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IMPACT OF DIFFERENT SOLVENTS ON PHYTOCHEMICAL PROFILING AND ANTIOXIDANT ACTIVITY OF *CARICA PAPAYA* LEAF FROM NORTHWESTERN HIMALAYAN REGION

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Abstract

Carica papaya an eminent medicinal plant used in the Asian and West countries to go through with a diverse group of diseases. This study was conducted to investigate the total phenolic, flavonoid and antioxidant potential of *Carica papaya* leaves with four different solvents *i.e.* aqueous, chloroform, ethanol and methanol. Phenolic and flavonoid content was found to be high in ethanol extract *i.e.* 73 mg GAE/g and 54 mg QE/g respectively. Ethanol extract of *Carica papaya* is also high in its antioxidant potential using DPPH and ABTS assay *i.e.* IC_{50} value 56.34 and 68.5 µg/ml. Ethanol was found to be the best solvent of choice for extracting natural compounds to obtain maximum medicinal benefits and could be used against various infectious diseases for medicinal formulation.

Key words : *Carica papaya*, Antioxidants, Phytochemicals, Total phenolic and flavonoid content.

Introduction

Medicinal plants are being used from ancient times in the Ayurvedic, Unani and other traditional medicine methods for treating the various diseases. These effects of plants are due to the presence of phytochemicals, such as phenolic acids, flavonoids, tannins, hydroxycinnamic acid and phenolic diterpenes, which are among the best known for their medicinal properties (Prakash *et al.,* 2017). WHO estimates that 80% of the world's population relies on medicinal plants for primary health care (Mothana *et al.,* 2008; WHO, 2002; Gupta *et al.,* 2010; Ngoci *et al.,* 2011; Prakash and Sandhu, 2012). Drugs produced from unmodified natural or semi-synthetic products derived from natural sources constituted 78% of new drugs approved by the Food and Drug Administration (FDA) of the United States (Suffredini *et al.,* 2006; Ngoci *et al.,* 2011). Plants are a valuable source of a wide variety of secondary metabolites used as pharmaceutical goods, agrochemicals, flavorings and additives (Alonso-Amelot, 2018). Due to the presence of different phytonutrients (Nostro *et al.,* 2000; Prakash *et al.,* 2016), medicinal plants are useful for the healing and cure of various human diseases.

Carica papaya belongs to family Caricaceae, commonly known as Papaya, Paw Paw, Kates, and Papaw. It is found in most tropical and subtropical countries of the world. *Carica papaya* is short-lived, single-stemmed/unbranched, hollow, perennial herbaceous with a varying height ranging from 10 to 30 ft. The papaya nuts, bark and leaves are used as medicinal items to treat various diseases such as warts, cancer cell growth, corns, constipation, amenorrhea, general debility, dyspepsia, sinuses, eczema, diabetes, malaria, cutaneous tubercles, glandular tumor, blood pressure, diabetes, malaria, expel worms and stimulate reproductive organs, syphilis, and gonorrhea (Aravind *et al.,* 2013). The literature suggests that fruit and leaf extracts from the *Carica papaya* are used to treat dengue fever. It is also stated that the *Carica papaya* leaf extract works to combat RBC sickling (Imaga *et al.,* 2009).

Therefore, the study aimed to determine the effects of aqueous and other organic solvents on the extraction yield, phytochemical and antioxidant potential of papaya

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leaves. This will help in determining its medicinal value, which may be useful in increasing the database for the medicinal plant or could be used as an antioxidant in food, pharmaceutical and medicinal preparations.

Materials and Methods

Collection of plant material

Leaves of *Carica papaya* were collected in March and April from the Solan district of Himachal Pradesh, India.

Morphological evaluation

In the morphological evaluation, various organoleptic characters such as color, odour, taste, size & shape were determined (Kokate *et al.,* 2005).

Preparation of extract

Extraction

Four different solvents were used for the extraction of bioactive compounds from the leaves of *Carica papaya* including aqueous, chloroform, ethanol and methanol. *Carica papaya* leaves were rinsed with distilled water. Afterward, the samples were dried under shade in the laboratory and then homogenized into a fine powder using a mortar and pestle. Powdered material was extracted with all the solvents. Then kept at the shaker for three days. Using Whatman filter paper No.1 (42µm), extracts were filtered and the filtrate was processed and used for further study (Turner, 2006).

