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PHYTOCHEMICAL PROFILING, GC-MS ANALYSIS, AND ANTIOXIDANT CAPACITY OF EXTRACTS OF *CARICA PAPAYA* LEAVES

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ABSTRACT

Carica papaya L. belongs to the family Caricaceae and is a well-known medicinal herb that is used all over the world to cure diseases such as malaria, dengue fever, inflammation, skin infections, etc. The present study is, therefore, aimed to investigate the phytochemical constituents, GC-MS analysis, antioxidant potential, total phenolic & flavonoid contents in the leaf extracts of *C. papaya*. Many secondary metabolites are present in both aaqueous and methanolic extracts. TLC showed total 5 spots in the methanolic extract with different Rf values. The presence of phenolics and flavonoids in plant extracts was also confirmed by using UV-visible spectroscopy. The mean values of total phenolic and flavonoids contents are 41.14 ± 3.99mg GAE/g &143.3 ± 29.57 mg QE/g respectively. The dose dependent total antioxidant activity was observed. Further, retention time, peak area, and molecular weight were used to perform GC-MS analysis and it revealed the presence of various bioactive compounds. However, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- was found to have the largest peak area (11.20%). The phytochemical, TLC and GC-MS profiling of methanolic extraction revealed the presence of many bioactive compounds with important medicinal properties. Hence, the presence of these phytochemicals could be responsible for the therapeutic effects of the plant leaves.

Keywords: *C. papaya*, Ascorbic acid, Antioxidant Activity, Total Phenolic Content, Total Flavonoids Contents

INTRODUCTION

Plants are the good sources for the discovery of pharmaceutical compounds and medicines. Natural products could be potential drugs for humans or live stock species. These products and their analogues can act as intermediates for synthesis of useful drugs **[1]**. Natural antioxidants haven't cause health problems that may arise from the use of synthetic antioxidants which have side effects **[2]**. Antioxidant substances block the action of free radicals. When generated in excess, free radicals can counteract the defense capability of the antioxidant system, impairing the essential biomolecules in the cell by oxidizing membrane lipids, cell proteins, carbohydrates, DNA, and enzymes.

Polyphenols, flavonoids, vitamin C and vitamin E are phytochemicals in foods that have been known as a natural antioxidant that has antioxidant activity. Reactive oxygen species (ROS) play a role in various pathological processes including cancer, aging, and atherosclerosis **[3]**. Chronic disease can be reduced by ROS scavenging. Some studies suggest that increased intake of foods containing polyphenols and flavonoids can reduce oxidative stress, inflammation, tumor and coronary heart disease **[4]**.

GC-MS is an analytical techniques mainly used to determine and identify compounds found in a plant sample. Papaya leaves used in the folk medicine to combat dengue fever, because it is considered to be an effective, safe and cheap remedy **[5]**. Therefore, we sought to analyze the phytochemicals present in methanolic as well as in aqueous extract of papaya leaves. Anti-oxidant activity was also evaluated. Moreover, total phenol and flavonoid content were estimated. Further, TLC was conducted to monitor the number of bioactive components (spots) present in the extracts.

MATERIALS AND METHODS

Sample Preparation

Leaves sample of *C. papaya* was collected from the Bundelkhand University campus, Jhansi (U.P.). 5 gm and 10 gm of dried sample with equal amount (100 ml) of de-ionized water were extracted in the water bath for 1 hour at 90°C, filtered and stored at 4°C. 80% of methanolic extract of dried leaves were also prepared in the soxhlet apparatus at 60-65°C.

Phytochemical Analysis

The presence or absence of secondary metabolites were carried out as described **[6]**.

Total antioxidant capacity (TAC) by Phosphomolybdenum assay

For the total antioxidant activity, phosphomolybdenum assay was carried out as described by (Prieto *et al*) **[7]** with some modifications. Different concentrations of

the test sample were combined with 2 mL of reagent solution. Reaction mixture was incubated at 95°C for 90 min. 80 % Methanol was used in the place of extract for the blank with same ratio. Ascorbic acid was used as reference control. The absorbance was measured at 695nm with the help of multi plate reader.

