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GC–MS and FTIR Analysis of Crude Extracts of *Carica Papaya* Seed

Reuben Agada[1], Wurochekke Abdullahi Usman[2], Sarkkiyayi Shehu[3]

[1]Modibbo Adama University of Technology Yola, Department of Biochemistry, Adamawa State, Nigeria
[2]Modibbo Adama University of Technology Yola, Department of Biochemistry, School of Life Sciences, Adamawa State, Nigeria
[3]University of Technology Yola, Department of Biochemistry, Adamawa State, Nigeria

Correspondence Author: Reuben Agada, Modibbo Adama University of Technology Yola, Department of Biochemistry, Adamawa State, Nigeria.
E-mail:- Agabaidu73@gmail.com

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Abstract

Background: Identification, quantitation, and characterization of bioactive principles from *Carica papaya* seed will further improve discoveries in therapeutic medicine and economic values.
Objective: This study aimed to identify the possible bioactive compounds present in methanol and aqueous extracts of *Carica papaya* seed using Gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopic (FTIR) analysis. Materials and method: The *Carica papaya* seed was obtained, air-dried and turned to powdered. Extraction was done with methanol and aqueous using a soxhlet extractor.
Results: The results confirmed the presence of 21 compounds including undecylenic acid (40.33%), oleic acid (30.21%), n-hexadecanoic acid (7.55%), 9-octadecenal (7.09%) and 9,17-octadecadienal, (Z)- (5.98%) in methanol extract while oleic acid (31.58%), 9,12-octadecadienoyl chloride, (Z,Z)- (13.18%), undecylenic acid (12.60%), n-hexadecanoic acid (7.83%), 10-undecenal (7.44 %), octadecanoic acid (5.68 %), 10-undecenoyl chloride (4.55%) and hexadecanoic acid, 2,3-dihydroxpropyl ester (4.08%) were found in aqueous extract. The FTIR spectroscopic investigation revealed the presence of these functional groups: C=C, -C-O, -O-H, -N-O, -C=O, -C=C=C, -S-H, -O=C=O, -C-H, and -N-H indicating the presence of alkanes, alkenes, ether compounds, alkyl aryl ether, carboxylic acid, nitro compounds, aliphatic ketones, halides, allenes, thiols, carbonates, amines and alcohols in methanol and aqueous extracts *Carica papaya* seed.
Conclusion: The present study revealed that extracts of *Carica papaya* seed contained a lot of metabolites and therapeutic active substances. These substances could be further isolated and investigated to confirm their pharmacological activities. Hence, this study supports the use of the *Carica papaya* seed in alternative/traditional medicine.

Keywords: Carica papaya seed; bioactive compounds; secondary metabolites; GC-MS and FTIR analysis; methanol and aqueous extracts

INTRODUCTION

Natural products from plants will continue to be extremely important as sources of new drugs with new modes of pharmacological action. These plants produce secondary metabolites that have played an essential role as medicine and lead compounds for new drug development (Castello *et al*., 2002). Through recent studies on medicinal plants, there have been great developments in the identification and isolation of new therapeutic compounds of medicinal importance from plants for most common and specific diseases (Ertuk *et al*., 2006, Kumar *et al*., 2007). Therapeutic agents, oil and gums are all constituents of plants, hence harnessing the constituents of plants will further improve discoveries in therapeutic medicine and economic values. The plants which have pharmacological properties possess bioactive constituents like saponins, alkaloids, flavonoids, tannins, vitamins, and phenolic compounds. In Nigeria, a large population of people depend on alternative/traditional medicine without the knowledge of their chemical constituents (Anaduaka *et al*., 2013; Ashokkumar and Ramaswamy, 2014). The analysis of these chemical constituents in plants would provide useful insights into their pharmacological properties.

