



STUDIES ON FTIR ANALYSIS OF FRACTION I AND II OF ANNONA SQUAMOSA METHANOL LEAF EXTRACT

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ABSTRACT

The plant *Annona squamosa* is native to tropical America and exotic to India. It is very common plant growing for its fruits. In this plant, all the parts such as leaves, stem, fruits, seeds and roots are used for medicinal preparations. Particularly in the rural village area of Tammilnadu state in India, this plant is used to cure the dandruff on the scalp and control the lies on the head, and to reduce the pain on the teeth. Hence, the present study has been carried out to find the functional group of phytochemicals present in the *Annona squamosa*. In this study, the methanol extract of the plant leaves of *Annona squamosa* were fractionated by using column chromatography and got

two fractions. These two fractions were analyzed for their functional group by conducting FTIR study. The obtained results indicated the presence of nitro compounds, aromatic, alkanes, alcohols, carboxylic acids, esters, ether, carboxylic acids, primary, secondary amines, alkynes, alkyl halides and unknown few compounds in these two fractions.

KEYWORDS: *Annona squamosa*, Phytochemicals, FTIR study, functional group, fractionation and methanol leaf extract.

INTRODUCTION

The art of herbal treatment has very deep root in Indian culture. India has a long heritage in using the curative properties of herbs. Even today one of the leading countries in Asia as well as to the entire world in terms of the wealth of traditional knowledge systems related to herbal medicine. The majority of the Vaithiyar family in rural India has been passing their traditional knowledge about the plants generation after generation to the sikhsiaparambarai as

a family secrete. These secrets are passing through oral communications and not through literal communications. Very few of them are documented in ancient manuscripts such as in Rig Veda, Garuda Purana and Agni Purana. But the methods of medicine Preparation and treatment, the rules and regulations followed before preparations are mentioned in these manuscripts and Vithiya parambara are not in scientific way. Hence, the present FTIR study has been carried out to find what types of functional group of phyto-compounds present in the plants *Annona squamosa*.

MATERIALS AND METHOD

Collection and processing the plant materials

Collection of plants

The plant leaves of *Annona squamosa* was mainly collected from the agricultural land just near to Thiruvalluvar University, Serkad, Vellore. The collected plants were identified with the help of traditional medical practitioners, compared with the herbarium (Voucher specimen) available in the Department of Botany, Thiagarajar College, and Madurai and is further confirmed by plant identification key available in the Literature. The leaves were picked out individually from the branches and washed thoroughly with tap water and shade dried.

Drying

The plant materials are processed for drying under ideal conditions. The leaves were spread on newspaper under shade at room temperature. They were exposed to air until completely dried. The leaves were turned over of atleast twice a day. This processes permits rapid and uniform drying of leaves.

Garbling

It is the process to prepare the plant material before packing for grinding in the Laboratory. By this process foreign materials adhering to the leaves and sand particles were removed.

Grinding

The dried plant leaves is made into fine powder by using maxi (grainding machine). The powder is weighed and used for plant extract preparation.

Plant extract preparation

Dried and finely powdered *Annona squamosa* (25g) was packed in soxhlation unit. The extraction was done by using the solvent methanol. The greenish extract was collected from round bottom flask poured into the petri-dish and freed openly for few days at room temperature to remove the water content and to get a sticky solid. These purified compounds were fractionated by running through column chromatography.

Fractionation of phytochemicals

Fractionation of phytochemical was carried out by column chromatography technique using silica gel (60-120 mesh size) of column chromatography grade (SLR Mumbai). Column (35x10mm) was cleaned well with chromic acid, then using water and rinsed with acetone. Dried it for some time, after drying, a small piece of sterilized cotton was placed at the bottom of the column. The silica powder poured into the column to pack it in a dry condition, there after the solvent petroleum ether: chloroform: methanol (60:20:20) mixture was used as a solvent system to moistened the silica powder to pack it in compactly without air bubbles. The plant extract compounds were loaded at top of the column and the same solvent mixture was used to elude the phyto- compounds. Two bands appeared on column were collected in a beaker and sent it to FTIR analysis.

FTIR Determination of functional group from *Annona squamosa* leaf extracts

Fourier Transform Infrared Spectrum (FTIR) spectra were recorded on JASCO model. The spectra were scanned in the range of 400-4000 cm^{-1} . The spectra were obtained using potassium bromide pellet technique. Hundred milligram of potassium bromide (AR grade) was dried under vacuum at 100°C for 48hr and then mixed with the 1mg sample. The mixture was taken to the FTIR analysis. The obtained spectra were plotted as intensity versus wave number.