Extraction yield

The extraction yield was determined from {(W1/W2)/ 100}, where W1 is the extract weight after solvent evaporation, and W2 is the plant sample dry weight.

Qualitative phytochemical analysis

Biochemical tests were done to check the presence of different phytochemicals such as alkaloids, flavonoids, saponins, steroids and tannins in *Carica papaya* leaves extract by following standard methods (Harborne, 1973; Sofowora, 1993; Roopashree *et al.,* 2008; Das *et al.,* 2010; Harborne, 2012).

Detection of alkaloids

Mayer's test

Extracts were dissolved in dilute hydrochloric acid and washed individually. The filtrate was treated with Mayer (Potassium Mercuric Iodide) reagent 2-3 drops. The development of a yellow color precipitate has indicated the presence of alkaloids.

Detection of carbohydrates

Benedict's test

Extracts were treated and heated gently with 2-3 drops of Benedict's reagent. Precipitate color yellow/ green/red showed the presence of reduced sugars.

Detection of flavonoids

Lead acetate test

Extracts of lead acetate were treated with a few drops of the solution. Precipitating the formation of yellow color indicated the presence of flavonoids.

Detection of tannins or phenols

Ferric chloride test

Extracts were treated with a solution of 3-4 drops of ferric chloride. The appearance of bluish or greenish-black coloration indicates the presence of pyrogallol or catechol tannins. The formation of a bluish-black color indicated the presence of phenols.

Detection of amino acid

Millon's test

3-4 drops of Millon's reagent were added to the extracts and heated. The presence of amino acids revealed the existence of white precipitate that changed to brick red on heating.

Total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu test following the updated procedure described by Singleton and Rossi, (1965). 5 mg of every extract was dissolved in 5 ml of methanol for the preparation of regular extract solutions. About 1 ml of Folin-Ciocalteu reagent was taken and diluted to 10 ml with distilled water. The working standards of each extract were prepared by mixing 1 ml standard solution with 9 ml distilled water. Added 1 ml of diluted Folin-Ciocalteu reagent to each test tube and allowed for 6 minutes to stand. Through adding distilled water and incubating at room temperature for 90 min, 10 ml of 7% sodium carbonate solution was then added to the reaction mixtures in each test tube and further diluted to 25 ml. The absorbance of the sample was measured with a 760 nm UV spectrophotometer. A calibration curve of the gallic acid (0 to 100 mg/ml) was drawn to determine the total phenolic content. The total phenolic content was measured as the equivalent milligrams of gallic acid (mg GAE / g) per gram of dry sample.

Total flavonoid content

Total flavonoid content was determined by the aluminum chloride colorimetric assay according to the method described by Meda *et al*., (2005). Approximately 5 mg of each of the extracts is dissolved in 5 ml methanol. 1 ml was combined with 9 ml distilled water from each

of these normal solutions and eventually with 1 ml $NaNO_2$ (5%). The mixtures were allowed to stand 6 min for a continued reaction. Then 2 ml of 10% aluminum chloride solution was applied to each and allowed to stand for 5 minutes. Then the mixtures were added with 2 ml of sodium hydroxide (1 M) in series. Eventually, a UV spectrophotometer was used to measure the absorbance of the mixture at 510 nm. Drawn for determining the normal quercetin curve of the total flavonoid material (0 to 100 mg/ml). The total content of flavonoids was measured as milligrams of equivalent quercetine (mg QE/ g) per gram of dry sample.

In-vitro antioxidant activity

DPPH radical scavenging activity

The method defined by Barros *et al*., (2007) calculated the radical scavenging behavior of the extract DPPH. DPPH solution was prepared by dissolving 20 mg DPPH in 100 ml (stock solution) methanol. 3 ml was taken from this solution, and its absorbance at 515 nm (control solution) was set to 0.75. To prevent free radicals, the DPPH stock solution was coated with aluminum foil and kept in the dark for 24 hours. 5 mg of each extract was dissolved in 5 ml methanol for the preparation of stock solutions. Different dilutions (25, 50, 75 and 100 µg / ml) were prepared from stock solutions through serial dilution. Approximately 2 ml of each dilution was mixed with a solution of 2 ml DPPH and incubated for 15 min in darkness. Ascorbic acid has been used as a typical antioxidant compound in all the assays for comparative analysis. The percentage inhibition of DPPH free radical by extracts was calculated using the following formula:

$$\% \text{ Inhibition} = (Ac\text{-}As/Ac) \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of the extract/standard. The free radical scavenging activity of samples was expressed as IC_{50} value, which represented the effective concentration of extract/standard required to scavenge 50% of DPPH radicals.

