

Identification of Phytochemicals from Seed Extract of Custard Apple, *Annona squamosa*

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ABSTRACT

Custard apple has many alkaloids, such as aporphine, roemerine, norcorydine, corydine, norisocorydine, glaucine and anonaine in different parts of the plant. The roots are used to treat acute dysentery, depression and spinal marrow diseases, while leaves have been used in cases of prolapse of the anus, sores and swelling. Ripe fruit is sweet and good tonic for human health and it is enriching the blood, increases muscular strength and lessens vomiting. Its seeds are used as abortifacients. Seed oil is used in paint and soap industry. This investigation involves preliminary screening, detection, and separation of secondary metabolites from the seed extract of *Annona squamosa* Linn. Oil was extracted from seeds of *Annona squamosa* Linn. by Soxhlet extraction method; methanol was used as a solvent for extraction. Absorbance and functional group detection were studied using FTIR, seed oil supernatant was used to detect the functional groups of secondary metabolites those were presented in sample. A potent Cyanidin-3-O-(2-O-beta-xylopyranosyl-6-O-acetyl)-beta-galactopyranoside, 4-[4-(2-aminoethyl)-2,6-Diodophenoxy]2-iodophenol, Amikacin. The study was done using atmospheric pressure chemical ionization (APCI) Liquid Chromatography mass Spectroscopy technique for identification of Phytochemical. Various phytochemicals were detected using this technique. The analysis by APCI-LC-MS reveals presence of several compounds like cyclopeptides and acetogenins. Cyclosqamosin A, Cyclosqamosin B, Cyclosqamosin H, Acetogenins (polyketides); Annonacin, Squamocin, Annonin VI, which were detected by peaks of m/z ratio between 605 to 640 positive ions shift and Tenacissoside F (steroid) at 667 m/z negative ion shift. Identified compounds were compared with reference of earlier investigations, it was clear that this compound played a major role as anti-diabetic, anticancer, anti-inflammatory and have insecticidal property. However, further Research is required to study phytochemicals.

KEY WORDS: FTIR, APCI-LC-MS, TENACISSOSIDE F, CYANIDIN-3-O-BETA-GALACTOPYRANOSIDE, SQUAMOCIN.

INTRODUCTION

Custard apple is tropical fruit which belongs to genus *Annona* and family *Annonaceae* and are collectively

known as annonaceous fruits. There are over 120 species of genus *Annona* and are commonly found in India as a fruit consuming plant. This plant is commonly known as custard apple in English and Sitafal in Gujarati. Custard apple contains alkaloids, such as aporphine, roemerine, norcorydine, corydine, norisocorydine, glaucine and anonaine in various parts of the plant (Kowalska and Puett, 1990; Pinto et al., 2005; Hiwale, 2015). The Custard apple roots are used in the treatment of acute dysentery, spinal marrow diseases and some cases of depression. The leaves of Custard apple are used in cases of prolapse of the anus, sores and swelling. Custard apple contains alkaloids, such as aporphine, roemerine, norcorydine, corydine, norisocorydine, glaucine and anonaine in various

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parts of the plant (Kowalska and Puett, 1990; Chao-Ming et al., 1997; Pinto et al., 2005). Seeds of Custard apple contains acetogenins namely; squamocins B to N, coumarinologans, annotemoyin-1, annotemoyin-2, squamocin and cholesteryl, glucopyranoside (Zahid et al., 2018).

Seeds of Custard apple are toxic, but they are used to treat head lice as they have insecticidal properties (its preparation causes eye irritation and can cause

blindness). Seeds of *Annona* also have insecticidal properties. Farmers use pesticides to protect their crops from pest infestation, using chemical pesticides is no longer preferable. Seeds of custard apple can be used as biopesticide which can be used as suitable alternatives for pest control. Oil content is high in seeds which can be used to make soap or, if treated to remove the toxic alkaloids, it can be used as a cooking oil (Das et al., 2016; Vetal and Pardeshi, 2019). Earlier studies have been done to study phytochemicals obtained from seeds, leaves.