Thin layer chromatography

TLC plate coated with silica gel-G of 0.2 mm thickness was used for the testing of methanolic extract of *C. papaya* leaves. The ratio of solvent mixture (Butanol-acetic acid-water) was 2:1:1 v/v. Spotting the methanolic extract above 4mm from the base of the plate, spots migrates with the solvent mixture on the silica coated plate by the capillary action. Fully developed silica coated plate was air dried followed by heating for 20-25 minutes. The plate was sprayed with 0.2% freshly prepared ninhydrin solution to detect the bands **[6]**. These spots were expressed by its retention factor (Rf).

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Total Phenolic Content (TPC)

The total phenolic content was determined by using the Folin- Ciocalteu method **[8]**. 100 µl of various concentrations were mixed with 500 µl of water followed by adding 100 µl of Folin-Ciocalteu reagent, this mixture was allowed to stand for 6 minutes. After incubation, 7% sodium carbonate (1ml) and distilled water (500 µl) were added to the reaction mixture tubes. All the tubes were incubated for 90 minutes at room temperature. The absorbance was measured at 760 nm. Gallic acid was used as standard control. The total phenolic content was calculated with respect to gallic acid equivalents (mg GAE/g). All the experiments were performed in duplicates.

Total Flavonoid Content (TFC)

The total flavonoids content of the methanolic extract of *C. papaya* leaves was performed by the aluminium chloride complex forming assay **[9]**. Flavonoid content was determined as quercetin equivalent (mg QE/g) because it was used as a standard. 100 µl of the quercetin dilution was mixed with 500 µl of distilled water and then with 100 µl of 5% sodium nitrate and allowed to stand for 6 minutes. Then 150 µl of 10% aluminium chloride solution was added and allowed to stand for 5 minutes after which 200 µl solution of 1M Sodium hydroxide was added sequentially. The absorbance of this reaction mixture was recorded at 510 nm. All the procedures were performed in duplicates.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of methanolic extract of *C. papaya* leaves was perfomed on a Perkin Elmer Turbo Mass Spectrophotometer with

a Perkin Elmer autosampler XLGC. A Perkin Elmer Elite- 5 capillary column with a 0.25 mm film thickness and a length of 30 m was used. It was made primarily of dimethylpolysiloxane, which recorded upto 95% of the total. Helium gas was used as carrier gas and was adjusted to column velocity flow of 0.5 ml/min. A 1 l sample was used as an injection length. The GC's inlet temperature was kept at 250 °C, with a programmed oven temperature of 110°C (isothermal for 2 min), then a 10°C/min increase to 200°C, then a 5°C/min increase to 280 °C, and a 5°C/min isothermal at 280°C. It took 30 minutes to complete the GC. The MS transfer line is kept at a temperature of 200°C, while the source is kept at 180°C. The GC-MS analysis used electron impact ionization at 70 eV, and Total Ion Count was used to evaluate the data for compound detection and quantification (TIC). The components' spectra were compared to the known components in the GC-MS library. Turbo-Mass OCPTVS-Demo SPL programme 19 was used for peak area measurement and data processing.

RESULTS AND DISCUSSION

Because medicinal plants are used all over the world for human healthcare, a systematic phytochemical evaluation is required for safety and medicinal purposes. The medicinal properties of papaya have made significant progress during the previous few decades **[10]**. Phytochemicals are naturally found in medicinal plants and according to the World Health Organization (WHO), are the finest sources for innovative medication discovery **[11]**. Many of these compounds are important in the treatment of various ailments **[12]**. These secondary metabolites plays vital role for both humans and animals. In the present investigation, screening of both the aqueous and methanolic extract of papaya leaves shows the presence of various secondary metabolites **(Table 1)**. However, the methanolic extraction seems better over aqueous extraction for the determination of phytoconstituents.