ABTS free radical scavenging assay

The 2, 2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) free radicals scavenging assay was used to determine the antioxidant potential of extracts (Re *et al.,* 1999). Solutions in 100 ml of methanol were prepared for ABTS (7 mM) and potassium persulphate (2.45 mM) These two solutions were thoroughly mixed for the formation of free radicals and kept in the dark overnight. Around 3 ml of this stock solution was taken and its absorbance at 745 nm was set to 0.76 (control solution). Approximately 300 µl of the test sample was mixed with 3 ml of ABTS solution and incubated at 25°C for 15 min. The absorbance of the mixture was measured using a spectrophotometer with a double beam of 745 nm. The same procedure was followed for the preparation of various ascorbic acid dilution (positive control). The data was collected in triplicates, and the formula used to measure the percentage of ABTS free radicals scavenging activity:

$$\% \text{ Inhibition} = (Ac\text{-}As/Ac) \times 100$$

FTIR analysis

Fourier transforms infrared (FTIR) spectroscopy is a technique used in the chemical investigation of substances. It is based on the measure of the vibration of a molecule by IR radiations at a specific range of wavelengths within chemical functional groups and generates a biochemical spectrum of the sample. FTIR spectra were recorded for the sample in the middle IR region (4000-4000 cm^{-1}) using an avatar-330 FTIR type instrument (David and Mauer, 2010; Ekpenyong, 2012).

Statistical analysis

Total phenolic, total flavonoid content and values of IC_{50} were calculated using linear regression analysis. In triplicates, each sample was evaluated independently and the results were expressed as mean value ± standard deviation (n=3).

Results and Discussion

Morphological features

Morphological features of the *Carica papaya* plant showed that leaves were 25-75 cm in diameter, the surface was fine, greenish or purplish-green, palmate, deeply 7-lobed, glabrous, prominently veined; lobes deeply and broadly toothed, with characteristic odour and bitter in taste (Table 1).

Table 1: Morphological characteristics of *Carica papaya* leaves.

Features	Observation
Color	Greenish or purple green
Odour	Unpleasant
Shape	Palmately lobed, veined
Size	25-75cm in width
Texture	Fine
Taste	Bitter

Extraction yield

The extracts from the dried leaves of the plant were made by using different solvents. Aqueous and other organic solvents were studied for their effects on the extraction yield of *Carica papaya*. Results showed a significant difference in the extraction yield using different solvents (Fig. 2). Among solvents tested, aqueous extract

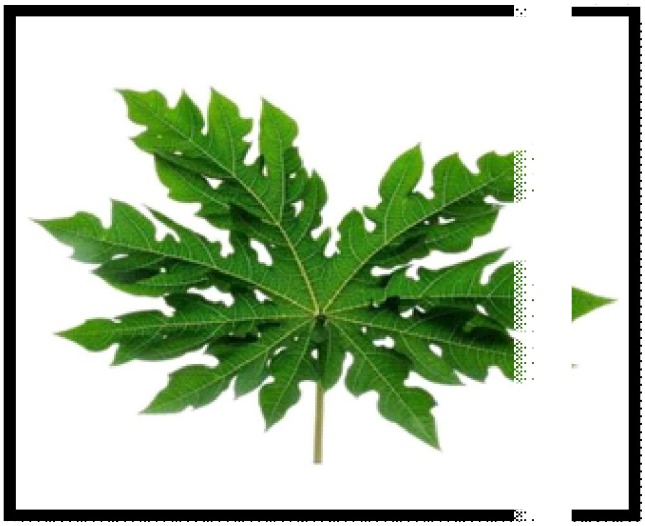

Fig. 1: *Carica papaya* leaf.

resulted in the highest extraction yield (14.98%), followed by methanol (11.88%), ethanol (9.33%) and chloroform (7.98%). The weight and percentage compositions are shown in (Table 2).