BACKGROUND INFORMATION ABOUT *ANNONA SQUAMOSA***Common name in various Indian Languages**

English: Sugar Apple, Custard apple, Sweetsop, Kaner'apra, Pomme Cannelle, Tapotapo, Fun Li Chi, Anon, Anona Blanca, Ati, Ates - Hindi: Sharifa , Sitaphal-Manipuri: Sitaphal-Assamese: Katal - Tamil: Sitapalam.

Classification

Kingdom: Plante, Order: Magnoliales, Family: Annonaceae, Genus: *Annona* Species: *A. squamosa*

Plant description

Annona squamosa is a small tree. The height of the tree ranges from 5-6 m high. The trees contain irregular branches with sparsely hairy in the young branches. Leaves are alternately arranged on short and hairy petioles. It is elliptic to oblong-obovate, 8-11 x 3-4.3 cm, acute to obtuse with cuneate to subrounded base, glabrous on both sides, dull-green on the upper side, pale, The fruit are compound, nearly round, ovoid, or conical; 2 1/3 to 4 in (6-10 cm) long; its thick rind composed of knobby segments, pale-green, gray-green, bluish-green, or, in one form, dull, deep-pink externally (nearly always with a bloom); separating when the fruit is ripe and revealing the mass of conically segmented, creamy-white, glistening, delightfully fragrant, juicy, sweet, delicious flesh. Seeds are dark brown to black. Trees start to bear fruit when 3-4 years old. Flowering and fruiting occur throughout the year. In India, the leaves fall in January-February and are renewed in April-May when the flowers appear, and fruiting is in July-Aug.

Medicinal Uses

Annonas quamosa has been used as a medicine to cure various diseases in world wide. In this plant, all parts from leaves to root are used in different medicinal preparations. In India the crushed leaves are sniffed to overcome hysteria and fainting spells; they are also applied on ulcers and wounds and a leaf decoction is taken in cases of dysentery. Throughout tropical America, a decoction of the leaves alone or with those of other plants is imbibed either as an emmenagogue, febrifuge, tonic, cold remedy, digestive, or to clarify the urine. The leaf decoction is also employed in baths to alleviate rheumatic pain. The fruits of *Annona* are haematinic, cooling, sedative, stimulant, and expectorant, maturant, tonic. They are useful in anemia, burning sensation. The green fruit, very astringent, is employed against diarrhea in El Salvador. In India, the crushed ripe fruit, mixed with salt, is applied on tumors. The seeds are used as abortifacient and insecticidal and are useful in destroying lice in the hair. The bark and roots are both highly astringent. The bark and leaves contain annonaine, an alkaloid. A bark decoction is used to stop diarrhea, while the root is used in the treatment of dysentery. The root, because of its strong purgative action, is administered as a drastic treatment for dysentery and other ailments.

RESULTS

FTIR analysis for fraction-I

The FITR spectra for fraction I of *Annona squamosa* have 20 peaks. The peaks at 3927 cm^{-1} , 3865 cm^{-1} , 3819 cm^{-1} , 3742 and cm^{-1} 3672 cm^{-1} indicates the presence of O-H stretch and the functional group free hydroxyl-alcohol and phenols. The peaks at 3364 cm^{-1} , 2947 cm^{-1} , 2901 cm^{-1} , 1705 cm^{-1} and 1643 cm^{-1} indicates the presence of O-H stretch, H bonded, C-H stretch, Unknown, C=O stretch and -C=C- stretch and the functional group such as Alcohols, phenols, alkanes, unknown, ketones, saturated aliphatic and alkenes respectively. The peak formation at 1551 cm^{-1} , 1512 cm^{-1} , 1366 cm^{-1} , 1234 cm^{-1} , 1041 cm^{-1} , 872 cm^{-1} , 602 cm^{-1} , 540 cm^{-1} , 478 cm^{-1} and 432 cm^{-1} specify the presence of N-O asymmetric stretch, -C stretch (in ring), C-H rock, O stretch and C-H bend, C-Br and Unknown bonds evidenced for the presence of nitro compounds, aromatic, alkanes, alcohols, carboxylic acids, esters, ether, carboxylic acids, primary, secondary amines, alkynes, alkyl halides and unknown compounds respectively (Table-I).