Table 1. Analysis of functional group present in first separated layer (Upper layer) from crude oil of *Annona squamosa* L. by FTIR peaks values.

Sr. No	Peak Value	Bond	Functional group
1	3625.19	O-H free hydroxyl, Aromatic O-H free	Alcohols, Phenols
2	3470.35	O-H stretch, Aromatic O-H H-bonded	Alcohols, Phenols
3	3389.38	Dimer OH, Aromatic O-H H-bonded	Carboxylic acids, Phenols
4	3271.59	#C-H stretch, Dimer OH, Aromatic O-H H-bonded	Alkenes, Carboxylic acids, Phenols
5	3123.41	=C-H stretch, Dimer OH	Alkenes, Carboxylic acids,
6	3088.30	=C-H stretch, Dimer OH, Aromatic-H stretch	Alkenes, Carboxylic acids, Aromatics
7	2924.11	C-H stretch, Dimer OH	Alkanes, Carboxylic acids
8	2854.84	C-H stretch, -CH ₂ , Dimer OH,	Alkanes, Carboxylic acids
9	1741.59	C=O stretch doublet, C=O stretch	Ketones, Amides
10	1650.64	C=C stretch, C=O stretch (H-Bond), C=N, =NOH	Alkenes, Amides, Quinone or conjugated ketone, Imine, Oxime
11	1519.49	N-O asymmetric stretch, N=O	Nitro compounds, Nitroso compounds
12	1457.11	Aromatic C-C stretch, -CH ₃ , -CH ₂	Aromatics, Alkanes
13	1372.31	-CH ₂ and -CH ₃ , S=O	Alkanes, Sulphate ester
14	1329.22	R-F (C-F stretch), S=O (sulfone), N-O Symmetric stretch	Alkyl halides, Sulfone, Nitro Compound
15	1245.07	R-F (C-F stretch), C-O stretch, P-H bending (phosphine), P=O (phosphonate), P=O (phosphoramidate), Si-CH ₃ , N-O (aromatic)	Alkyl halides, Ethers, Phosphine, Phosphonate, Phosphoramidate, Trimethylsilyl, Amine Oxide, Carboxylic acids, Esters
16	1167.22	R-F (C-F stretch), C=S (thiocarbonyl), P-H (phosphine), P=O (phosphine oxide), P=O (phosphate), C-O stretch	Alkyl halides, Thiocarbonyl, Phosphine, Phosphine oxide, Phosphate, carboxylic acid, esters
17	1027.96	R-F, P-H phosphine, P-OR esters, Si-OR, C-O Stretch	Alkyl halides, Phosphine, Phosphite Esters, Organosilicon, Carboxylic acids, Esters
18	869.11	C-H out of Plan, S-OR, RNH ₂ , RNH	Aromatics, esters, Amines
19	818.44	R ₂ C=CHR (=CH out of plan), R-Cl, C-H out of Plan, S-OR, RNH ₂ , RNH	Alkenes, Alkyl halides, Aromatics, esters, Amines
20	779.00	R-Cl, C-H out of Plan, S-OR, RNH ₂ , RNH	Alkyl halides, Aromatics, esters, Amines

Investigations done in past suggests that the alkaloids from *Annona* species have rarely been explored for their medicinal applications (Nugraha et al., 2019). Many volatile components have been isolated