Asghar *et al*., (2016) reported that the extraction yield of papaya leaf extract was obtained in the following descending order; water (28%), methanol (15.90%), ethanol (11.47%), ethyl acetate (18.89%), dichloromethane (13.85%), n-butanol (08.25%) and n-hexane (08.56%). Solvent polarity, the structure of the extracted products, and method of extraction greatly affect the antioxidant and antibacterial activities of the plant extracts.

Table 2: The total yield of different extracts.

Extract	Amount of dried plants used (g)	Yield (in %)
Aqueous	5g	7.98%
Chloroform	5g	14.98%
Ethanol	5g	9.33%
Methanol	5g	11.88%

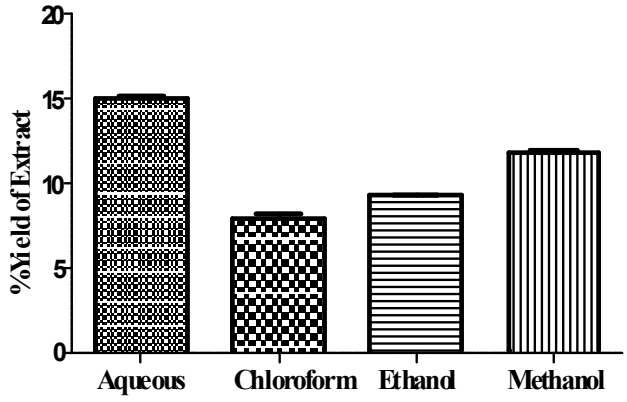

Fig. 2: Effect of different solvents on extract yield.

Phytochemical profiling

Phytochemical screening tests revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, and proteins. Similar findings were also reported by (Ayoola *et al.,* 2010; Sherwani *et al.,* 2013; Akhila *et al.,* 2015; Baskaran *et al.,* 2012).

Total phenolic and flavonoid content

The total phenolic content from the standard gallic acid curve was calculated using the equation: y=0.741x+0.1326, while the total flavonoid content was calculated using the standard quercetin curve using the equation: y=0.965x+0.0362. The extracts of different organic solvents of *Carica papaya* had a prominent yield. The most extractable solvents of the highest phenolic contents were ethanol and methanol (Fig. 3). The highest phenolic compound was achieved by ethanol extract (73.7 ± 2.77) followed by methanol (45.0 ± 2.04) and aqueous extract (36.3 ± 2.38). Whereas lowest phenolic content was obtained with chloroform extract (15.0 ± 2.08 mg GAE/g dry leave powder). The total flavonoid content was highest in ethanol extract (54.9 ± 2.38) followed by methanol (24.4 ± 3.10), aqueous (16.5 ± 3.11) and chloroform extract (11.9 ± 2.24) (Fig. 4).

Asghar *et al*. (2016) reported that the highest phenolic content was achieved with ethanol extract (65.12 mg GAE/g dry leave powder) followed by methanol extract (54.28 mg GAE/g dry leave powder). Dichloromethane extract (1.2 mg GAE/g dry root powder) provided the lower phenolic content. The highest flavonoid content was obtained by extracting ethanol (21.88 mg QE/g dry powder), followed by extracting methanol. The lowest content was in the dichloromethane extract (0.13 mg QE / g dry powder), followed by extracts of n-hexane and n-butanol.

Vuong *et al*., (2013) reported that the total phenolic content of *Carica papaya* fruit extracts with methanol and ethanol extract was found to be 15.03 and 9.43 mg GAE/g dry powder, respectively. Those contents were found to be lower than those obtained in this report. The total phenolic content of the ethanol solvent release extract showed 63.59 mg GAE/g crude powder which is in good agreement with the present study (73.7 mg GAE/ g dry leave powder).

Mandal *et al*., (2015) also estimated the total phenolic content of papaya leaf and it was found to be 57.6 ± 4.69 mg GAE/g dry extract. On the other hand, methanol extract produced the highest flavonoid content, *i.e.* an extract of 0.34 ± 1.34 mg QE/g.

Table 3: Total phenolic content of different extracts of *Carica papaya* leaves.