FTIR analysis for fraction-II

The FITR spectra for fraction II of *Annona squamosa* have 18 peaks. The peaks formation at 3865 cm^{-1} , 3834 cm^{-1} , 3747 cm^{-1} , 3672 cm^{-1} , 3364 cm^{-1} , 2947 cm^{-1} , 2893 cm^{-1} , 1705 cm^{-1} , 1643 cm^{-1} , 1551 cm^{-1} , 1512 cm^{-1} , 1366 cm^{-1} , 1227 cm^{-1} , 1041 cm^{-1} , 864 cm^{-1} , 617 cm^{-1} , 555 cm^{-1} and 517 cm^{-1} indicates the existence of O-H stretch, O-H stretch, H-bond, C-H stretch, C=O stretch, N-O stretch asymmetric stretch, C-C stretch (in-ring), C-H rock, C-N stretch, O-H bend, N-H wag, C-Cl, C (triple bond), C-H:C-H bend, C-Br stretch and Unknown bonds and the presence of free hydroxyl-alcohol and phenols, alcohol, phenols, alkanes, alpha, beta unsaturated aldehyde, ketones, nitro compounds, aromatics, alkanes, aliphatic amines, carboxylic acids, primary, secondary amines, Alkyl halides, alkynes, alkyl halides and unknown respectively (Table-II).

Table 1. FTIR peak values and functional groups of methanol extracts of *Annona squamosa*

(Fraction-I)

| No. | Peak value | Bonds | Functional group |
|------------|-------------------|------------------------|---|
| 1. | 3927 | | |
| 2. | 3865 | OH stretch | Free hydroxyl-Alcohol and Phenols |
| 3. | 3819 | | |
| 4. | 3742 | | |
| 5. | 3672 | | |
| 6. | 3364 | N-H stretch | Primary, secondary amines and amides |
| 7. | 2947 | N-H stretch | Alkenes |
| 8. | 2901 | | |
| 9. | 1705 | C=O stretch | Alpha,betaunsaturated aldehyde ketones |
| 10. | 1643 | | |
| 11. | 1551 | N-O asymmetric stretch | Nitro compounds |
| 12. | 1512 | | |
| 13. | 1366 | C-H rock | Alkanes |
| 14. | 1234 | C-O stretch | Alcohol, carboxylic acid, esters ethers |
| 15. | 1041 | C-N stretch | Aliphatic amines |
| 16. | 872 | O-H bend | Carboxylic acids |
| 17. | 602 | C-Br | Alkyl halides |
| 18. | 540 | | |
| 19. | 478 | Unknown | Unknown |
| 20. | 432 | | |

