponins (mg of DE/g of extract)</th>
<th>Alkaloids(mg of AE/g of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetone</td>
<td>18.11</td>
<td>0.766</td>
<td>0.081</td>
</tr>
<tr>
<td>2</td>
<td>Dichloromethane + Methanol(1:1)</td>
<td>20.32</td>
<td>0.89</td>
<td>0.114</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>21.41</td>
<td>0.931</td>
<td>0.110</td>
</tr>
</tbody>
</table>

Figure 1: Quantitative estimation of flavonoids and saponins.
The quantitative phytochemical analysis of the various extracts of *Artocarpus heterophyllus* exhibited the presence of total flavonoids and saponins in considerable quantity. Among the three extracts, highest levels of flavonoids and saponins was found to be present in the ethanolic extract.

**DPPH free radical scavenging assay**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% inhibition</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol extract</td>
<td>Dichloromethane + methanol extract</td>
<td>Acetone extract</td>
<td>Quercetin (standard)</td>
</tr>
<tr>
<td>200</td>
<td>23.06±0.02</td>
<td>20.35±0.04</td>
<td>17.61±0.05</td>
<td>61.95±0.03</td>
</tr>
<tr>
<td>400</td>
<td>63.87±0.02</td>
<td>44.07±0.03</td>
<td>57.70±0.04</td>
<td>70.53±0.04</td>
</tr>
<tr>
<td>600</td>
<td>68.44±0.02</td>
<td>60.30±0.03</td>
<td>60.13±0.03</td>
<td>80.48±0.02</td>
</tr>
<tr>
<td>800</td>
<td>77.58±0.02</td>
<td>56.26±0.08</td>
<td>65.30±0.11</td>
<td>85.23±0.04</td>
</tr>
<tr>
<td>1000</td>
<td>77.82±0.03</td>
<td>70.22±0.02</td>
<td>67.34±0.04</td>
<td>96.22±0.02</td>
</tr>
<tr>
<td>IC50 value (µg/ml)</td>
<td>320</td>
<td>470</td>
<td>362</td>
<td>175</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SEM (n=3).

Figure 2: DPPH Free radical scavenging assay.

The ethanol extract showed higher radical scavenging activity than the other two extracts. However, standard quercetin was more active in scavenging the DPPH radicals as compared to the seed extracts. The IC50 values of ethanol, dichloromethane: methanol (1:1), acetone and quercetin was found to be 320 µg, 470 µg, 362 µg, 175 µg/ml respectively.
DISCUSSION

The phytochemicals in plants have shown plenty of health promoting effects in both in vitro and in vivo studies. Some of these are antioxidant[12-13], hypoglycemic[14], hypotensive[15] and hypolipidemic effects.[16-18] Studies have shown that risk of heart diseases can be reduced through consumption of flavonoid rich diets.[19] Flavonoids may inhibit the vascular diseases development through alteration in endothelial cell eicosanoid production.[20] Flavonoids may augment the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays a key role in the incorporation of free cholesterol into HDL and transferring it back to VLDL and LDL which are taken back later into the liver cells. Several studies have showed that increase in HDL is associated with decrease in Coronary Heart Disease. They also have beneficial actions in obesity due to their capacity to regulate fatty oxidation and improve adipocyte functionality.[21]

Food derived flavonol such as quercetin has been proven to improve dyslipidemia, decrease oxidative stress through stimulation of lipolysis activity.[22] Also, Kaempferol isolated from Moringa oleifera is known to possess anti-atherosclerotic and hypolipidemic properties.[23] Saponins also act as hypolipidemics by binding with cholesterol in intestinal lumen, so that cholesterol is less readily absorbed. They are also known to bind with bile acids causing reduction in its extra hepatic circulation and increasing metabolism of cholesterol to sterols leading to fecal excretion. Saponins are also reported to increase the Lipoprotein lipase activity, which is beneficial in faster removal of free fatty acid from circulation that in turn causes a decrease in total cholesterol.[24]

The present study carried out on Artocarpus heterophyllus seeds revealed the presence of active phytochemical constituents such as tannins, flavonoids, saponins, alkaloids, steroids, phenols in all the three extracts (ethanol, dichloromethane: methanol, acetone). Quantitative studies of flavonoids, saponins and alkaloids were carried out. Among the three extracts, higher content of flavonoids and saponins was found to be present in the ethanolic extract. Higher alkaloid content was found in the DCM: methanol (1:1) extract.

Antioxidants are substances that neutralize reactive oxygen species before they are able to react with cellular components and alter their structure or function. Oxidative modification of LDL in the vascular wall seems to be a key factor in the development of atherosclerosis. Oxidized LDL might recruit monocytes and favour their transformation into foam cells through a receptor mediated intake. Following the oxidation hypothesis of atherosclerosis, the
role of natural antioxidants i.e. Vitamin C, E and carotenoids has been investigated in a large number of studies. Animal studies indicate that antioxidants may reduce atherosclerosis progression.\cite{25} Hence DPPH free radical scavenging assay was conducted with all the three extracts and were found to possess antioxidant property. The IC$_{50}$ of the ethanolic extract was the least indicating that it possesses maximum antioxidant property.

CONCLUSION
The presence of saponins and flavonoids in *Artocarpus heterophyllus* seeds are indicative that it may have a promising role in treatment of hyperlipidemia. Higher level of antioxidant activity was observed in the ethanolic extract of *Artocarpus heterophyllus* seeds when compared with other extracts. This extract can be considered as a new source of natural antioxidants and a promising antihyperlipidemic agent.

REFERENCE