Phenolic content	(mg GAE/g)
Aqueous	15.5±2.08
Chloroform	36.3±2.38
Ethanol	73.7±2.77
Methanol	45.0±2.04

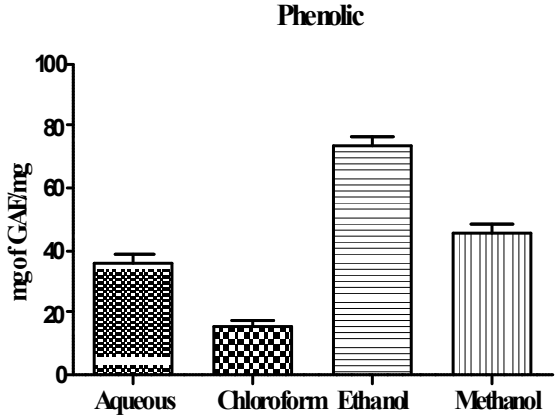

Fig. 3: Quantification of total phenolic content in different extracts of *Carica papaya.*

Table 4: Total flavonoid content of different extracts of *Carica papaya* leaves.

Flavonoid content	(mg QE/g)
Aqueous	11.9±2.24
Chloroform	16.5±3.41
Ethanol	56.9±2.38
Methanol	24.4±3.10

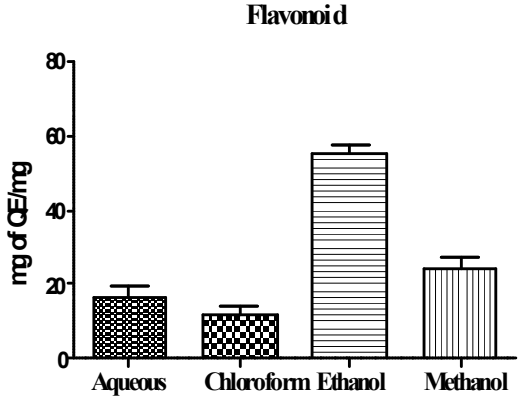

Fig. 4: Quantification of total flavonoid content in different extracts of *Carica papaya.*

Furthermore, Maisarah *et al*., (2013) recorded the highest phenolic and flavonoid content of young papaya leaves, *i.e.* 424.89 ± 0.22 mg GAE/100 g dry weight, and 333.14 ± 1.03 mg RE/100 g dry weight, respectively. The variance in solvent polarities will explain those variations.

***In vitro* antioxidant activity**

In the case of the DPPH assay, the percent free radical scavenging potential in ethanol extract was most potent with an IC_{50} value of 56.34 µg/ml. The IC_{50} values of other extracts; such as methanol, chloroform and aqueous were 86.48, 134.5 and 187.4 µg/ml respectively (Fig. 5). Ascorbic acid was used as a standard and its IC_{50} value was 33.0 µg/ml (Table 5). In the case of ABTS scavenging assay again ethanol extract was more potent with an IC_{50} value of 68.5 µg/ml. (Fig. 6) The IC_{50} values of other extracts; such as methanol, chloroform and aqueous were 72.5, 102.2 and 162.8 µg/ml respectively (Table 6). Ascorbic acid was used as a standard and its IC_{50} value was 28.2 µg/ml.

Fidrianny *et al*., (2016) reported that the lowest IC_{50} value of DPPH scavenging activity was found to be 0.84 µg/ml which was given by ethanol leaf extract of *Calina papaya*. The ethanol leaf extract of *Bangkok papaya* showed the lowest IC_{50} of ABTS scavenging activity i.e 1.79 µg/ml.

Asghar *et al*., (2016) recorded that the highest potential for free DPPH radical scavenging was found with *Carica papaya* ethanol leaf extract (75.05%) followed by pulp extract (68.07 %) with the same solvent. *Carica papaya* bark and root extract also showed promising potential for radical scavenging of DPPH; particularly for extracts of ethanol and methanol, where bark extract superseded the pulp's scavenging ability. Bark's highest potential for free DPPH radical scavenging could be attributed to the promising amount of phenolic and flavonoid content in their extracts.

Mandal *et al*., (2015) examined *Carica papaya* DPPH scavenging capacity for aqueous, methanol, and

Table 5: IC_{50} values of different extracts of *Carica papaya* leaves.

DPPH assay	IC_{50} (µg/ml)
Ascorbic acid	33.0
Aqueous	187.4
Chloroform	134.5
Ethanol	56.34
Methanol	86.48

Table 6: IC_{50} values of different extracts of *Carica papaya* leaves.