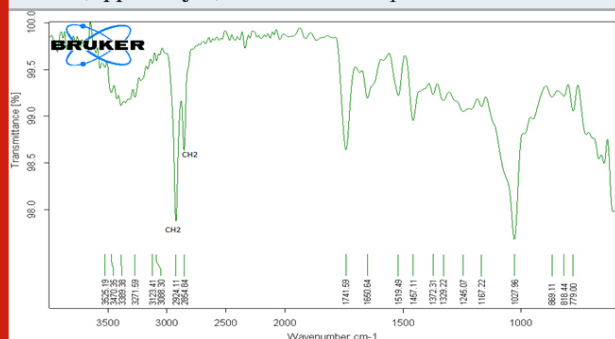
from *A. squamosa* such as bullatacin, 12,15-cis-squamostatin-A, α -Pinene, β -caryophyllene, camphene, β -pinene, myrcene, anonaine, spathulenol, germacrene Duvariamicin-III, annonacin, squamocin, liriiodenine,

and molvizarin (Alkazman, Harnett and Hanrahan, 2020). This investigation involves preliminary screening, detection, and separation of secondary metabolites from the seed extract of *Annona squamosa* Linn. By comparing with reference of earlier studies, it was clear that seed extract contains compound that played a major role as anti-diabetic, anticancer, anti-inflammatory and have insecticidal property (Mangal et al., 2015; Ribeiro et al., 2018). The present investigation involves identification of such compounds that can be used for medicinal purposes.

MATERIAL AND METHODS

Seeds of *Annona squamosa* were collected from fruit of *Annona squamosa* L. seeds were tested and determined viability, viable seeds were selected for identification of phytochemicals as previous study (Patel et al., 2019). Seeds were crushed using mixer grinder and powdered seeds were filled in a paper cup for further oil extraction. Finely crushed seed powder packed in paper cup was taken for oil extraction using Soxhlet extraction method, methanol was used as a solvent for extraction. After few cycles the crude oil was obtained and stored in dark bottles for further phytochemical analysis.

Figure 1: FTIR Spectral pattern of Methanolic extracts of seeds (Upper Layer) from *Annona squamosa* L.



FTIR spectral analysis was conceded from the *Annona squamosa* seed oil supernatant to detect the functional groups of secondary metabolites those were presented in sample. It was performed on BRUKER-FITR instrument in Department of chemistry, P. S. Science & H. D. Patel Arts College, Kadi. We used atmospheric pressure chemical ionization (APCI) Liquid Chromatography mass Spectroscopy technique for identification of Phytochemicals. According to APCI-LC-MS technique we have obtained major peaks, APCI (Positive) m/z at 623, 605, 587, 639 and APCI (Negative), we have obtained major peaks of m/z at 667, 683. The oil was used to check antibacterial activity against *Bacillus subtilis* & *Staphylococcus aureus*, (Chavan, 2006).

Table 2. Analysis of functional group present in Second separated (lower level) layer from crude oil of *Annona squamosa* L. by FTIR peaks values.

Sr. NO	Peak Value	Bonds	Functional Group
1	3275.46	#C-H stretch, Dimer OH, Aromatic O-H H-bonded	Alkynes, Carboxylic acids, Phenols
2	2831.96	Dimer OH	Carboxylic acids
3	1794.53	Unknown	Acid halide, Aryl carbonate, Five-membered ring Anhydride
4	1634.20	NH out of plan, C=N	Amides, Imine
5	1404.74	C-O stretch, S=O (Sulfate ester), Aromatic C-C stretch	Carboxylic acids, Sulfate esters, Aromatics
6	1107.91	R-F (C-F stretch), C-O stretch, C=S (thiocarbonyl), P-H bending (phosphine), P=O (phosphine oxide), P=O (phosphate), Si-OR,	Alkyl halides, carboxylic acid, esters, ethers, Thiocarbonyl, Phosphine, Phosphine oxide, Phosphate, Organosilicon
7	1011.65	R-F (C-F stretch), P-H bending (phosphine), P-OR (esters), Si-OR, C-O stretch	Alkyl halides, Phosphine, Phosphite Esters, Organosilicon, Carboxylic acids, Esters
8	925.24	P-OR (esters), RCOOH O-H bend	Phosphite Esters, Carboxylic acids

