Identification of Bioactive Constituents in Peel, Pulp of Prickly Custard Apple (*Annona muricata*) and its Antimicrobial Activity

K Karthikeyan¹*, S Abitha², V G Saravanan Kumar³

¹Corresponding author Assistant Professor, Department of Food Science and Nutrition, Periyar University, Salem - 636 011, TamilNadu, India.

²M. Phil Scholar, Department of Food Science and Nutrition, Periyar University, Salem - 636 011, TamilNadu, India.

³V.G. Saravanan Kumar, Associate Professor, Department of Biotechnology, St. Michael College of Engineering & Technology, St. Santhiagappar Nagar, Kalayarkoil - 630 551, Sivagangai district, Tamil Nadu, India.

ABSTRACT

The present intent of the study to identify the bioactive components and its antimicrobial activity in the prickly custard apple (*Annona muricata*), it is a common fruit in tropical Asia of annona species, is occasionally met under cultivation in Tamilnadu, Karnataka, Andharapradesh, Maharashtra, Assam and Andaman and Nicobar Islands¹. The prickly custard apple has with glossy, dark green leaves. It produces a large, heart shaped fruit that is 15 – 20 cm in diameter which is yellowish when ripped but whitish inside². It belongs to the annonaceae family and is commonly known as Sour-sop. It is growing 5-6m in height it is one of the easily found plants used as traditional medicine³. Prickly custard skin is a dark green, but later yellowish-green and finally all yellow when over-ripe. The soft spines on the skin, the fully mature fruit are good or light greenish yellow. The ripe, mature fruit is soft to the touch; ripeness is better detected by touch than by colour. The outside of the fruit is thorny while the pulp is white and juicy with brownish seeds⁴. It is one of the most nutritious fruit in the tropical region. The white cottony pulp is very juicy, varying in flavour from acid to sweet and highly regarded because of its distinctive aroma and flavour. The flash or pulp that is creamy white in colour and has the texture of juicy floss interlaced with black seeds, the flavour was tropical, strong and pungent on entry but with a delicate ending a soothing creamy after taste⁵. This unique flavour of soursop have the role of some compounds like Esters were found to be the dominant flavour compounds regardless of the soursop’s origin, with methyl (E)-2-hexenoate, methyl (E)-2-butenoate, methyl butanoate and methyl hexanoate being the four principal compounds involved the fruit development and other role⁶. In addition, health benefits of the crushed seeds are used to treat against internal and external parasites, head lice and worms. The use of complementary traditional medicine which includes herbal medicines in the treatment of various diseases has expanded rapidly in developing countries. Herbal remedies from medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited. There are several reasons why the use of medicinal plants should be studied: herbal remedies may have recognizable therapeutic effects⁷. Although, the use of medicinal plants provides an indication of illness and its treatment that may conflict with beliefs of workers in the formal healthcare system⁸. The juice of the fruits is taken orally as herb remedy of arthritis, haematuria and liver ailments, pulverizing the Annona seed and mixing it with soap and water is used as effective spray against caterpillar⁹. Generally, the fruit and fruit juice are taken to eliminate worms and parasites, cool fever, increase mother’s milk after child birth, and is as an astringent for diarrhoea and dysentery¹⁰. The crushed seeds are used against internal and external parasites, head lice and worms¹¹. Hence, in Jamaica, Haiti and the west indies, the fruit and or fruit juice is used for fever, parasites and

