Phenolics, proteins, antioxidant activity and GC – MS analysis of Artocarpus heterophyllus Lam seeds

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Abstract
Artocarpus heterophyllus seeds are available in larger quantity and are good source for many phytochemicals. The seeds are rich in proteins and low in fat content. The present study was carried out to analyse the phenolics, proteins and antioxidant activity of A. heterophyllus seeds. Different aqueous solvents such as acetate buffer pH 5.0, phosphate buffer pH 7.0, tris buffer pH 8.5, different concentrations of sodium hydroxide (0.05M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M), sodium chloride, water and organic solvents like ethanol, methanol and acetone were used for extraction.

Sodium hydroxide (0.1M) showed maximum extraction efficiency. Among the organic solvents, ethanol showed maximum phytochemical extraction. Different concentrations of ethanol (20%, 40%, 60%, 80%) containing 0.1M NaOH were also used for extraction and maximum extraction was found in 40% ethanol containing 0.1M NaOH. GC – MS analysis was carried to identify various bioactive compounds present in the extracts (hexane, ethyl acetate, methanol and water) of the seeds.

Keywords: Artocarpus heterophyllus, antioxidants, GC-MS analysis, phenolics, proteins.

Introduction
Artocarpus heterophyllus Lam, also called Jackfruit or Ceylon Jack tree, is an integral part of Indian diet. It is very popular in Bangladesh and considered as their National Fruit. The term Jackfruit is derived from the Portuguese term Jaca and Malayalam an Indian regional language chakka. This fruit is considered as “poor man’s food” as it is cheap and easily available in large quantities. Fruits belong to the family Moraceae which constitutes about 37 to 43 genera and 1100 to 1400 species.

Plants are natural sources which are increasingly used in the modern medicine. According to WHO, 80% of the people rely on traditional medicines, mostly plants, since they have fewer side effects. The phytonutrients such as flavonoids, alkaloids, saponins, glycosides, terpenoids, tannins, xanthoproteins and phenols add for the medicinal properties. Polyphenols possess anti-apoptotic, anti-aging, anti-carcinogenic, anti-atherosclerotic and anti-inflammatory properties. They also help in cardiovascular protection, improvement of the endothelial function, inhibition of angiogenesis and cell proliferation. The antioxidant capacities of plants are related to total phenolics making them major contributor to antioxidant capacity and better health promoting agents. They have found applications in pharmaceuticals in treating cancer, atherosclerosis and diabetes when used with formulations.

GC – MS analysis reveals the lipid composition and different bioactive compounds present in the seeds. A. heterophyllus fruit comprises of seeds (8 – 15%) which are the storage source of polysaccharides like galactomannans, glucomannans and xyloglucans apart from proteins and phenols. The seed oil content is 11.39%. The seeds have not received much attention as nutritive supplements because of lack of commercial importance and popularity. The present study was designed to explore the protein, phenolics and antioxidant activity of various extracts of A. heterophyllus seeds and analyse the bioactive components using GC–MS.

Material and Methods
Seeds: The seeds of Jackfruit (A. heterophyllus) were collected from different parts of Kannur District, Kerala in the month of April.

Chemicals: Acetic acid, tris, sodium hydroxide, sodium chloride, sodium carbonate, sodium dihydrogen phosphate, ethanol, methanol, acetone, gallic acid, potassium ferricyanide, ferric chloride, trichoroacetic acid, Folin–Ciocalteau reagent and Bovine Serum Albumin were purchased from SRL Chemicals.

GC/MS Clarus 500 (Perkin Elmer) was used with a column – Restek Rtx®–5, (30 meter X 0.25 mm) (5 % diphenyl / 95% dimethyl polysiloxane) and an initial oven temperature of 45°C for 5 min Ramp 1:5°C / min to 280°C and 280°C for 15 min, injector temperature of 280°C, injection volume of 1.0 μl and run time 60 min.

Preparation of Seed Extracts: The seeds were de-pulped from fruits, washed with water followed by distilled water and shade dried at room temperature.

Defatting of the Seeds: The seeds were crushed to small pieces and defatted using hexane with continuous shaking for 60 minutes. The hexane was filtered and the residue was dried at 60°C and powdered.

Extraction of defatted seed powder
1) Different aqueous solvent systems: 5% defatted seed extracts (500 mg / 10 ml) were prepared using 50 mM acetate buffer pH 5.0, 50 mM phosphate buffer pH 7.0, 50 mM tris...
– HCl buffer pH 8.5, different concentrations of sodium hydroxide (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 M), sodium chloride and distilled water for 30 mins and centrifuged at 10,000 rpm for 20 mins. The pellets were discarded, the supernatant made upto 10 ml and used for the estimation of phenolics, proteins and antioxidant activity.