RESULTS AND DISCUSSION

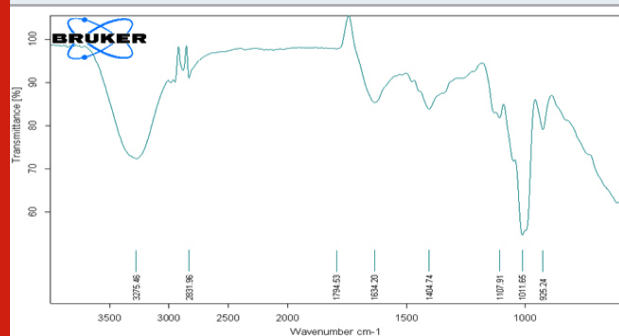
The Upper layer of Methanolic extract FTIR spectra had 20 peaks. The Peaks at 3625.19 cm^{-1} , 3470.35 cm^{-1} , 3389.38 cm^{-1} , 3271.59 cm^{-1} , 3123.41 cm^{-1} , 3088.30 cm^{-1} , 2924.11 cm^{-1} , 2854.84 cm^{-1} , 1741.59 cm^{-1}

indicates the presence of O-H stretch, O-H free hydroxyl, Aromatic O-H free, Aromatic O-H (H-bonded), Dimer OH, #CH stretch, =CH stretch, CH stretch, C=O stretch doublet, Aromatic H stretch and Functional group free hydroxy Alcohol, Phenols, Carboxylic acids, Alkanes, Ketones and Aromatics. The Peaks formed at 1650.64

cm⁻¹, 1519.49 cm⁻¹, 1457.11 cm⁻¹, 1372.31 cm⁻¹, 1329.22 cm⁻¹, 1245.07 cm⁻¹, 1167.22 cm⁻¹, 1027.96 cm⁻¹, 869.11 cm⁻¹, 818.44 cm⁻¹, 779.00 cm⁻¹ specify the presence of C=C stretch, C=O stretch (H-Bond), C=N, =NOH, N-O asymmetric stretch, N=O, Aromatic C-C stretch, -CH₃, -CH₂, S=O, R-F (C-F stretch), S=O (sulfone), N-O Symmetric stretch, P-H bending (phosphine), P=O (phosphonate), P=O (phosphoramidate), Si-CH₃, N-O (aromatic), C-O stretch, R-F (C-F stretch), C=S (thiocarbonyl), P-H (phosphine), P=O (phosphine

oxide), P=O (phosphate), C-O stretch, P-OR esters, Si-OR, C-H out of Plan, S-OR, RNH₂, RNH, R₂C=CHR (=CH out of plan), R-Cldemonstrated for the presence of Alkenes, Amides, Quinone or conjugated ketone, Imine, Oxime, Nitro compounds, Nitroso compounds, Aromatics, Sulphate ester, Alkyl halides, Sulfone, Ethers, Phosphine, Phosphonate, Phosphoramidate, Trimethylsilyl, Amine Oxide, Carboxylic acids, Esters, Thiocarbonyl, Phosphine oxide, Phosphate, Phosphite Esters, Organosilicon, Amines respectively (Table-I) (Harnett and Hanrahan, 2020).

Figure 2: FTIR Spectral pattern of Methanolic extracts of seeds (Lower Layer) from *Annona squamosa* L.



The Second separated (Lower layer) layer of Methanolic extract FTIR spectra had 8 peaks. The Peaks at 3275.46 cm⁻¹, 2831.96 cm⁻¹, 1794.53 cm⁻¹, 1634.20 cm⁻¹, 1404.74 cm⁻¹, 1107.91 cm⁻¹, 1011.65 cm⁻¹, 925.24 cm⁻¹ indicated the presence of Alkynes, Carboxylic acids, Phenols, Acid halide, Aryl carbonate, Five-membered ring Anhydride, Amides, Imine, Sulfate esters, Aromatics, Alkyl halides, esters, ethers, Thiocarbonyl, Phosphine, Phosphine oxide, Phosphate, Organosilicon, Alkyl halides, Phosphine, Phosphite Esters respectively (Table-II) (Harnett and Hanrahan, 2020). The third separated (Crystal) layer of Methanolic extract FTIR spectra had 11 peaks.