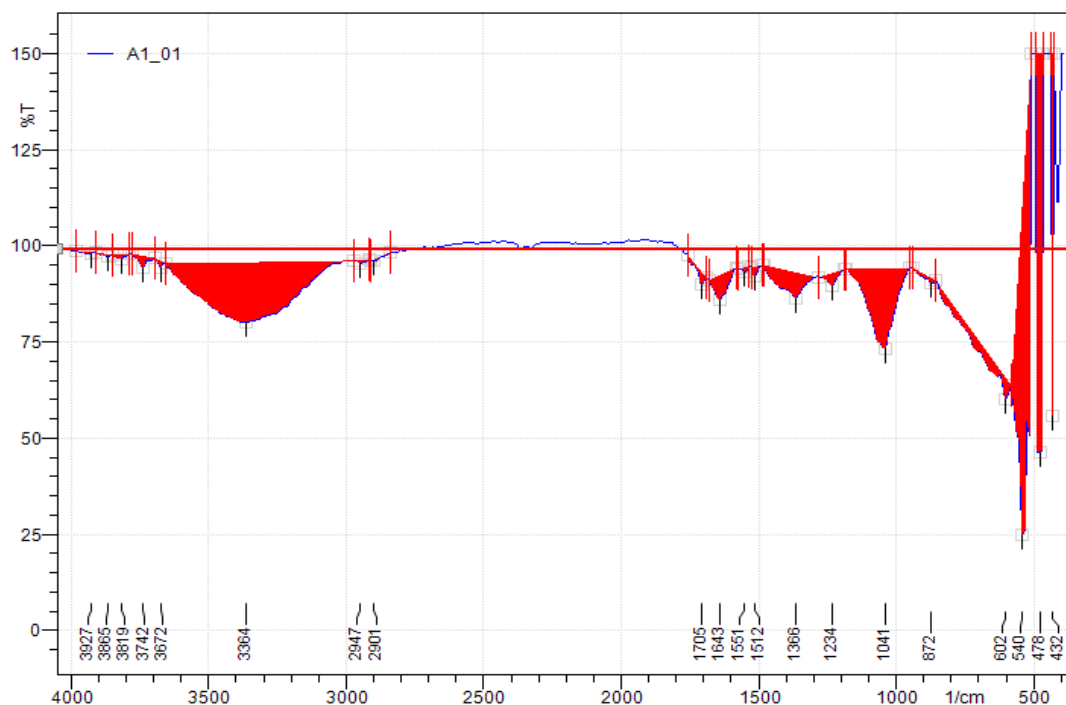


Fig 1. FTIR Spectral pattern of methanol extracts of *Annona squamosa* (Fraction-I)

Table 1. FTIR peak values and functional groups of methanol extracts of *Annona squamosa*

(Fraction-II)

| .No | Peak value | Bonds | Functional group |
|-----|------------|------------------------|--------------------------------------|
| 1. | 3865 | | |
| 2. | 3834 | OH Stretch | Free hydroxyl-Alcocol and Phenols |
| 3. | 3747 | | |
| 4. | 3672 | | |
| 5. | 3364 | N-H Stretch | Primary, secondary amines and amides |
| | | C-H stretch | Alkenes |
| 6. | 2947 | | |
| 7. | 2893 | | |
| | | Unknown | Unknown |
| 8. | 1705 | -C=C- stretch | Alkenes |
| 9. | 1643 | | |
| | | N-O asymmetric stretch | nitro compounds |
| 10. | 1551 | | |
| 11. | 1512 | | |
| | | C-H rock | Alkanes |
| 12. | 1366 | | |
| | | C-N stretch | Aliphatic amines |
| 13. | 1227 | | |
| | | C-N bend | Alkanes |
| 14. | 1041 | | |

| | | | |
|-----|-----|--------------|---------------------------|
| 15. | 864 | N-H wag | Primary, secondary amines |
| 16. | 617 | C-Cl stretch | Alkyl halides |
| 17. | 555 | C-Br stretch | Alkyl halides |
| 18. | 517 | | |

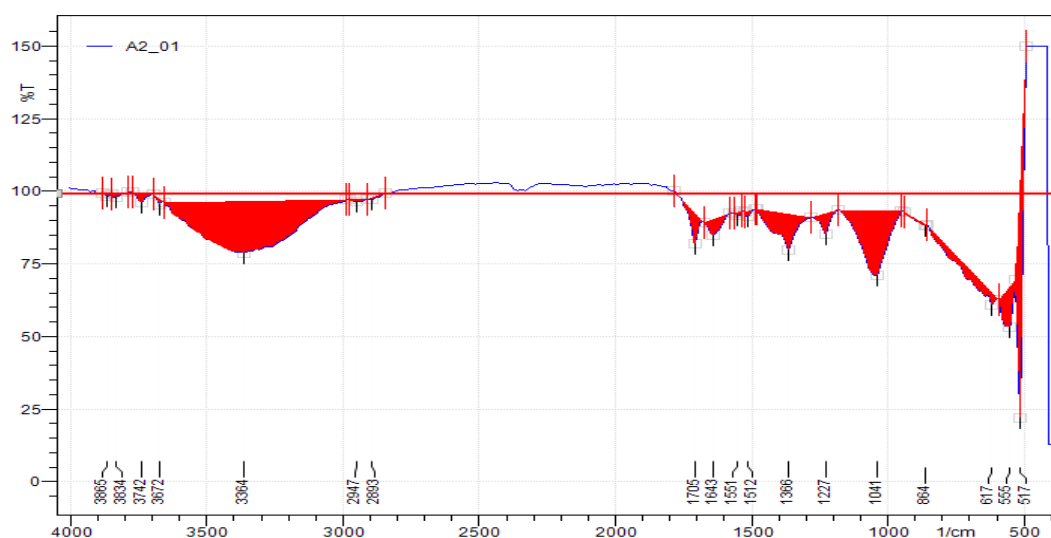


Fig 1. FTIR Spectral pattern of methanol extracts of *Annona squamosa* (Fraction-II)