2) Different organic solvents: 10% extract of defatted seed powder was prepared using 80% aqueous solutions of methanol, ethanol and acetone. The sample was extracted for 30 mins and centrifuged at 10,000 rpm for 20 mins. The supernatants were collected and the residues obtained were re-extracted with five volumes of respective solvents. The supernatants were pooled, evaporated to dryness and the residue was dissolved and made up to 25 ml with distilled water. The solutions were used for the estimation of antioxidants, phenolics and proteins.

3) Combination of alkali and alcohol: A 10% extract of defatted seed powder (1 g / 10 ml) was extracted with different concentrations of ethanol (20%, 40%, 60% and 80%) containing 0.1M NaOH for 30 mins and centrifuged at 10,000 rpm for 20 mins. The supernatants were collected and the residues re-extracted with five volumes of respective solvents. The supernatants were pooled, evaporated to dryness, dissolved and made up to 25 ml with distilled water. The solutions were used for the estimation of antioxidants, phenolics and proteins.

Estimation of antioxidants
Reducing Power Assay: The total reducing power of the extract was determined according to the method of Hinneburg et al8 with slight modifications. The extract (0.1 ml) was mixed with 0.9 ml of distilled water, 0.5 ml of 1% potassium ferricyanide and incubated at 50° C for 20 mins. 0.5 ml of 10% trichloroacetic acid was added and centrifuged at 5,000 rpm for 10 mins. The supernatant was collected and 1 ml of 0.1% ferric chloride was added. The absorbance was measured at 700 nm and antioxidant activity was expressed as ascorbic acid equivalents in gm/100gm dry weight of the sample.

Estimation of Phenolics: Total soluble phenolics were determined according to the Folin – Ciocalteau (FC) method with slight modifications11. The reaction mixture consists of 0.1 ml of extract, 7.9 ml of water and 0.5 ml of FC reagent. The tubes were allowed to stand for 3 mins, 2.0 ml of 20% sodium carbonate was added and allowed to stand for 60 mins. The absorbance was read at 650 nm and total soluble phenolic was expressed as gallic acid equivalents (GAE) as gm/100gm dry weight of sample5.

Estimation of Proteins: The estimation of proteins was carried out by Lowry et al method11 with slight modifications. The reaction mixture consists of 0.1 ml of extract, 0.9 ml of buffer and 5 ml of copper reagent. After 10 mins, 0.5 ml of FC reagent was added and after 30 mins, the absorbance was read at 660 nm. The total protein was expressed as Bovine Serum Albumin equivalents as gm/100gm dry weight of sample as12.

GC – MS analysis: The seed powder (1 g / 10 ml) was extracted with solvents of different polarity (hexane, methanol, ethyl acetate and water) and centrifuged for 20 mins at 10,000 rpm. The extracts (1 ml) were injected for GC – MS analysis of volatile and semi-volatile bioactive compounds6.

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Results and Discussion
Plants are abundant sources of polysaccharides, proteins, flavanoid, alkaloid, terpenoid and steroid derivatives which contribute to their antioxidant and pharmacological functions. The amounts of proteins, antioxidants and phenolics extracted with different solvents are shown in fig. 1, 2 and 3. Maximum amounts of proteins (8.60g/100g), antioxidants (0.66g/100g) and phenolics (0.59g/100g) were extracted with 0.1M NaOH when compared to other aqueous solvents.

Among the different organic solvents, 80% ethanol showed maximum extraction of proteins (6.28g/100g), antioxidants (0.30g/100g) and phenolics (0.44g/100g). A combination of 40% ethanol in 0.1M NaOH showed maximum extraction of proteins (5.19g/100g), antioxidants (1.37/100g) and phenolics (0.49 /100g). The result indicates that ethanol at lower concentration does not drastically affect the extraction whereas at higher concentrations, it decreases the extraction. This decline in extraction with increasing concentration of ethanol can be due to the insolubility at higher concentration.

Bioactive component screening using GC – MS analysis:
GC – MS analysis was carried out with four different solvents (hexane, ethyl acetate, methanol and water). Among the solvents used, hexane extract showed maximum number of 11 peaks (Table 1) and exhibited various biological properties like anti-diabetic, antitumor, allelopathic and antimicrobial activity (Tricontane)13,14, thickener or binder (good cleansing agents) and are used in treatment of acne, soaps and cosmetics17. Phenol 2, 6 – di – tert- butyl derivatives are used as UV stabilizers, antioxidants and light-protection agents for the stabilization of polymers. Phenol 2, 6 – di – tert- butyl is the major compound (21.51%) present in hexane extract.