Table 3. Analysis of functional group present in Crystal (lower level) from crude oil of *Annona squamosa* L. by FTIR peaks values.

Sr. NO	Peak Value	Bonds	Functional Group
1	3040.94	=C-H stretch, Dimer OH, Aromatic H stretch	Alkenes, Carboxylic acids, Aromatics
2	2878.65	C-H stretch, Dimer OH	Alkanes, Carboxylic acids
3	2378.22	P-H (Phosphine)	Phosphine
4	1700.72	C=O stretch, C=O stretch, C=N	Aldehydes, Amides, Imine
5	1571.66	C=C stretch, C-O stretch, N=O	Alkenes, Carboxylic acids, Nitroso Compounds
6	1424.56	S=O (Sulfate ester), Aromatic C-C stretch	Sulfate esters, Aromatics
7	1341.64	R-F (C-F stretch), S=O (sulfone 1), S=O, N-O symmetric stretch,	Alkyl halides, Sulfone, Sulfonic acid, Nitro Compounds
8	1303.88	R-F (C-F stretch), S=O (sulfone 1), N-O symmetric stretch, C-O stretch	Alkyl halides, Sulfone, Nitro Compounds, Carboxylic acids, Esters
9	1158.56	R-F (C-F stretch), C=S (thiocarbonyl), S=O (sulfone 2), P-H bending (phosphine), P=O (phosphine oxide), P=O (phosphate), C-O stretch,	Alkyl halides, thiocarbonyl, Sulfone, Phosphine, Phosphine oxide, Phosphate, Carboxylic acids, Eesters
10	1050.54	R-F (C-F stretch), C-O stretch, C=S (thiocarbonyl), P-H bending (phosphine), P-OR (esters), Si-OR,	Alkyl halides, Alcohols, carboxylic acids, esters, ethers, Thiocarbonyl, Phosphine, PhosphiteEsters, Organosilicon,
11	908.56	=CH out of plan, P-OR (esters)	Alkenes, PhosphiteEsters

Figure 3: FTIR Spectral pattern of Methanolic extracts of seeds (Crystal) from *Annona squamosa* L.

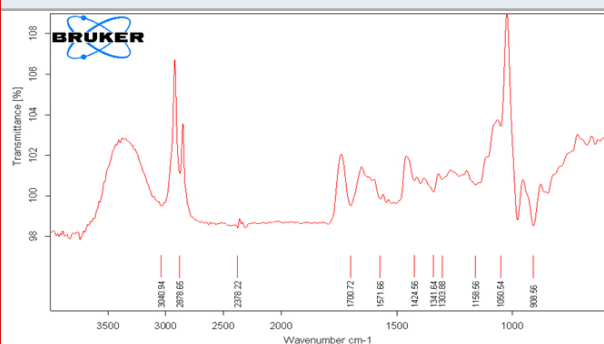


Figure 4: MS analysis of *Annona squamosa* L. seed oil. (positive ion shift)