ABTS assay	IC_{50} (µg/ml)
Ascorbic acid	28.2
Aqueous	162.8
Chloroform	102.2
Ethanol	68.5
Methanol	72.5

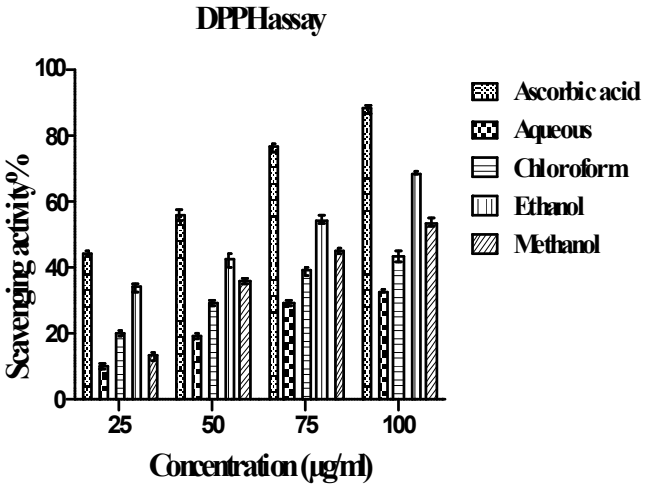

Fig. 5: DPPH free radical scavenging assay in different extracts of *Carica papaya.*

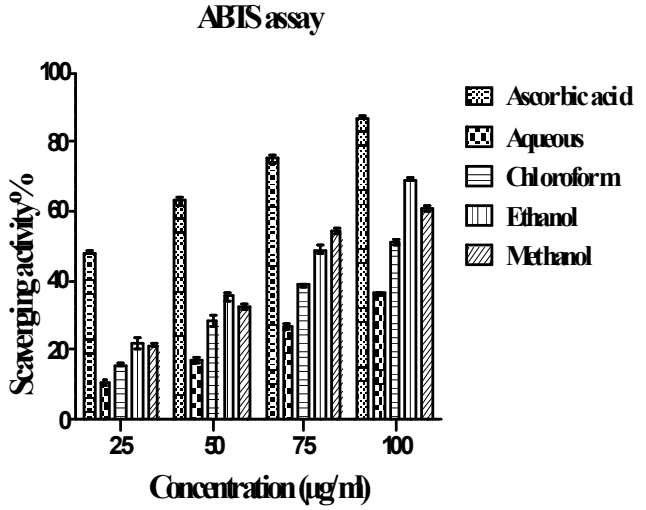

Fig. 6: ABTS free radical scavenging assay in different extracts of *Carica papaya.*

petroleum ether solvent method at different concentrations (25–325 µg / ml). All three extracts had been found to have possible scavenging activity. The *Carica papaya* plant IC_{50} value for aqueous, methanol and petroleum ether was 247, 262.18 and 171.52 µg/ml, respectively.

FTIR analysis

The FTIR spectrum can be used to identify the functional groups of the active components based on the absorption band values in the region of infrared radiations. The spectra obtained in this study showed that the *Carica papaya* leaf extract peak was observed at 3574, 2242, 2166, 2011, 1600 and 935 cm^{-1}, which were associated with O-H, C≡ N, S-CN, N=C=S, C-H, and C=C stretching. There was the presence of alcohol, nitrile, thiocyanate, isothiocyanate, aromatic compounds and alkenes respectively (Fig.7).

Setyawati *et al*., (2016) reported that the FTIR spectrum was 3463,958 cm^{-1}, respectively associated with N-H stretching, O-H groups, H-bonded alcohols, phenols, and carboxylate acid. We observed peaks of 2927,351 cm^{-1}, related to aldehyde, alkane C-H stretching and ketones, 1737,584 cm^{-1} associated with alkane C-H stretching, 1642,675 cm^{-1} associated with alkene C=C stretching, C=N stretching, N-H primary amine stretching,

Table 7: FTIR peak of *Carica papaya* leaf extract.

