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Full Length Research Article

Evaluation of Antimicrobial and Antioxidant Activity of Bioactives in *Carica papaya*

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Abstract

The main aim of the current study is to investigate the ability of extract from *Carica papaya* leaves. Various sections of papaya have been used as ethnomedicine for a variety of disorders including cancer and dengue. There are many patients with advanced cancers achieving suspension followed by drinking the tea extract of papaya leaves. Initially, extract of the leaves was subjected to phytochemical tests, Gas chromatography- mass spectroscopy (GC-MS) and Fourier transform infrared spectroscopy (FTIR) analysis. Phytochemical tests indicate the presence and absence of the compounds like alkaloids, flavanoids, terpenoids and many more. GCMS classifies ions based on their mass to charge ratio and ionizes atoms, molecules, ions and other chemical compounds to estimate their bio-actives and molecular weights. FTIR confirmed the structural groups by peak values indicating groups such as alcohol, phenol, alkanes, proteins and isopropyl were found. Studies shows that *Carica papaya* leaves are a potent antimicrobial agent with wide variety of medicinal bio-actives. The extract of the papaya leaves are also used to cure a lot of illness such as eye infections, gastrointestinal problems, urinary tract infections, joint pain and the papaya leaves also have the properties to treat dengue and malarial fever. *Carica papaya* is rich in tannin, saponin, flavanoids, protein etc. The papaya leaf extract holds numerous nutritional values like magnesium, calcium, iron, manganese, zinc and vitamin A, B, C, E and K. It also contains good anti-oxidant and anti-bacterial properties.

***Key words*:** *Carica papaya*, GC-MS, FTIR, Dengue fever, Cancer, Leaves extract

Carica papaya is commonly known as papaya and is part of *Caricaceae* family. It is a tropical fruit plant and it is mostly grown in tropical and sub-tropical countries. The papaya leaves are huge, averaging 50-70 cm in diameter and has seven lobes which are deeply palmately lobed and at times it may contain more than seven lobes. All the parts of the tree including leaves, stem, seeds are edible and having great medicinal properties. The juice made from the papaya leaves is used to cure a lot of illness such as eye infections, gastro-intestinal problems, urinary tract infections, joint pain and by smoking the dried leaves it treats asthma and are used as a substitute of tobacco. The papaya leaves are also having the properties to treat dengue and malarial fever. The papaya leaves are able to fight against the symptoms of malaria. As papaya leaves are rich in anti-oxidants and anti-cancerous, it can help cancer patients with benefits. In comparison with the enzymes of ripened papaya, unripened papaya holds higher enzyme concentration. In most of the countries, the mashed papaya stems are served with sugar and salt as a healthy good dessert.

The papaya leaves holds numerous nutritional values like magnesium, calcium, iron, manganese, zinc and vitamins A, B, C, E and K. Plants of papaya grow in three genera: male, female and hermaphrodite. The male part never yields fruit but produce pollens only [1]. The female part produces small fruits which are inedible unless pollinated. But the hermaphrodite part is different from the other two because its flowers contain both female ovaries and male stamens which help in self-pollination. The papaya leaf extract is also used to treat liver diseases, jaundice and liver cirrhosis thereby acts as a liver cleanser. The papaya juice kills *Helicobactor pylori* which causes peptic ulcer and it shows the anti-microbial properties of papaya leaves. The papaya leaf juice can heal the excruciating menstrual pain in women. Because the papaya leaf is rich in vitamin A and C it improves the protection of the skin and protects against pimples, acne, scar and freckles.

Acetogenin is a compound found in papaya leaves which causes papaya leaves to treat cancers such as lung cancer, hepatic cancer, pancreatic cancer, breast cancer. Papaya leaves also has side effects like it causes allergic reactions in some sensitive people and the papaya latex can be terrible irritant on skin. It may lead to abortion in women depending on the part of the plant and dosage taken. As the papaya seeds contain benzyl isothiocyanate, it may be toxic if taken in high doses.