Tetracosane showed cytotoxicity against Atypical Glandular Cells (AGS), M.D Anderson-Metastasis Breast cancer cells (MDA-MB-231), Human colonic adenocarcinoma (HT29) and NIH 3T3 cells18.
Fig. 1: Proteins extracted with different solvents.

Fig. 2: Antioxidants extracted with various solvents.

Fig. 3: Phenolics extracted with different solvents.

Table 1
Phytochemical analysis of Hexane extract

<table>
<thead>
<tr>
<th>Extract</th>
<th>Apex R.T</th>
<th>Name of compound</th>
<th>MF</th>
<th>MW</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>8.69</td>
<td>Cyclodecasiloxane, eicosamethyl</td>
<td>C_{20}H_{60}Si_{10}</td>
<td>740</td>
<td>20.39</td>
</tr>
<tr>
<td>2.</td>
<td>9.23</td>
<td>Cyclodecasiloxane, eicosamethyl</td>
<td>C_{20}H_{60}Si_{10}</td>
<td>740</td>
<td>18.22</td>
</tr>
<tr>
<td>3.</td>
<td>9.87</td>
<td>Stearic acid, 3-(octadecyloxy) propyl ester</td>
<td>C_{24}H_{40}N_{2}O_{3}Si</td>
<td>432</td>
<td>1.98</td>
</tr>
<tr>
<td>4.</td>
<td>10.56</td>
<td>Octasiloxane</td>
<td>C_{16}H_{30}O_{2}Si_{8}</td>
<td>578</td>
<td>0.71</td>
</tr>
<tr>
<td>5.</td>
<td>25.99</td>
<td>Phenol, 2,6-di-tert-butyl</td>
<td>C_{14}H_{22}O</td>
<td>206</td>
<td>21.51</td>
</tr>
<tr>
<td>6.</td>
<td>42.24</td>
<td>Heptacosane</td>
<td>C_{27}H_{56}</td>
<td>380</td>
<td>1.38</td>
</tr>
<tr>
<td>7.</td>
<td>43.48</td>
<td>Tetracosane</td>
<td>C_{26}H_{50}</td>
<td>338</td>
<td>2.48</td>
</tr>
<tr>
<td>8.</td>
<td>44.39</td>
<td>Heptacosane</td>
<td>C_{27}H_{56}</td>
<td>380</td>
<td>1.39</td>
</tr>
<tr>
<td>9.</td>
<td>45.11</td>
<td>Hexacosane</td>
<td>C_{26}H_{54}</td>
<td>366</td>
<td>1.02</td>
</tr>
<tr>
<td>10.</td>
<td>45.78</td>
<td>Tricontane</td>
<td>C_{30}H_{62}</td>
<td>422</td>
<td>4.45</td>
</tr>
<tr>
<td>11.</td>
<td>46.78</td>
<td>Octacosane</td>
<td>C_{28}H_{58}</td>
<td>394</td>
<td>7.4</td>
</tr>
</tbody>
</table>
The ethyl acetate extract showed 3 peaks (Table 2) and they possessed various biological activities like anti-microbial, anti-bacterial, anti-diabetic, allelopathic and anti-tumor properties (Tricontane). Tetramethoxyflavanone, a plant derivative of quercetin, protects chondrocytes from ER stress-induced apoptosis. Tricontane (36.15%) is the major compound in ethyl acetate extract.

The methanol extract showed 5 peaks (Table 3) eliciting the presence of some bioactive compounds which are used in the treatment of acne (Dodecanoic acid) and other antioxidants (2, 4-Di-tert-butylphenol). Dodecanoic acid 1, 2, 3-propanetriyl ester (67.43%) is the major compound in methanol extract. The water extract showed 3 peaks and the compound which is present (2, 4-Di-tert-butylphenol) is a good antioxidant. Tris (2, 4-di-tert-butylphenyl) phosphate (50.81%) is the major compound.

**Conclusion**

Phytochemical constituents extracted from a plant are dependent on solvents, the extraction methods, storage conditions and reagents used. The results obtained in the present study indicate that the seed extracts of *A. heterophyllus* contain large amounts of phenolic compounds, exhibit moderate antioxidant activity which can contribute for their medicinal properties. They also contains large amount of protein.
The GC-MS analysis carried out using various solvents of increasing polarity revealed the presence of various medicinally important secondary metabolites.

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References


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