Papaya (from Carib via Spanish), papaw or pawpaw (English) is the fruit of the plant *Carica papaya* and is one of the 23 accepted species in the genus *Carica* of the plant family *Caricaceae*. The *Carica papaya* is consumed worldwide, either in natural or processed as jam, sweets, and pulp, and to aggregate the nutritional value, other parts of the plant (leaves and seeds) are

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added to some products in the form of teas and flours (Nwofia *et al*., 20012). The black seeds of the papaya are usually numerous small, black, ovoid, corrugated, peppery seeds about 3/16 in (5 mm) long, each coated with a transparent, gelatinous aril as presented in Figure 1. They are edible, non-toxic and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper (Aravind, 2013). In Nigeria, different parts of the plant are used traditionally in the treatment of some diseases by different cultural groups (Oboh *et al*., 20013) and the seed contains phenolic compounds, such as benzyl isothiocyanate, glucosinolates, tocopherols (α and δ), β-cryptoxanthin, β-carotene and carotenoids (Kermanshai., 2001, Tang, 1971), while the seed oil contains oleic fatty acid, followed by palmitic, linoleic and stearic acids (Van Breemen and Pajkovic, 2008). The present study aims to identify the bioactive phytochemical components of the methanol and aqueous extracts with the aid of GC-MS and FTIR techniques.

MATERIALS AND METHODS

Collection of plant material

The ripe fruits of *Carica papaya* (red royal) were collected from farmland at Ofuorachi Igalamela-Odolu, Kogi state during dry season. The plant was authenticated by a botanist at the department of Plant Sciences, School of Life Sciences, Moddibo Adama University of Technology Yola, Nigeria. The ripe seeds were removed and rinsed under running tap water and then shade dried at ambient temperature. Thereafter the dried seed sample was pulverized into coarse powder using laboratory blender, ready for extraction.

Preparation of plant extract

The powdered *Carica papaya* seed about (200 g), was extracted with methanol using a soxhlet extractor. The extract obtained was filtered using Whatman filter paper (15 cm) after which the filtrates were concentrated on a rotary evaporator to obtain the methanol extract. The residue from the methanol extract was soaked in distilled water for 24 hours after which it was filtered using a muslin cloth and Whatman filter paper to obtain the aqueous extract. The methanol and aqueous extracts were subjected to GC/MS and FT-IR analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Methanol and aqueous extracts of *Carica papaya* seed were analyzed with the help of a GC-MS analyzer (GC-MS-QP 2010 plus Shimadzu, Japan). The carrier gas helium (99.999 %) was used at a flow rate of 1 ml per min in split mode (10:1) v/v. Methanol and aqueous extracts (8 µl) were injected into the column at 250 °C injector temperature. The temperature of the oven started at 70 °C and held for 5 min. It was then raised at a rate of 10 °C per min to 280 °C without holding. Holding was allowed for 6 min at a programmed rate of 5 °C per min. The temperature of ion sources was maintained at 200 °C. The injector temperature was set at 250 °C and the detector temperature was set at 250 °C. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and the detector operates in scan mode 50 to 600 Da atomic units. The MS Table was generated through an ACQ mode scan within 0.5 seconds of scan interval at the speed of 666 and fragments from 30 to 350 Da were maintained according to the method reported by Ibrahim *et al.,* (2013). The total running was 21 minutes.

Identification of components

Interpretation of the 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. The name of the chemical component, molecular weight, and the chemical structure contain in *Carica papaya* seed extracts were identified.

Fourier Transformed Infrared (FTIR) Spectroscopic Analysis

Fourier transform infrared spectroscopic (FTIR) analysis of the extracts was carried out using Shimadzu FTIR– 8400s Fourier transform infrared spectrophotometer, Japan. Methanol and aqueous extracts of *Carica papaya* seed were oven-dried to get powders of the different solvent extracts used for FTIR analysis. The dried extracts powder (10 mg) were encapsulated in 100 mg of KBr pellet, to prepare translucent sample disc and analysis was carried out by scanning the samples through a wave number range of 400 to 4000 cm-1 with a resolution of 2 cm-1. FTIR analyses were performed and the different peaks present and possible chemical interactions were examined.

Statistical analysis

The results are presented as Mean ± SEM (Standard Error of Mean). Comparisons between the groups were performed by one-way analysis of variance using Statistical Package for Social Sciences (SPSS) for windows version 28.0 (SPSS Inc., Chicago, IL, USA). Significant differences were compared by Duncan's Multiple Range test; a probability level of less than 5% ($P< 0.05$) was considered significant.