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The papaya seeds are similar to black pepper and used as an adulterant of black pepper. The papaya leaf extract contains a variety of phytochemicals including phenols, flavanoids, triterpenoids, terpenoids, steroids, glycosides, saponin. The phytocomponents present in the papaya leaf extract is analyzed by gas chromatography mass spectrometer (GC-MS). And the Fourier Transform Infrared Spectroscopy (FTIR) is performed to analyze the organic and inorganic compounds in the papaya leaf extract by using infrared light to scan the samples. The papaya leaves are also having the immunomodulatory and anti-inflammatory properties. The chronic inflammation is associated with generation and at times it may be the result of various diseases like diabetes, cancer, arthritis, auto immune diseases, asthma, hepatitis and in case of any transplant rejection and the papaya leaves naturally has the power to treat these diseases.

MATERIALS AND METHODS

Plant sample

The samples of *Carica papaya* obtained from Bannari Amman Institute of Technology, Sathyamangalam was taken to laboratory. The leaves were washed properly and made into small pieces. The chopped pieces were then made to shadow dry at room temperature for 72 hours. The dried leaves were later converted by using mixie into fine powder form, and then placed in the airtight sample box.

Preparation of the leaf extract

The fine sample powder of about 8g was taken and packed in the filter paper. Then the Soxhlet's extraction process was carried out at 8 °C for 5 cycles and the solvent used is acetone. After completing the extraction, the obtained extract was concentrated to 15ml through simple boiling process.

Preliminary qualitative phytochemical tests
The presence or absence of the phytochemical components was examined by making use of the following procedures

Protein test: Biuret's test- The leaf extract of 1ml was added with 2% NaOH and 0.3% $CuSO_4$. The presence of protein is confirmed by the occurrence of pink colour in the solution.

Tannin test: Ferric Chloride test- The leaf extract of 1ml and 5% ferric chloride were merged together and the occurrence of green colour indicates the presence of tannins.

Saponin test: Foam test- The leaf extract was mixed strongly with 5ml distilled water. The tenacity of foam after 15 minutes shows the presence of Saponins.

Flavonoid test: Alkaline Reagent Test- 1ml of the leaf extract was allowed to mix properly with 1ml of 2N NaOH and the presence of Flavonoids is confirmed by the occurrence of yellow colour.

Alkaloid test: Mayer's Test- The leaf extract of 1ml and 2ml of conc. HCl were agitated together and then few drops of Mayer's reagent were added. The occurrence of white colour precipitate confirms the presence of Alkaloids.

Glycoside test: Borntrager's Reagent- 1ml of the leaf extract was taken and added to 3ml chloroform and 10% ammonium solution. The appearance of pinkish red colour indicates the presence of Glycosides.

Terpenoid test: The leaf extract of 1ml was mixed with 2ml of chloroform and then few drops of conc.H2SO4 were added. The formation of red brown colour at the interface indicates the presence of Terpenoids.

Phenol test: Ferric Chloride Test- 1ml of leaf extract and 2ml of distilled water were agitated together and then few drops of 10% ferric chloride were added. The formation of green colour shows the presence of Phenol.

Steroid test: Equal volume of acetic acid and chloroform was added to 1ml of the leaf extract followed by a few drops of conc. H_2SO_4. The occurrence of violet brown ring indicates the presence of Steroids.

Lipid test: 1 ml of the leaf extract was added with 0.1N alcoholic potassium hydroxide accompanying the drops of phenolphthalein which is then heated in the boiling water bath for 1 hour. The formation of soapy appearance shows the presence of lipids [2].

GC-MS analysis

The chemical configuration of the leaf sample was examined by Gas chromatography mass spectrometry. The analysis was attained using GC-MS Perkin Elmer model: Clarus 680 which is fitted with mass spectrometer Clarus 600 (EI) and examined using Turbo Massver 5.4.2 software. The Fused silica capillary column which is composed of Elite-5MS (5% biphenyl 95% dimethylpolysiloxane 30m * 0.25mm ID *250 µm df). The constant flow rate is about 1ml/min and carrier gas such as helium was used to separate the components. The temperature of the injector was maintained at 260 °C. The extract sample of 1µl was injected into the equipment and the temperatures of the oven were 60 °C for about 2 minutes and 300 °C at the rate of 10 °C/min and 300 °C for 6 minutes [3]. The conditions of the mass detector were: the temperature of transfer line was 240 °C and ionization mode of electron impact at 70eV, the duration time of interval of scanning is 0.2sec and scan interval is 0.1sec. The fragments are from 40 to 600Da. The spectrum of the particles present was comparable to the database of the spectrum of established particles gathered in the GC-MS NIST library [4].