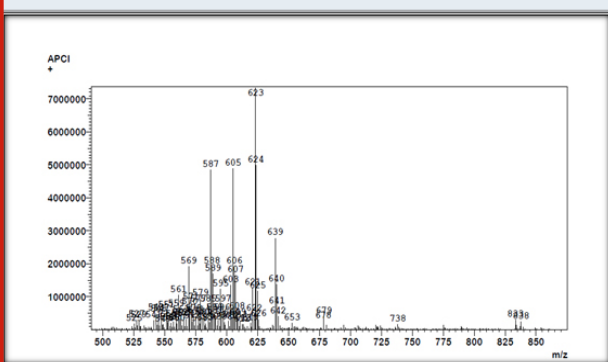
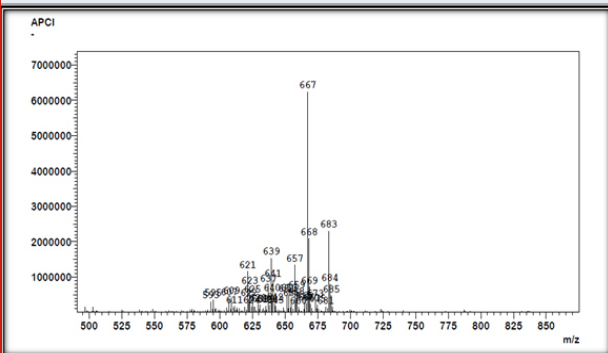


Figure 5: MS analysis of *Annona squamosa* L. seed oil. (Negative ion shift)



The Peaks at 3040.94 cm⁻¹, 2878.65 cm⁻¹, 2378.22 cm⁻¹, 1700 cm⁻¹, 1571.66 cm⁻¹, 1424.56 cm⁻¹, 1341.64 cm⁻¹, 1303.88 cm⁻¹, 1158.56 cm⁻¹, 1050.54 cm⁻¹, 908.56 cm⁻¹ shows the presence of Alkenes, Carboxylic acids, Aromatics, Phosphine, Aldehydes, Amides, Imine, Nitroso Compounds, Sulfate esters, Alkyl halides, Sulfone, Sulfonic acid, Nitro Compounds, Esters, thiocarbonyl, Phosphine oxide, Phosphate, Phosphite Esters, Organosilicon (Harnett and Hanrahan, 2020).

According to LC-APCI-MS technique we have obtained major peaks, APCI (Positive) m/z at 623, 605, 587, 639 (Fig 4) and APCI (Negative), we have obtained major peaks of m/z at 667, 683 (Fig 5). The analysis by APCI-

LC-MS revealed presence of several compounds namely cyclopeptides and acetogenins (Zahid et al., 2013). Cyclopeptides like Cyclosqamosin A, Cyclosqamosin B, Cyclosqamosin H, Acetogenins (polyketides) like Annonacin, Squamocin, Annonin VI, which were detected by peaks of m/z ratio between 605 to 640 positive ions shift and Tenacissoside F (steroid) at 667 m/z negative ion shift. Investigations done in past suggests that the alkaloids from *Annona* species have rarely been explored for their medicinal applications. Many volatile components have been isolated earlier from *A. squamosa* such as Annonacin, Squamocin which suggest that these chemicals have medicinal properties (Nugraha et al., 2019; Alkazman, Harnett and Hanrahan, 2020). By comparing with reference of earlier studies, it was clear that this compound played a major role as anti-diabetic, anticancer, anti-inflammatory and have insecticidal property (Mangal et al., 2015; Ribeiro et al., 2018).

CONCLUSION

The present study explicates the therapeutic application of the seeds which is a rich source of antioxidants such as phenols and flavonoids. The analysis by APCI-LC-MS and FTIR reveals presence of several compounds; cyclopeptides and acetogenins. Cyclopeptides like Cyclosqamosin A, Cyclosqamosin B, Cyclosqamosin H and other groups of cyclopeptides. Acetogenins (polyketides), Annonacin, Squamocin, Annonin VI and Tenacissoside F. By comparing with reference of earlier studies, it was clear that this compound played a major role as anti-bacterial, anti-diabetic, anticancer, anti-inflammatory and have insecticidal property. Research and development would be an important area to focus on medicinal importance of plant. The isolated compounds can be used in future to make antidiabetic, anticancerous, anti-inflammatory medicines, further in future this compound can be used in anti-leaching cream, anti-dandruff shampoo, hair oil and seed oil can also be used as biopesticide.

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Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

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