RESULTS

The GC-MS chromatogram of methanol and aqueous extracts are shown in Figures 2 and 3. The present study has identified the presence of 8 and 10 bioactive compounds in methanol and aqueous extracts of *Carica papaya* seed as shown in Table 2 and 3 with their corresponding retention time, molecular formula, molecular weight and structure as well as their relative abundance, which was expressed in terms of peak area (%). The major compounds contained in methanol and aqueous extracts of *Carica*

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papaya seed in terms of their relative abundance were undecylenic acid, 9-octadecanoic acid (oleic acid), 9,12-octadecadienoyl chloride, (Z, Z)-, n-hexadecanoic acid (palmitic acid)**,** 10-undecenal, 9-undecenal, 9,17-Octadecadienal, (Z)- and octadecanoic acid (stearic acid) while the minor compounds contained in methanol and aqueous extracts of *Carica papaya* seed were 13-hexyloxacyclotridec-10-en-one, hexadecanoic acid, ethyl ester, 1-octanol, 2-butyl-, Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphinyl]oxy]metyl]-1,2-ethanediyl ester, hexadecanoic acid, 2,3-dihydroxpropyl ester and 10-Undecenoyl chloride.

The FTIR chromatogram of methanol and aqueous extracts are shown in Figures 4 and 5 and the identified functional groups are presented in Tables 4 and 5. The functional groups found in methanol and aqueous extracts of *Carica papaya* seed are -C=C, -C-O, -O-H, -N-O, -C=O, -C=C=C, -S-H, -O=C=O, -C-H, and -N-H indicating the presence of alkanes, alkenes, ether compounds, alkyl aryl ethers, carboxylic acids, nitro compounds, aliphatic ketones, halides, allenes, thiols, carbonates, amines, and alcohols.

Table 1: Compounds identified in methanol extract of *Carica papaya* seed

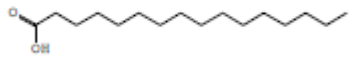
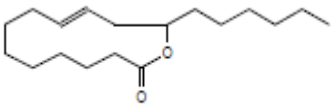
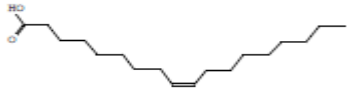
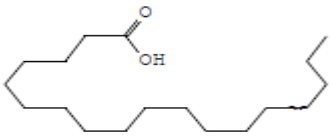
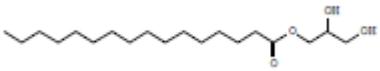
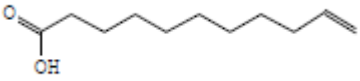
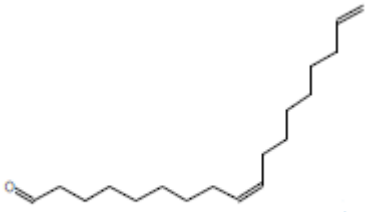
Peak No	Retention time	Formula	Molecular weight	Compound Name	Area%	Structure
1	15.974	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid	7.55	
2	16.966	$C_{18}H_{32}O_2$	280	13-Hexyloxacyclotridec-10-en-one	1.19	
3	17.718	$C_{18}H_{34}O_2$	282	Oleic acid	30.21	
4	17.868	$C_{18}H_{36}O_2$	284	Oleic acid	5.28	
5	18.905	$C_{19}H_{38}O_4$	330	Hexadecanoic acid, 2,3-dihydroxpropyl ester	2.37	
6	19.512	$C_{11}H_{20}O_2$	184	Undecylenic acid	40.33	
7	20.412	$C_{18}H_{34}O$	266	9-Octadecenal	7.09	
8	22.345	$C_{18}H_{32}O$	264	9,17-Octadecadienal, (Z)-	5.98	

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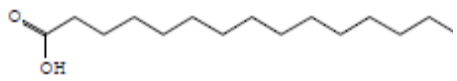
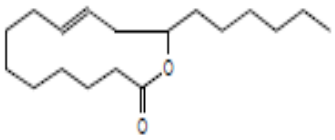
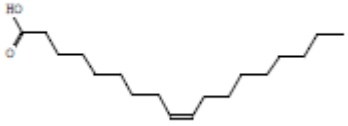
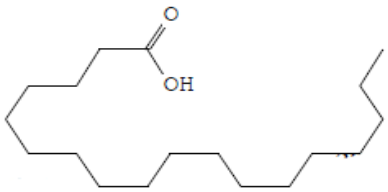
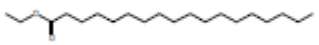
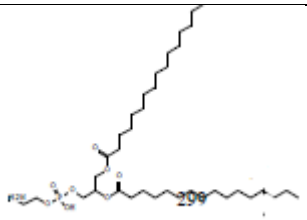
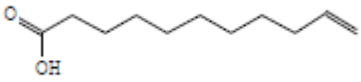
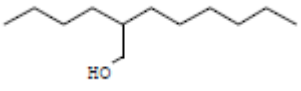
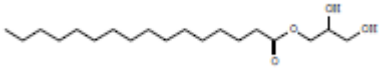
Table 2: Compounds identified in aqueous extract of *Carica papaya* seed