FTIR analysis

The FTIR technique was used to examine the extract of papaya leaves. The extracted material was mixed with KBr in a 1:9 ratio and pelletized using a KBr hydraulic press at a pressure of around 10 tonnes. The functional moieties in the pellet were investigated using FTIR in the 400-4000 cm^{-1} infrared region. The C-H stretching of the alkaloids (Z, Z-6,13-Octadecadien-1-ol Acetate) contained in the extract caused the stretching at 3304.44 cm^{-1}. The aldehyde C-H stretch- ing (6,11-Undecadiene,1-Acetoxy-3,7-Dimethyl) of the ex- tract caused the stretching at 2923.38 cm^{-1} and 2852.71 cm^{-1}. C=O stretching caused the area between 1700 and 1600 cm^{-1}, whereas C=C and acyl C=O stretching caused the area between 1500 and 1300 cm^{-1}. The alkoxy C-O stretching found in the extracted compound ranges from 1200 cm^{-1} to 1000 cm^{-1} (1,9-Nonanediol, Dimethanesulfonate). The presence of Z, Z-6,13-Octadecadien-1-ol Acetate, 6,11-Undecadiene,1-Acetoxy-3,7-Dimethyl, 6,8-Dodecadien-1-ol(6Z,8E) and 1,9-Nonanediol Dimethanesulfonate was determined by FTIR analysis.

Antibacterial assay

The indicator organisms were procured from Microbial Type Culture Collection, Chandigarh and National Collection

of Industrial Microorganisms, Pune, for antibacterial testing. The antibacterial assay of *Carica papaya* was performed using five gram-positive bacteria like *Listeria monocytogenes*, *Bacillus subtilis*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus faecalis* and 3 gram-negative bacteria like *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella aerogenes* by following well diffusion agar method [5]. The following bacteria was inoculated in sterile nutrient plates individually in triplicates by using swabbing technique. The wells were made in the diameter of 3.5 mm using the sterile agar well puncher. The leaf extract sample was diluted at different concentration (20%, 40%, 60%, 80% and 100%) using distilled water. Then the agar plates were incubated at ±37 °C. After 24 hours of incubation, the zone of inhibition was measured in mm [6].

Antioxidant activity

The antioxidant property of the extract was done by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Both samples and standards were taken in different concentrations and the volume was adjusted to about 100 l using methanol. The DPPH solution of 0.1mM was prepared by using methanol as solvent [7]. About 3ml of 0.1mM solution of DPPH was mixed with samples of different concentrations and the negative control was prepared by mixing 100 l of methanol and 3ml of the solution. The tubes along with mixtures are allowed to stand in dark at room temperature for 30mins. The change in shading from violet to yellow shows the nearness of cancer prevention agents and the evaluation was by estimating its absorbance at 517nm against the clear [8]. The IC_{50} esteem (inhibitory focus) was determined for both sample and standard. The level of hindrance was determined utilizing the accompanying formula:

$$\% \text{ of hindrance} = [A_0 - A_1 / A_0] * 100$$

Where A_0 is the absorbance of control (for example DPPH arrangement without test) and A1 is absorbance of test or on the other hand standard (for example DPPH arrangement with test/standard) [9].