Table 1: Qualitative phytochemical analysis of the aqueous and methanolic extracts of *C. papaya* leaves

S No.	Phytochemical Test	(Papaya) Aqueous Extract		(Papaya) Methanolic Extract
		10 gm	5 gm	
1.	Alkaloids Test			
	Mayer's	- ve	- ve	+ ve
	Wagner's	+ ve	+ ve	+ ve
	Hager's	+ ve	+ ve	+ ve
2.	Carbohydrates Test			
	Molisch	+ ve	+ ve	+ ve
	Barfoed's	- ve	- ve	- ve
3.	Reducing Sugars Test			
	Fehling's	+ ve	+ ve	+ ve
	Benedict's	+ ve	+ ve	+ ve
4.	Flavonoids Test			
	Alkaline Reagent	- ve	- ve	- ve
	Lead Acetate	+ ve	+ ve	+ ve
5.	Glycosides Test			

	Borntrager's	**- ve**	**+ ve**	**- ve**
	Legal's	**+ ve**	**+ ve**	**+ ve**
	Keller-killiani	**- ve**	**- ve**	**- ve**
6.	**Tannin & phenolic Test**			
	Ferric Chloride	**- ve**	**- ve**	**+ ve**
	Lead Acetate	**+ ve**	**+ ve**	**+ ve**
	Dilute iodine solution	**+ ve**	**+ ve**	**+ ve**
7.	**Saponin Test**			
	Froth	**- ve**	**- ve**	**+ ve**
8.	**Protein & A.A. Test**			
	Ninhydrin	**+ ve**	**+ ve**	**+ ve**
	Biuret	**+ ve**	**+ ve**	**- ve**
9.	**Triterpenoids &Steroids Test**			
	Salkowski's	**- ve**	**- ve**	**+ ve**
10.	**Hydrolysable tannin Test**	**+ ve**	**+ve**	**+ ve**
11.	**Glycosides**	**+ ve**	**+ ve**	**+ ve**

(+) indicates presence while, (–) indicates the absence of the components

Alkaloids are a type of nitrogenous chemical compounds generated by plants, and there are over 10,000 identified alkaloids **[13]**. There are about 8000 flavonoids found in various plant species **[14]**. They are important for stimulation, defence, flavour, communication, and pigmentation **[15]**. Saponins and tannin have antibacterial, anti-malarial, anti-allergic, anti-diabetic, insecticidal, and anti-inflammatory properties **[16]**. The presence of all of these substances in the papaya leaf emphasises its importance in the treatment of a variety of diseases. The health advantages of papaya leaf are widely known among tribal people, who use it in a variety of conditions.

The thin layer chromatography of methanolic extract of *C. papaya* leaves shows the presence of total five spots with Rf values 0.49, 0.62, 0.71, 0.79 and 0.86 respectively **(Figure 1)**. We used alanine amino acid as a reference to make sure the correct procedure and its Rf value was 0.65.

Figure 1: TLC Plate showing spots having different Rf values (0.49, 0.62, 0.71, 0.79, 0.86) of Methanolic extract of *C. papaya* leaves

Total antioxidant capacity of *C. papaya* leaves shown the dose-dependent **(Figure 2)**. The mean values of total phenolic and flavonoids content are 41.14 ± 3.99mg GAE/g &143.3 ± 29.57 mg QE/g respectively **(Table 2)**. The presence of phenolic compounds, flavonoid compounds, and their derivatives, in the current investigation of extracts, indicating that *C. papaya* has antioxidant paotential.