Peak No	Retention time	Formula	Molecular weight	Compound Name	Area %	Structure
1	15.987	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid	7.83	
2	16.109	$C_{18}H_{36}O_2$	284	Hexadecanoic acid, ethyl ester	3.65	
3	16.970	$C_{18}H_{32}O_2$	280	13-Hexyloxacyclotridec-10-en-one	2.72	
4	17.733	$C_{18}H_{34}O_2$	282	Oleic acid	31.58	
5	17.877	$C_{18}H_{36}O_2$	284	Octadecanoic acid	5.68	
6	17.983	$C_{20}H_{40}O_2$	312	Octadecanoic acid, ethyl ester	2.73	
7	18.910	$C_{37}H_{74}NO_8P$	691	Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphinyl]oxy] metyl]-1,2-ethanediyl ester	2.26	
8	19.439	$C_{11}H_{20}O_2$	184	Undecylenic acid	12.60	
9	20.408	$C_{11}H_{20}O$	168	10-undecenal	7.44	
10	20.591	$C_{12}H_{26}O$	186	1-Octanol, 2-butyl-	1.72	
11	20.777	$C_{19}H_{38}O_4$	330	Hexadecanoic acid, 2,3-dihydroxpropyl ester	4.08	

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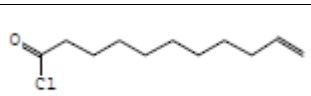
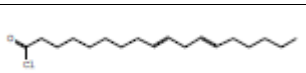
12	22.091	$C_{11}H_{19}ClO$	202	10-Undecenoyl chloride	4.55	
13	22.369	$C_{18}H_{31}ClO$	298	9,12-Octadecadienoyl chloride, (Z,Z)-	13.18	

Table 4: FTIR Interpretation of the methanol extract of the *Carica papaya* seed

S/NO	Test sample (cm^{-1})	Reference standard (cm^{-1})	Functional group Assignment	Identified Compounds
1	709.83	665-730	C=C bend	Alkene
2	941.29	915-995	C=C bend	Alkene
3	1095.6	1150-1085	C-O stretch	Ether
4	1226.77	1200-1275	C-O stretch	alkyl aryl ether
5	1435.09	1395-1440	O-H bend	carboxylic acid
6	1543.1	1500-1550	N-O stretch	nitro compound
7	1712.85	1705-1725	C=O stretch	aliphatic ketone
8	1797.72	1770-1800	C=O stretch	Halide
9	1944.31	1900-2000	C=C=C stretch	Allene
10	1982.89	1900-2000	C=C=C stretch	Allene
11	2337.8	2275-2349	O=C=O stretch	carbonate
12	2692.72	2500-3000	O-H stretch	carboxylic acid
13	2916.47	2840-3000	C-H stretch	Alkene
14	3086.21	3080-3140	C-H stretch	Alkene
15	3255.95	3250-3330	N-H stretch	Amine
16	3425.69	3400-3500	N-H stretch	Amine
17	3495.13	3400-3500	N-H stretch	Amine
18	3595.43	>3500	O-H stretch	Alcohol
19	3796.04	>3500	O-H stretch	Alcohol
20	3880.91	>3500	O-H stretch	Alcohol
21	3965.78	>3500	O-H stretch	Alcohol