RESULTS AND DISCUSSION

It is shown by many researches that the fruit, root, bark, latex and leaves of papaya tree are very effective and used as ethno medicine [10]. They are used for the treatment of Stomach Ache, Dengue, Cancer like Liver Cancer, Lung Cancer, Breast Cancer and Pancreatic Cancer and they are used as anti-cancerous agents. Consuming papaya help to reduce risk of heart disease, aiding in digestion, lowering blood pressure, diabetes and improving wound healing [11]. There are so many chemical compositions of different parts of papaya plant. Papaya fruit contains carbohydrate, protein, fat, fibre, minerals, calcium, phosphorus, iron, vitamin C and many more. Seed contains crude proteins, crude fibre, fatty acids, papaya oil, benzylisothiocynate, benzylglucosinolate etc. [12]. Bark contains glucose, sucrose, fructose, and galactose. Leaves mainly contain alkaloids carpain, pseudocarpain and dehydrocarpaine, choline, vitamin C and E. Papaya is termed as a rich source of antioxidants, phytonutrients and flavanoids which prevent our body cells from undergoing free radical damage keeping wrinkles and other signs of ageing at bay [13]. The leaf extract treats skin diseases. Unripe fruit is harmful and even lead to allergic response [14].

The phytochemical studies displayed the presence of compounds such as tannins, phenols, glycosides, triterpenoids, steroids, proteins, flavanoids, saponins, alkaloids and lipids whereas, terpenoids were absent in the aqueous extract of our leaf sample. The compounds present in *Carica papaya* which were identified by phytochemical analysis have anti-oxidant, anti-microbial and anti-bacterial properties. The result of the phytochemical analysis is given in (Table 1).

Table 1 The phytochemical components of *Carica papaya*

Phytochemical constituents	Presence / Absence
Tannins	+
Saponins	+
Lipids	+
Flavonoids	+
Alkaloids	+
Glycosides	+
Proteins	+
Terpenoids	-
Phenols	+
Steroids	+

+: Presence; -:Absence

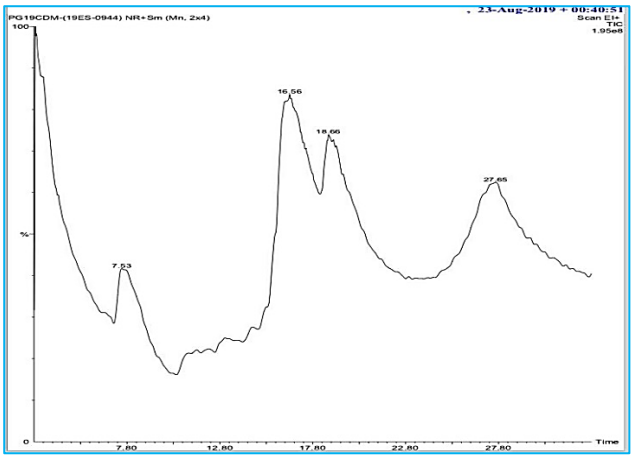


Fig 1 The chromatogram of compounds present in the *Carica papaya*

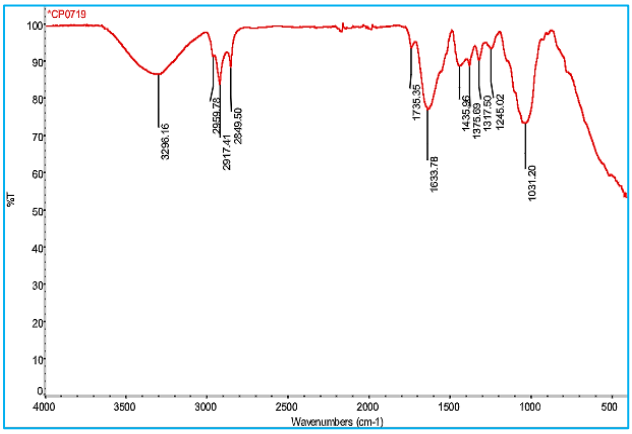
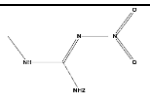
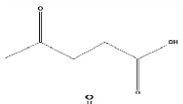
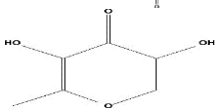
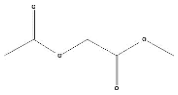
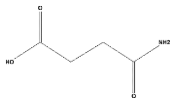
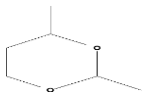
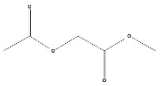
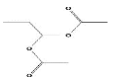
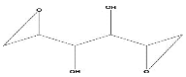
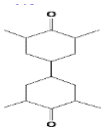
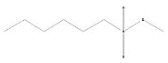