INTRODUCTION

Prickly custard apple is most tropical annona species, is occasionally met under cultivation in Tamilnadu, Karnataka, Andharapradesh, Maharashtra, Assam and Andaman and Nicobar Islands¹. The prickly custard apple has with glossy, dark green leaves. It produces a large, heart shaped fruit that is 15 – 20 cm in diameter which is yellowish when ripped but whitish inside². It belongs to the annonaceae family and is commonly known as Sour-sop. It is growing 5-6m in height it is one of the easily found plants used as traditional medicine³. Prickly custard skin is a dark green, but later yellowish-green and finally all yellow when over-ripe. The soft spines on the skin, the fully mature fruit are good or light greenish yellow. The ripe, mature fruit is soft to the touch; ripeness is better detected by touch than by colour. The outside of the fruit is thorny while the pulp is white and juicy with brownish seeds⁴. It is one of the most nutritious fruit in the tropical region. The white cottony pulp is very juicy, varying in flavour from acid to sweet and highly regarded because of its distinctive aroma and flavour. The flash or pulp that is creamy white in colour and has the texture of juicy floss interlaced with black seeds, the flavour was tropical, strong and pungent on entry but with a delicate ending a soothing creamy after taste⁵. This unique flavour of soursop have the role of some compounds like Esters were found to be the dominant flavour compounds regardless of the

*Author for Correspondence: vgsbiotech@gmail.com
diarrhea. Recent studies concentrate only in leaves and the leaves medicinal properties but no more studies available in relate with the seed and other part of the annona fruit, basis of the previous studies the present study focus on the other part of annona fruit were select and the study revealed that the antimicrobial activity. This fruit have been focus to several studies carried out around the world, reporting in their nutritional values, and data especially in relation to the evaluation of antimicrobial activity. Based on the previous reports the present study aims to identify the bioactive compounds in Annona muricata peel and pulp extract and their effect of the antimicrobial activity.

**methodology**

**collection and processing of samples**

Prickly custard apple fruit was collected from Jalagandapuram village. The sample was collected by hand picking. The collected fruit prickly custard apple was washed with fresh water to separate contaminants such as adhering impurities, sand particles and dust. Then cut the fruit separated the peel, pulp and seed. The peel, pulp and seed were allowed to shadow dry in a room temperature for four days. The dried peel, pulp and seed were taken out after four days. The dried peel, pulp and seed samples were uniform make it in powder using mixer grinder machine. The powder was stored at until further analysis.

**aqueous extraction of prepared samples peel and pulp**

The powder obtained was extracted with distilled water. 5g of powered sample added 100ml of distilled water and boiled to 100 C for about 15 min. then the resulting curd was filtered through normal filter paper and stored in refrigerator.

**estimation of total phenol content**

The determination of total phenol content in each sample (12 no samples) was estimated by Folin-Ciocalteu (F-C) assay. Briefly a volume of 100μl of sample was added to 2ml micro centriuge tube followed by 860μl distilled water, 50μl F-C reagents, mixed and allowed to react for 5 min before adding 100μl 20% Na2CO3, 890μl distilled water. All the added solutions were mixed and allowed to stand 60 min at room temperature than measured absorbance at 725nm.

**estimation of total flavanoid content**

Total flavanoids were estimated by Aluminium chloride colorimetric assay. 1 ml aliquot of appropriately diluted each sample or standard solution of quercetin (10, 20, 40, 60, 80,100μg/ml). The results were expressed as milligram of Gallic acid equivalents (GAE) per gram of extract.

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The powder was prepared in similar manner without sample and standard. Calibration curve was plotted using Gallic acid as standard (10, 20, 40, 60, 80,100μg/ml). The results were expressed as milligram of Gallic acid equivalents (GAE) per gram of extract.

**results**

The GCMS analysis showed that the compound fed between 09 to 25 minutes’ figure 1.2 and 3 show the mass spectrum of compound identified in prickly custard apple peel pulp respectively. There are twenty-seven compounds were identified in the peel and pulp of prickly custard and the identified compound shown in the (Table 1). Sixteen were noted to be aliphatic compounds and fifteen were aromatic compounds and the eight compounds are identified from peel that are namely, Dasyacarpid-1-methanol, acetate(ester), 6-Octadecenonic acid, (Z), Eicosanoic acid, Ethanol,2-(9-octadecenolxy)., (Z), Z-(13,14-Epoxo) tetradec-11-en-1-ol acetate, Dasyacarpid-1-methanol, acetate(ester), 4-Hexyl-1-(7-ethoxycarbamyloxy) bicyl (4,4,0) deca-2,5,7-triene, Ethanol, 2, (9-octadecenolxy)., (Z). There are eight compounds were identified from pulp namely 1,2,12-Nonadecatrine,5,14-diol, Gibb.3.ene.1,10-dicarboxylic acid. 