Table 5: FTIR Interpretation of the aqueous extract of the *Carica papaya* seed

S/NO	Test sample (cm^{-1})	Reference standard (cm^{-1})	Functional group Assignment	Identified Compounds
1	694.4	665-730	C=C bend	Alkene
2	1095.6	1070-1150	C-O stretch	ether compound
3	1234.48	1200-1275	C-O stretch	alkyl aryl ether
4	1435.09	1395-1440	O-H bend	carboxylic acid
5	1535.39	1500-1550	N-O stretch	nitro compound
6	1643.41	1638-1648	C=C stretch	Alkene
7	1712.85	1705-1725	C=O stretch	aliphatic ketone
8	2337.8	2275-2349	O=C=O stretch	Carbonate
9	2584.7	2550-2600	S-H stretch	Thiol
10	2685	2500-3000	O-H stretch	carboxylic acid
11	2862.46	2850-3000	C-H stretch	alkane
12	2924.18	2850-3000	C-H stretch	alkane
13	3086.21	3000-3100	C-H stretch	Alkene
14	3132.5	3080-3140	C-H stretch	Alkene
15	3255.95	3250-3330	N-H stretch	Amine
16	3363.97	3300-3400	N-H stretch	Amine
17	3510.56	>3500	O-H stretch	Alcohol
18	3618.58	>3500	O-H stretch	Alcohol
19	3742.03	>3500	O-H stretch	Alcohol
20	3826.9	>3500	O-H stretch	Alcohol
21	3873.19	>3500	O-H stretch	Alcohol
22	3950.35	>3500	O-H stretch	Alcohol

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DISCUSSION

GC–MS analysis has been employed as a powerful tool for the identification of novel compounds in plant extracts as revealed in Table 1 to 2. Some of the identified compounds have been reported to exhibit a lot of biological activities. For instance, 9-octadecanoic acid (oleic acid) was reported to exhibit anti-inflammatory, antitumor, immunostimulatory, antiandrogenic, antibacterial, antifungal, lipoxygenase inhibitory, hypocholesterolemic and cancer preventive activities Anyasor *et al.*, (2014) and Omotoso *et al.*, (2014). Similarly, Gnanavel and Saral, (2013) also observed the antioxidant activity of this compound. Additionally, hexadecanoic acid (palmitic acid) was the most abundant saturated fatty acid found in the plant. According to Rajeswari *et al.*, (2012), Anyasor *et al.*, (2014) and Omotosho *et al.*, (2014), this compound has been noted to have antioxidant, anti-inflammatory, hypocholesterolemic, antiandrogenic, 5-α reductase inhibitor and hemolytic activities. Gobalakrishnan *et al.*, (2014); Jiang *et al.*, 2013; Mgbeji *et al.*, (2016) also observed its anticancer and antimicrobial activities respectively. The anti-inflammatory activity of n-hexadecanoic was revealed from structure and kinetic study carried out by Aparna *et al.*, (2012) due to its ability to inhibit PLA2 competitively.

Other compounds identified in the extracts in terms of relative abundance include undecylenic acid, 9,12-octadecadienoyl chloride, (Z, Z)-, 10-undecenal, 9-undecenal, and 9,17-Octadecadienal, (Z)- which were present in noticeable quantities in *Carica* papaya seeds, were novel compounds in that their therapeutic attributes and biologic activities have not been previously reported elsewhere. The number of peak values revealed by FTIR spectroscopic analysis of *Carica papaya* seed extracts demonstrated the presence of functional groups which are indicative of secondary metabolites and other bioactive compounds. The presence of these compounds in *Carica papaya* seed extract underscores its ability to possess biological activity. This is in line with the work of Maobe and Nyarango, (2013) who reported that these functional groups confirm the presence of secondary metabolites and other phytochemical components present in plants.

CONCLUSION

The present study revealed that extracts of *Carica papaya* seed contained a lot of metabolites and therapeutic active substances. These substances could be further isolated and investigated to confirm their pharmacological activities. Hence, this study supports the use of the *Carica papaya* seed in alternative/traditional medicine. Further research is ongoing to examine the pharmacological activities of the seed extracts especially in the treatment of oxidative stress resulting from hyperglycemia.