Fig 2 The structural compounds present in the extract were predicted by using FTIR analysis is shown in the graph

The GCMS results exhibited the components present in the extract and that is given in the below (Table 2). The below attached chromatogram shows that the molecules 1,3-DIOXANE,2,4- DIMETHYL- and 4,4'-BISCYCLOHEXANE,2,2',6,6'-TETRAMETHYL- own maximum peaks. The molecules ETHYL ACETATE and GUANIDINE, METHYL- possess highest peaks. Except the molecule GUANIDINE, METHYL- all the molecules are carrying oxygen group in it and so the molecules are having their anti-oxidant behaviour. Also, the molecules are comprised of anti-microbial, anti-cancer, anti-bacterial properties [15].

Table 2 GCMS analysis result for major phytochemical components in *Carica papaya* extract

Serial No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structure
1	15.734	N-Methyl-N'-Nitroguanidine	$C_2H_6O_2N4$	118	5.347	
2	15.954	Pentanoic Acid,4-Oxo-	$C_5H_8O_3$	116	6.082	
3	16.074	4h-Pyran-4-One,2,3-Dihydro-3,5-Dihydroxy-6-Methy-	$C_5H_8O_3$	116	8.821	
4	16.339	Methyl Acetoxy Acetate	$C_5H_8O_3$	116	35.511	
5	16.474	Succinamic Acid	$C_5H_8O_3$	116	3.652	
6	16.584	1,3-Dioxane,2,4- Dimethyl-	$C_5H_8O_3$	116	8.574	
7	16.649	Methyl Acetoxy Acetate	$C_5H_8O_3$	116	9.590	
8	16.784	2-Propene-1,1-Diol, Diacetate	$C_5H_8O_3$	116	4.538	
9	16.889	1,2:5,6-Dianhydrogalactit Ol	$C_5H_8O_3$	116	4.793	
10	17.004	1,3-Dioxane,2,4- Dimmethyl-	$C_5H_8O_3$	116	3.097	
11	18.935	4,4'- Biscyclohexanone,2,2',6,6'-Tetramethyl-	$C_{16}H_{26}O_2$	250	2.474	
12	19.055	1-Hexanesulfonic Acid, Methyl Ester	$C_7H_{10}O_2$	126	3.519	
13	19.165	Decyl Trifluoroacetate	$C_{16}H_{26}O_2$	250	4.003	
14	27.614	Guanidine, Methyl-	$C_2H_7N_3$	73		
15	7.530	Ethyl Acetate	$C_4H_8O_2$	88		