**summary**

An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250μ I.D., 0.25μ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C, hold for 2 minutes, then ramp at 20° C per minute to 300°C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).
The total phenol and flavanoid content in prickly custard apple (Annona muricata). The total phenol and flavanoid content were determined by the Folin-Ciocalteu reagent and the absorbance was measured at 765 nm. The total phenol content was calculated from prickly custard apple (peel 261mg/gm, pulp= 227mg/gm) and the flavanoid content was calculated from 1.059, 0.795, 0.567, 0.334, 0.009, 0.026, 0.149, 0.237, 0.319, 0.398, and 0.319 mg/gm, respectively. The higher value of phenol and flavanoid content in the peel when compared to that of pulp. The presence of higher phenol and flavanoid content in the peel stimulates the single compound may act as a better stimulator for the antioxidant activity.

### Table 1: Bioactive compounds identified in the peel and pulp of prickly custard apple (Annona muricata).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>Peel</th>
<th>Pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dasycarpidan-1-methanol,acetate(ester)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6-Octadecenoic acid,(Z)</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Eicosanoic acid</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol,2-(9-octadecenyloxy),(Z)</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate.</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Dasycarpidan-1-methanol,acetate(ester)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>4-Hexyl-1-(7-methoxy-carbonyl)heptyl) bicyclic(4,4,0)decatriene</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ethanol,2-(9-octadecenyloxy)-(Z)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1,2,12 NONADECATRIENEL5,14-diol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Gibb.3.ene.1,10-dicarboxyl acid,2,4a, dihydroxy-1-methyl-8-methylene, 1,4a-lactone,10-methyl ester, Corynan-17-ol,18,19-didehydro-10-methoxy, 5,8,11-heptadecatrien-1-ol, Chromon,5-8,11-dimethoxy-6,7,8-trimethoxy-2,3-dimethyl, Dasycapidan-1-Methanol,acetate(ester), Isomethone, Methyl 2-chlorohexadecanoate Interestingly, one of the compound present in both peel and pulp namely Dasycapidan-1-Methanol,acetate(ester) and Seven compound unique to pulp and seven compound unique to peel and eleven compound unique to seed samples. Based on the present investigation the single compound may act as a better stimulator for the antioxidant activity.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Absorbance of Gallic acid standard

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration µg/ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.026</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.068</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>0.149</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>0.237</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>0.319</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>0.398</td>
</tr>
</tbody>
</table>

### Table 3: Absorbance of quercetin standard

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration µg/ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0.009</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>0.106</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>0.334</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
<td>0.567</td>
</tr>
<tr>
<td>5</td>
<td>800</td>
<td>0.795</td>
</tr>
<tr>
<td>6</td>
<td>1000</td>
<td>1.059</td>
</tr>
</tbody>
</table>

The total phenol and flavanoid content that is Gallic acid was calculated from prickly custard apple peel and pulp, totally the phenolic content in anonna peel 261mg/gm, Pulp= 227mg/gm respectively. In the case of Gallic acid higher in peel when compared to that of pulp. The Gallic acid was used as standard and concentration of the standard 10 to 100µg/ml and the absorbance are 0.029, 0.068, 0.149, 0.257, 0.319, and 0.396 respectively it was shown in (Table.2 and Graph 1). The total quercetin was calculated from prickly custard apple (peel 261mg/gm, Pulp= 227mg/gm) interestingly, it was higher than the
prickly custard apple peel it was shown in (Table.3 and Graph.2). The comparison of Gallic acid and the quercetin the great difference was noted in the peel and pulp of prickly custard apple. It may give the various antioxidant effects to the fruit quality.