ACKNOWLEDGMENT

We sincerely wish to appreciate and thank Mr. Ibrahim Ahmed Hayatu for his valuable contribution towards the success of this research.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

Figure 1: *Carica papaya* fruit showing the seed and dried seed

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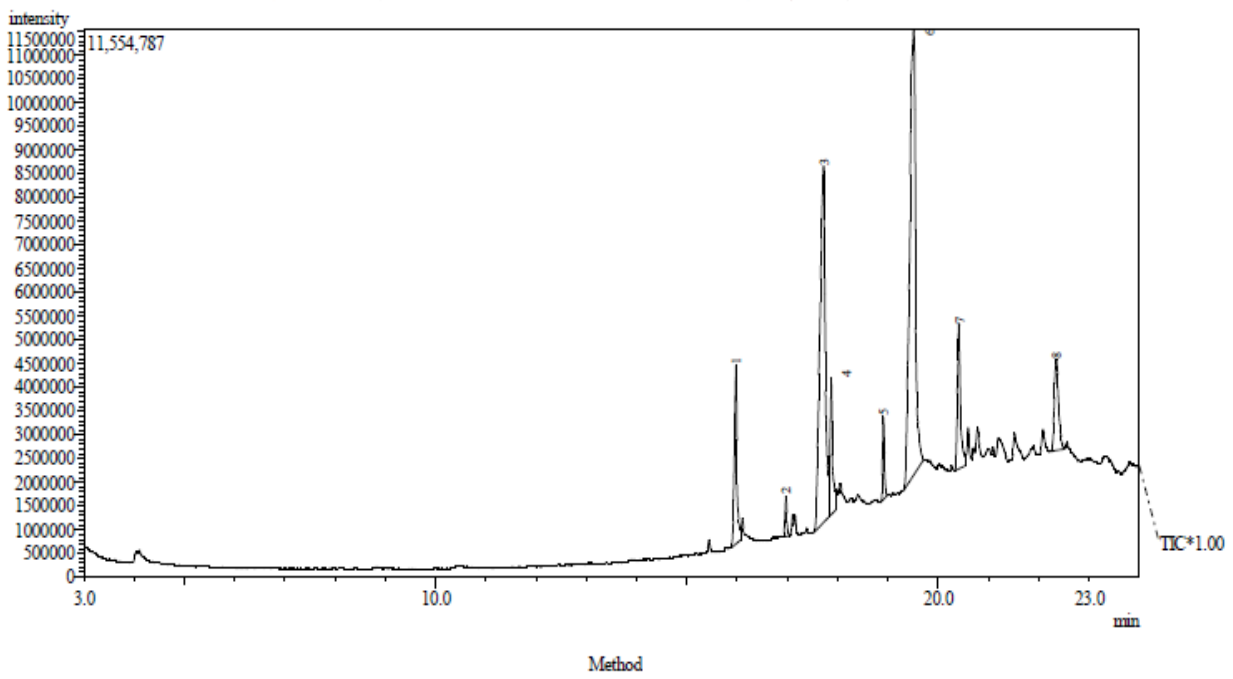