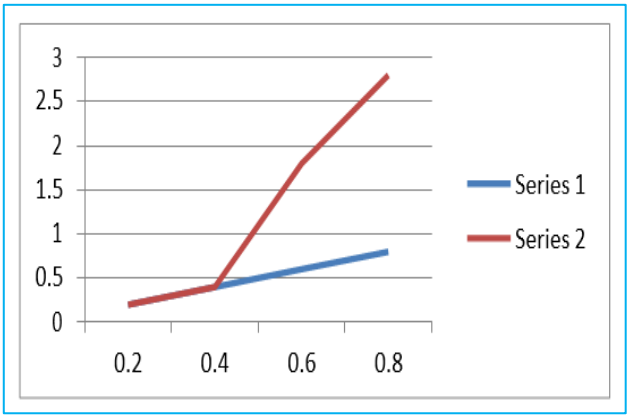
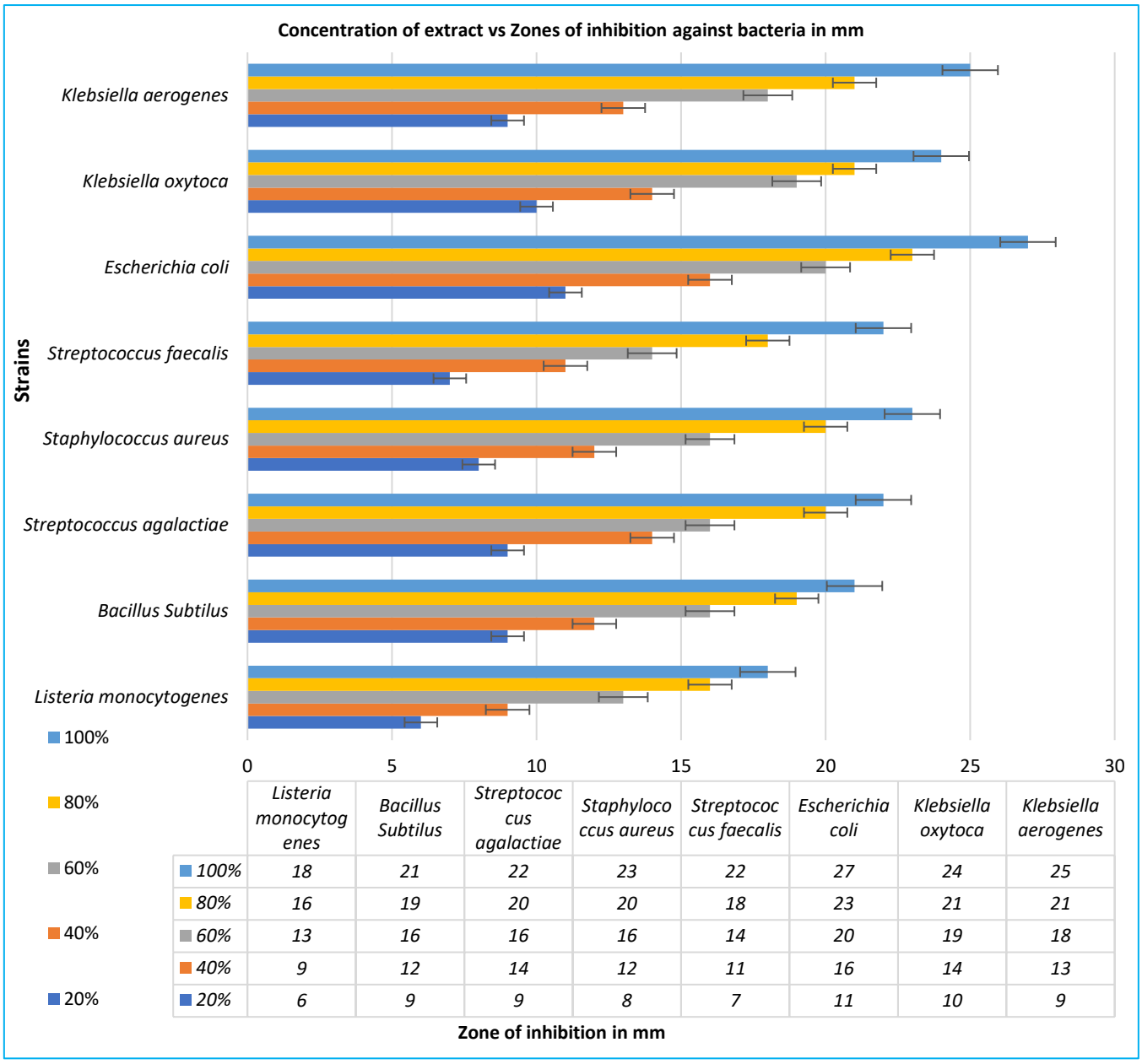


Fig 3 The above graph is the result of anti-oxidant activity which is provided with the IC50 values for both the standard and the sample

The antimicrobialproperties were carried out to identify whether the extract undergoes antibacterial activity [16]. The well diffusion assay was also performed to find the antibacterial properties. The clear region around the well exhibits no bacterial growth and this is called the zone of inhibition [17]. The different variety of bacterial strains which were used to perform the antibacterial activity are *Listeria monocytogenes, Bacillus subtilis, Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli, Streptococcus faecalis, Klebsiella aerogenes, Klebsiella oxytoca*. Among all these strains, *Escherichia coli* and *Klebsiella* have shown the highest susceptibility to the plant extract, (Fig 4) *Streptococcus faecalis* has exhibited moderate sensitivity. The bacterial species *Listeria monocytogenes* showed lower susceptibility [18].