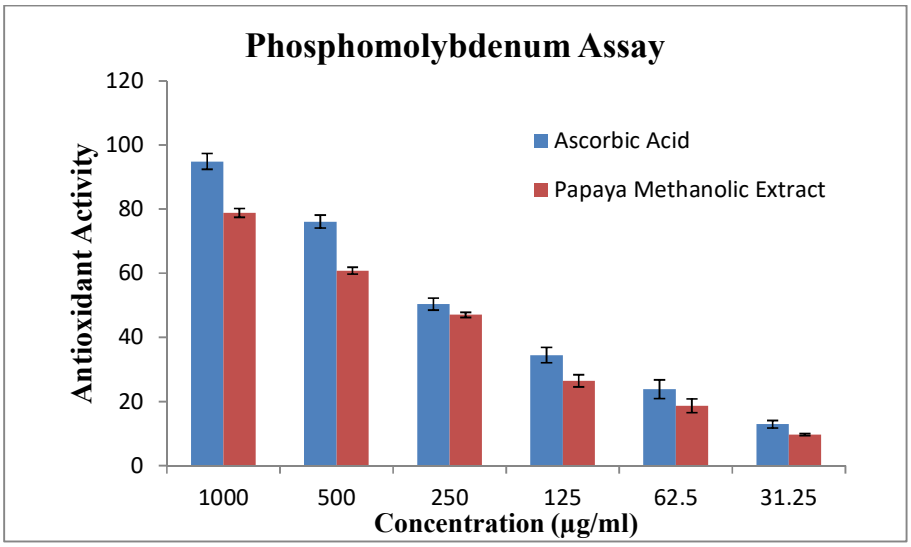

Figure 2: The Antioxidant activity of methanolic extract of *C. papaya* leaves

Table 2: Total Flavonoid & Phenolic Content of methanolic extract of *C. papaya* leaves

Total Flavonoid Content (mgQE/g)		Total Phenolic Content (mgGAE/g)	
Conc.(µg/ml)	Papaya (Leaves)	Conc. (µg/ml)	Papaya (Leaves)
1000	67	150	39.33
500	102	120	30.83
250	140	90	35.55
125	168	60	46.66
62.5	240	30	53.33
Mean ± SE	143.3 ± 29.57	Mean ± SE	41.14 ± 3.99

Natural antioxidants, which provide a variety of health benefits, have recently received a lot of attention. Many complex diseases are known to be prevented or treated by antioxidant chemicals. Redox characteristics that promote activity as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators contribute for the antioxidant potential **[17]**. We observe comparable antioxidant activity with different concentrations of papaya leaf extracts as compare to ascorbic acid **(Figure 3)**. These findings highlight the usefulness of organic extracts in the fight against bacterial pathogens **[18]**. Therefore, we sought to perform GC-MS analysis of *C. papaya* leaves. The GC-MS chromatogram of the identified compounds is shown in **Figure 3**. Many biologically active components were observed. However, the highest peak area of 9,12,15-ctadecatrienoic acid, (Z,Z,Z)- Linolenic acid (11.208%), followed by 1,2-Benzenedimethanol -(3.563%), Benzaldehyde, 4-methyl- (3.563%), Nonadecanoic acid (2.751%), Sucrose (2.610%), d-Glycero-d-galacto-heptose (2.610%), Rhodopin (2.431%), Benzyl nitrile (1.720%), Ethyl 9,12,15-octadecatrienoate (1.650%). Antioxidant, anti-inflammatory, anticancerous, antimalerial, nematicidal and antileishmanial activities are shown by various identified compounds **(Table 3)**.