Figure 2: GC-MS Chromatogram of methanol extract of *Carica papaya* seed

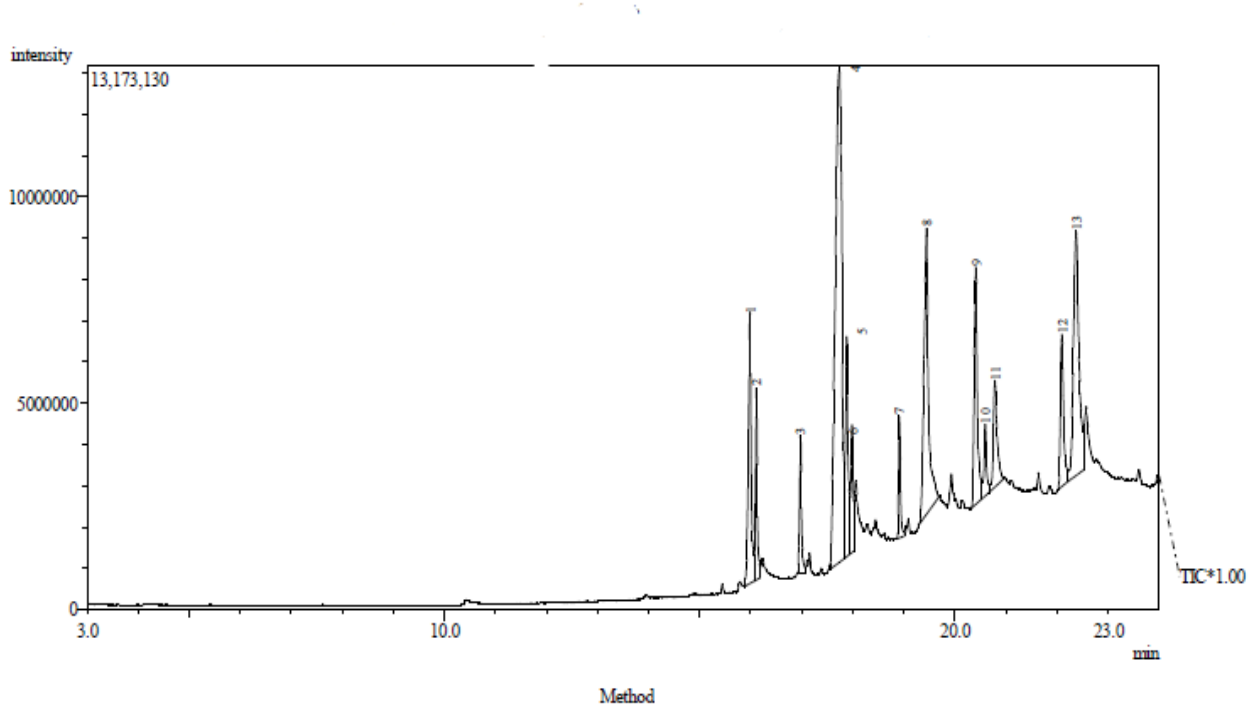

Figure 3: GC-MS Chromatogram of aqueous extract of *Carica papaya* seed

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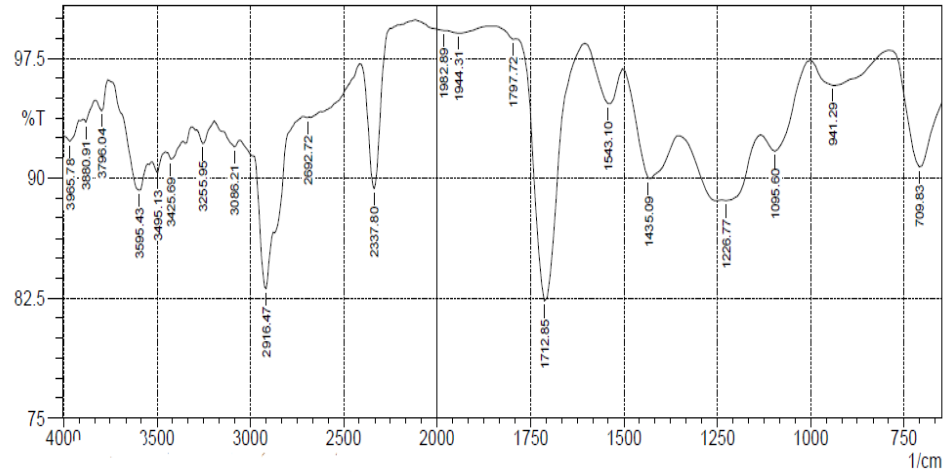

Figure 4: FTIR spectrum of methanol extract of *C. papaya* se

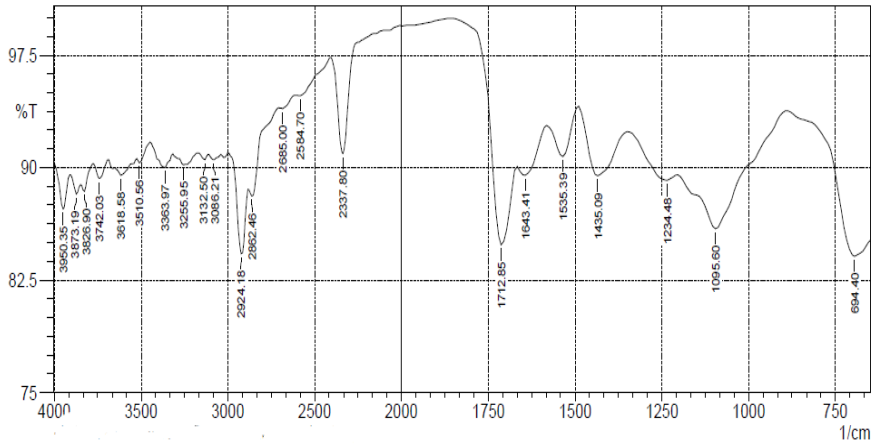

Figure 5: FTIR Spectrum of aqueous extract of *C. papaya* seed

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