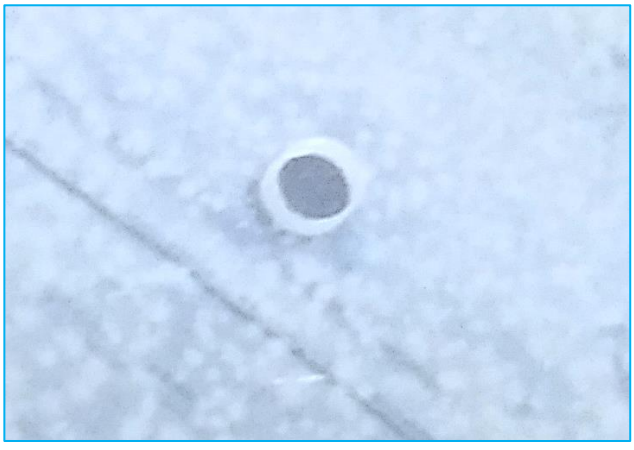
DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) is a process to detect the antioxidant activity. Generally, all the

plants have antioxidant activity which is responsible for the arrest of various reactive species as a result of DNA damage [19]. Ascorbic acid was used as the reference for comparison with the samples of different concentration and IC_{50} (inhibitory concentration) was measured for both sample and standard graph shows the anti-oxidation characteristics versus *C. papaya* (sample) concentration [20]. The DPPH has given the strong results for the various concentrations with the curvy rising graph.

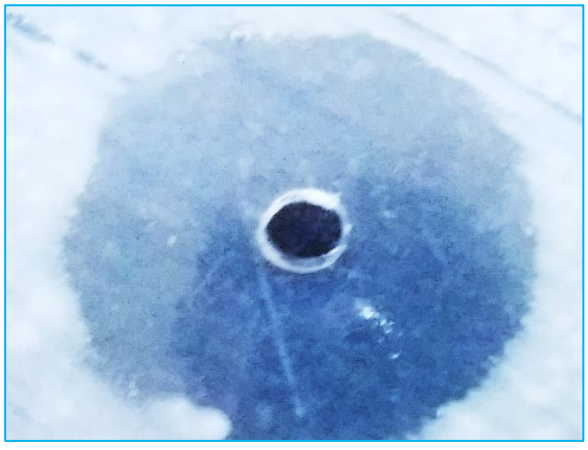


	Listeria monocytogenes	Bacillus Subtilus	Streptococcus agalactiae	Staphylococcus aureus	Streptococcus faecalis	Escherichia coli	Klebsiella oxytoca	Klebsiella aerogenes
100%	18	21	22	23	22	27	24	25
80%	16	19	20	20	18	23	21	21
60%	13	16	16	16	14	20	19	18
40%	9	12	14	12	11	16	14	13
20%	6	9	9	8	7	11	10	9

Fig 4 Antibacterial activity by well diffusion method, concentration of extract vs Zones of inhibition against bacteria in mm



Listeria showing least zone of inhibition



E. coli showing the highest zone of inhibition

Fig 5 Formation of zone of inhibition by the extract of *Carica papaya*

CONCLUSION

The phytochemical analysis of the papaya leaves indicates the presence of tannin, flavanoids, proteins etc. The antimicrobial activity of the leaf sample shows higher activity in *Streptococcus faecalis* and *Listeria monocytogenes* of the zone of inhibition at different concentrations. The antioxidant compound in the *Carica papaya* leaves extract can be used to inhibit oxidation, which may produce free radicals and chain reaction that may cause some diseases such as heart disease, cancer and other diseases.

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Ethical Clearance: Nil

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