Frequency	Bond	Functional group
3574	O-H	Alcohol
2242	CN	Nitrile
2166	S- CN	Thiocyanate
2011	N=C=S	Isothiocynate
1600	C-H	Aromatic
985	C=C	Alkenes

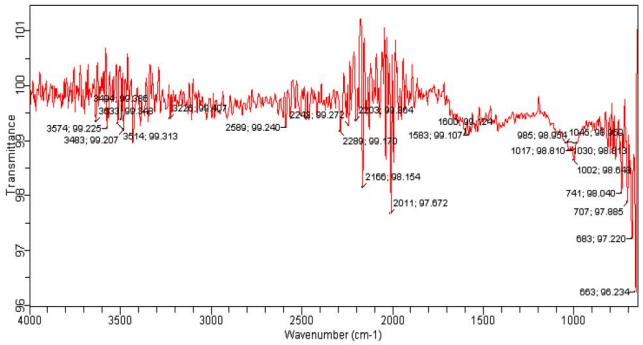

Fig. 7: FTIR peak of *Carica papaya* leaf extract .

and amide stretching. Meanwhile, the peak was observed at 1574,113 cm^{-1}, with C-H stretching alkanes, C=C stretching alkenes, C=N stretching, C-N stretching primary and secondary amines, and amide. 1384,386 cm^{-1}, joined to classes of O-H, H-bonded alcohol, phenolic and carboxylate acids.

Conclusion

This study was carried out to test the medicinal profile of the *Carica papaya* leaves by extracting secondary metabolites with aqueous and organic solvents. It can be adjudicated that total phenolic and flavonoid contents were high in ethanol extract. The study proved that the ethanol leaf extract of *Carica papaya* was also comparably high in antioxidant activity. From the results, it was concluded that this plant could be useful in the future for drug development for treating or preventing diseases.

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References

Akhila, S. and N.G. Vijayalakshmi (2015). Phytochemical Studies on *Carica Papaya* Leaf Juice. *International Journal of Pharmaceutical Science and Research,* **6:** 880-883.

Alonso-Amelot, M.E. (2018). Milk as a Natural Product: Foreign Natural and Anthropogenic Organic Compounds in it. Studies in *Natural products chemistry,* **56:** 335-435.

Aravind, G., B. Debjit, S. Duraivel and G. Harish (2013). Traditional and medicinal uses of *Carica papaya. Journal of Medicinal Plants Studies*, **1:** 7-15.

Asghar, N., S.A.R. Naqvi, Z.H.N. Rasool, Z.A. Khan, S.A. Shahzad, A. Sherazi and H.Z. Jaafar (2016). The compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. *Chemistry Central Journal*, **10:** 5.

Ayoola, P.B. and A. Adeyeye (2010). Phytochemical and nutrient evaluation of *Carica papaya* (pawpaw) leaves. *International Journal of Recent Research and Applied Studies,* **5:** 325-328.

Barros, L., M.J. Ferreira, B. Queiros, I.R. Ferreira and P. Baptista (2007). Total phenols, ascorbic acid, b-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry,* **103:** 413-419.

Baskaran, C., V. Ratha bai, S. Velu and K. Kumaran (2012). The efficacy of *Carica Papaya* leaf extract on some bacterial and a fungal strain by well diffusion method. *Asian Pacific Journal of Tropical Disease*, **2:** 658-S662.

Das, K., R.K.S. Tiwari and D.K. Shrivastava (2010). Techniques for evaluation of medicinal plants as antimicrobial agents: Current methods and future trends. *Journal of Medicinal Plants Research,* **4:** 104-111.

Davis, R. and L.J. Mauer (2010). Fourier transform infrared (FTIR) spectroscopy: A rapid tool for detection and analysis of foodborne pathogenic bacteria. *Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 1582-1590.

Ekpenyong, C.E., E.E Akpan and N.S. Udoh (2012). Phytochemistry and Toxicity Studies of *Telfairia occidentalis*. Aqueous Leaves Extract on Liver Biochemical Indices in Wistar Rats, **2:** 103.

Fidrianny, I., K.A. Paramitha and S. Kusmardiyani (2016). Antioxidant activities from various leaves extracts of three cultivars of papaya from west java-Indonesia. *Asian Journal of Pharmaceutical and Clinical Research,* **9:** 299-303.

Gupta, V.K., C. Shukla, G.R.S. Bisht, S. Kumar and R.L. Thakur (2010). Detection of anti-tuberculosis activity in some folklore plants by radiometric BACTEC assay. *Letters in Applied Microbiology,* **52:** 33-40.

Harborne, J. (2012). Phytochemical methods: A guide to modern techniques of plant analysis, Springer Science & Business Media.

Harborne, J.B. (1973). Phytochemical Methods. Chapman and Hall Ltd., London, 49-188.