DISCUSSION

Annona squamosa is very common in India because it is cultivated for fruits. Mostly in the rural areas of Tamilnadu states, the leaves of this plant are used to cure the pain of infected teeth. The paste and extract prepared from this plant is also used to cure the dandruffs. Every parts of *A. squamosa* have various medicinal property proved by many research works.^[1] The bark and leaves contain the phytochemicals annonaine and acetogenin. Among these, the annonaine is an alkaloid possess the hypoglycemic and antidiabetic effects^[2-4] and acetogenin possess anticancer activity.^[5-6] This plant is used as an antioxidant, antidiabetics, hepato protective, cytotoxic activity, genotoxicity, antitumour activity, anti lice agent because of the presence of phytochemicals alkaloids, carbohydrates, fixed oils, tannins and phenolic^[7]. The phytochemical analysis was carried out in this *Annona squamosa* indicates the presence of antitumour properties contain flavonoids, coumarines, alkaloids, terpenoids^[8] and antioxidant compounds terpenoids, flavonoids, alkaloids and tannins^[9]. In the present investigation, FTIR analysis was done for fraction –I *Annona squamosa* methanol leaf extract reveals the presence of characteristic absorption of 20 peaks. The peaks at 3927 cm^{-1} , 3865

cm^{-1} 3819 cm^{-1} , 3742 and cm^{-1} 3672 cm^{-1} indicates the presence of O-H stretch and the functional group free hydroxyl-alcohol and phenols. The peaks at 3364 cm^{-1} , 2947 cm^{-1} , 2901 cm^{-1} , 1705 cm^{-1} and 1643 cm^{-1} indicates the presence of O-H stretch, H bonded, C-H stretch, unknown, C=O stretch and -C=C- stretch and the functional group such as Alcohols, phenols, alkanes, unknown, ketones, saturated aliphatic and alkenes respectively. The peak formation at 1551 cm^{-1} , 1512 cm^{-1} , 1366 cm^{-1} , 1234 cm^{-1} , 1041 cm^{-1} , 872 cm^{-1} , 602 cm^{-1} , 540 cm^{-1} , 478 cm^{-1} and 432 cm^{-1} specify the presence of N-O asymmetric stretch, -C stretch (in ring), C-H rock-O stretch and C-H bend, C-Br and unknown bonds evidenced for the presence of nitro compounds, aromatic, alkanes, alcohols, carboxylic acids, esters, ether, carboxylic acids, primary, secondary amines, alkynes, alkyl halides and unknown compounds respectively. The above observed results showed a strong agreement with the spectral peak at 3511, 2925, 2859, 2738 and 1075 cm^{-1} , which corresponds to hydroxyl, methoxy, alkyl, carboxylic and glycosidic groups investigated in the leaf of *Annona squamosa*.^[10] and presence of N-H group at 3389.47 (amines), $\equiv\text{C}-\text{H}$ group, at 3000.77 and 962.21 (alkenes), C-H group at 2915.49 (alkanes), C=O and $\equiv\text{C}-\text{H}$ at 1656.22 (esters and alkenes), N-H and C-H at 1433.38 (secondary amines and alkanes), N=O at 1315.28 (nitro groups), C=O at 1021.25 (esters) and C \equiv C at 704.98 (alkynes)^[11]. The fraction-II of methanol extract of *Annona squamosa* form the peaks at 3865 cm^{-1} , 3834 cm^{-1} , 3747 cm^{-1} , 3672 cm^{-1} , 3364 cm^{-1} , 2947 cm^{-1} , 2893 cm^{-1} , 1705 cm^{-1} , 1643 cm^{-1} , 1551 cm^{-1} , 1512 cm^{-1} , 1366 cm^{-1} , 1227 cm^{-1} , 1041 cm^{-1} , 864 cm^{-1} , 617 cm^{-1} , 555 cm^{-1} and 517 cm^{-1} indicates the existence of O-H stretch, O-H stretch, H-bond, C-H stretch, C=O stretch, N-O stretch asymmetric stretch, C-C stretch, (in-ring), C-H rock, C-N stretch, O-H bend, N-H wag, C-Cl, C (triple bond), C-H:C-H bend, C-Br stretch and Unknown bonds and the presence of free hydroxyl-alcohol and phenols, alcohol, phenols, alkanes, alpha, beta unsaturated aldehyde, ketones, nitro compounds, aromatics, alkanes, aliphatic amines, carboxylic acids, primary, secondary amines, alkyl halides, alkynes, alkyl halides compounds. These results showed some concordance with the FT-IR bands observed at 1039 cm^{-1} , and 584.9 cm^{-1} may be ascribed to aliphatic RCH₂-NH₂ and amide, C-C(=O)-NH₂, respectively^[12].

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