effect of antibacterial activities

The antibacterial activities test was conducted in various concentrations to investigate their relationship. The results of antibacterial activities examination showed that methanol extract fraction was identified maximum antibacterial effect against *Escherichia coli*, *S. Sereus*, *Bacillus Sp*, they were denoted by the existence of inhibition zone around the disc it was shown in the Fig.4,5,6 and 7. The effect was no changes in the peel fraction against *Proteus Sp*. Hence the presence of *Klebsiella Sp* in peel and seed there is no zone effect was observed the results of the average of inhibition zone diameter of methanol extract fractions of sourp peel, pulp and seed can completely be seen on Table. 3. These studies were well documented by the agar double diffusion method.
DISCUSSION

The present investigation is carried out in the fruit of Soursop, the methanolic extract showed antibacterial activity it concluded that the identified phenolic and flavonoids compounds is found to be effective antibacterial activity against *E. coli, S.aureus, Bacillus, proteus Sp. Klebsiella Sp.* Well documented by agar diffusion method. The bioactive compound Dasycarpidan

![Figure 4](image1.png)  
**Figure 4:** Antimicrobial activity of *Annona muricata* peel, pulp and seed against *Staphylococcus*

![Figure 5](image2.png)  
**Figure 5:** Antimicrobial activity of *Annona muricata* peel, pulp and seed against *Bacillus*

![Figure 6](image3.png)  
**Figure 6:** Antimicrobial activity of *Annona muricata* peel, pulp and seed against *E.coli*

![Figure 7](image4.png)  
**Figure 7:** Antimicrobial activity of *Annona muricata* peel, pulp and seed against *Proteus Sp.*

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Graph 1: Absorbance of Gallic acid standard Absorbance: Peel= 0.453, Pulp= 0.437; Content Peel= 222.952mg/gm, Pulp= 215.33mg/gm

\[
y = 0.0042x - 0.0152 \\
R^2 = 0.9998
\]

Graph 2: Absorbance of quercetin standard Absorbance: Peel= 0.138, Pulp= 0.104 Content Peel= 261mg/gm, Pulp= 227mg/gm

\[
y = 0.0012x - 0.123 \\
R^2 = 0.9989
\]
such as Lepidoptera, Coleoptera, Homoptera and Diptera. In addition some of the research group reported that Annonaceae family contains acetogenin that are larvicidal, acetogenin also acts as an insecticide, acaricide, antiparasitic and bactericidal. Other variety like A. muricata Linn. (Soursop) seed extract contain annonacin, bullatacin, annonin VI, goniothalamin and sylvaticum act as insecticides. Based on the previous reports the present study was done the antimicrobial activity against the microbes. It was revealed that the Annona muricata extract have a wide range of activity against a microbes responsible for the most common diseases. These promissory extract open the possibility of finding new clinically effective antibacterial compounds. The present study of peel and pulp of Annona muricata forms a primary platform for further phytochemical and pharmacological studies.

CONCLUSION

Prickly custard apple (Annona muricata) peel and pulp extracted used by methanol and ethyl acetate. Methanolic extract was given good result compared to ethyl acetate extract. This study identified bio active compounds in specifically Dasycapidan – 1- methanol, acetate (ester) present in both peel and pulp and it was identified as best antibacterial agent by double diffusion method. The present study analyzed total phenol and flavonoids content in prickly custard apple give better antimicrobial activity of peel and pulp extract against E. coli, S. aureus, Bacillus Sp., Proteus Sp., Klebsiella Sp. The present study to reveal that the microbes’ reduction test was confirmed by agar well diffusion method. The present finding supports the peel and pulp of prickly custard apple have effective antimicrobial activity and also the coordination of the bioactive compound may play the important role for the antimicrobial activity. Hence, the presence of the Dasycapidan-1-Methanol, acetate (ester) compound may involve the stimulator for the antioxidant properties. Hence the recommendation of the study needs further confirmation to evaluate the additional importance of the part of the fruit and therapeuticeffect of the prickly custard apple and also to determine their full spectrum of efficacy.

REFERENCE