Imaga, N.O., G.O. Gbenle, V.I. Okochi, S.O. Akanbi, S.O. Edeoghon and V Oigbochie (2009). Antisickling property of *Carica papaya* leaf extract. *African Journal of Biochemistry Research,* **3:** 102-106.

Khan, S.S., T.Z. Bhokri, K. Nazeem and S.A. Gilani (2013). Qualitative phytochemical screening and antifungal activity of *Carica Papaya* leaf extract against human and plant pathogenic fungi. *International Research Journal of Pharmacy*, **4:** 83- 86.

Kokate, C.K. (2005). Practical Pharmacognosy. 4th ed. Delhi, Published by Vallabh Prakashan, 22-23.

Maisarah, A.M., B. Nurul Amira, R. Asmah and O. Fauziah (2013). Antioxidant analysis of different parts of *Carica papaya. International Food Research Journal*, **20:** 1043-1048.

Mandal, S.D., R. Lalmawizuala, M. Vabeiryureilai, N.S. Kumar and E. Lalnunmawii (2015). An Investigation of the Antioxidant Property of *Carica papaya* Leaf Extracts from Mizoram, Northeast India. Res*earch and Reviews: Journal of Botanical Sciences,* **4:** 42-46

Meda, A., C.E. Lamien, M. Romito, J. Millogo and O.G. Nacoulma (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry,* **91:** 571-577.

Mothana, R.A., S.A. Abdo, S. Hasson and F.M. Althawab (2008). Antimicrobial, Antioxidant and Cytotoxic Activities and Phytochemical Screening of Some Yemeni Medicinal Plants. *Journal of Evidence-based Complementary and alternative medicine*, **7:** 323-330.

Ngoci, S.N., C.M. Mwendia and C.G. Mwaniki (2011). Phytochemical and cytotoxicity testing of *Indigofera lupatana* Baker F. *Journal of Animal & Plant Sciences,* **11:** 1364-1373.

Nostro, A., L. Cellini, S. Bartolomeo, M. A. Cannatelli, E. Campli, F. Procopio, R. Grande, L. Marzio and V. Alonzo (2006). Effects of combining extracts (from *Zingiber officinale*) with clarithromycin on *Helicobacter pylori. Phytotherapy Research*, **20:** 187-190.

Prakash, V. and P. Sandhu (2012). In vitro antimycobiotic and antibacterial action of seed extract of *Celastrus paniculatus* Willd. (Jyotismati). *Journal of Antimicrobials-Photon*, **127:** 123-132.

Prakash, V., S. Rana and A. Sagar (2016). Studies on antioxidant activity of some medicinal plants. *International Journal of Pharma and Biosciences*, **7:** 246-253.

Prakash, V., S. Rana and A. Sagar (2017). Studies on analysis of antioxidant and enzyme inhibitory activity of *Vitex negundo* Linn. *International. Journal of Pharmacognosy and Phytochemical Research*, **9:** 833-839.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Eas (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine,* **26:** 1231-7.

Roopashree, T.S., D. Raman, S.R.H. Rani and C. Narendra (2008). Antibacterial activity of antipsoriatic herbs: *Cassia tora, Momordica charantia* and *Calendula officinalis*. *International Journal of Applied Research in Natural Products*, **1:** 20-28.

Setyawati, D., S. Andayani and U. Yanuhar (2016). Characterization of Fraction of *Carica papaya* L. Leaves Ethyl Acetate Extract to African Catfish *Clarias gariepinus* Leucocytes Using UV-Vis, FTIR and GC-MS Methods. *International Journal of Chem Tech Research,* **9:** 247-253.

Singleton, V.L. and J.A. Rossi (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *The American Journal of Enology and Viticulture*, **16:** 144-158.

Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, 191-289.

Suffredini, B.I., M.L.B. Paciencia, D.C. Nepomuceno, R.N. Younes and A.D. Varella (2006). Antibacterial and cytotoxic activity of Brazilian plant extracts- Clusiaceae. Memórias do Instituto Oswaldo Cruz, **101:** 1590-1598.

Turner, C. (2006). Overview of modern extraction techniques for food and agriculture samples.

World Health Organization (WHO) (2002). Traditional Medicine Strategy. 2004-2005. *WHO Publication,* 1-6.