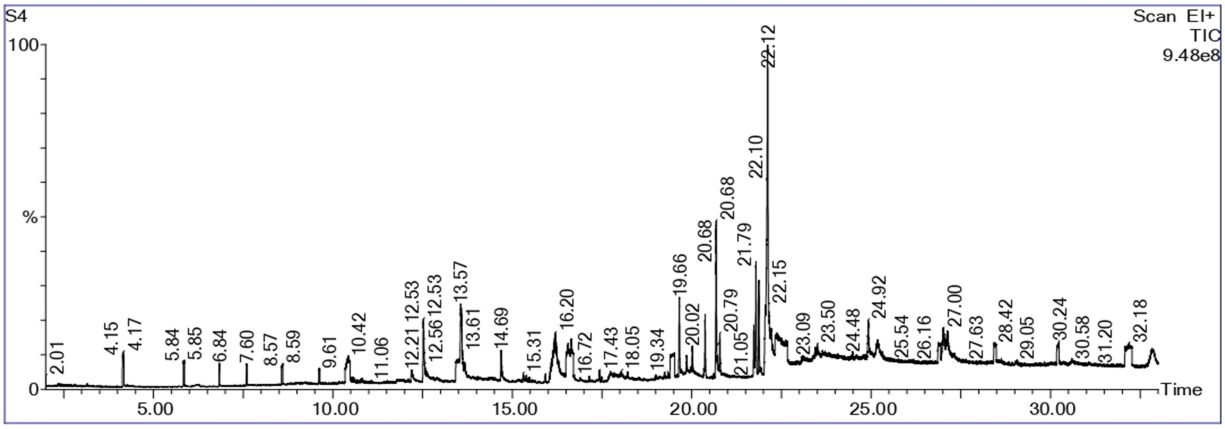

Figure 3: GC-MS Chromatogram of methanolic extract of *C. papaya* leaves

Table 3: Compounds identified by GC-MS analysis of methanolic extract of *C. papaya* leaves

Sr. No.	RT	PP %	Compound name	M.F.	M.W.	Biological Activities
1.	22.559	1.245	Prednisolone Acetate	$C_{23}H_{30}O_6$	402	Anti-inflammatory and immunomodulating properties, Stimulates apoptosis in sensitive tumor cell populations [19]
2.	10.427	1.293	Hematoporphyrin	$C_{34}H_{38}N_4O_6$	598	Phototherapy of malignant neoplasms [20]
3.	25.188	1.381	dl-à-Tocopherol	$C_{29}H_{50}O_2$	430	Potent peroxyl radical scavenger and inhibits noncompetitively cyclooxygenase activity, Antivirals Antioxidant activity, Anticancer [21]
4.	22.054	1.389	9-Octadecynoic acid	$C_{18}H_{30}O_3$	294	Anticancer [22]
5.	27.130	1.464	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	352	Anticancer [23]
6.	16.556	1.482	Perylo[1,12-def]-1,3-dioxepin-5,11-dione, 6,12-dihydroxy-8,9-bis(2-hydroxypropyl)-7,10-dimethoxy- stereoisomer	$C_{29}H_{26}O_{10}$	534	Antimalarial, Antileishmanial activity against Leishmania donovani, Antimicrobial activity [24]
7.	27.021	1.600	Oleic Acid	$C_{18}H_{34}O_2$	282l	An EC 3.1.1.1 (carboxylesterase) inhibitor, An Escherichia coli metabolite, Plant metabolite, Anantioxidant and a mouse metaboliten [25]
8.	21.793	1.650	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306	Antiviral,Anticancer [26]
9.	12.528	1.720	Benzyl nitrile	C_8H_7N	117	Pheromone, Animal metabolite, Anticancer [27]
10.	22.406	2.431	Rhodopin	$C_{40}H_{58}O$	554	Bacterial metabolite [28]
11.	16.197	2.610	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$	210	Anticancer [29]
12.	16.197	2.610	Sucrose	$C_{12}H_{22}O_{11}$	342	An osmolyte, Sweetening agent, Human metabolite [30]
13.	20.685	2.751	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	Human metabolite, algal metabolite, Nematicidal activity [31]
14.	20.685	2.751	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Nematicidal activity, Antibacterial, Antiviral [32]
15.	20.685	2.751	Nonadecanoic acid	$C_{19}H_{38}O_2$	298	Anticance, Fungal metabolite [33]
16.	13.569	3.563	Benzaldehyde, 4-methyl-	C_8H_8O	120	plant metabolite, Anticancer, Anti-inflammatory, Antiviral [34]
17.	13.569	3.563	1,2-Benzenedimethanol	$C_8H_{10}O_2$	138	Anticancer [35]
18.	22.120	11.208	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-Linolenic acid	$C_{18}H_{30}O_2$	278	Reduced inflammation, Antibacterial, Antiviral, Anticancer [36]

CONCLUSIONS

In the present investigation, *C. papaya* leaves shown various secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, triterpenoids, steroids, etc. which possess many pharmacological activities including antioxidant potential. The GC-MS analysis revealed the presence of many phytoconstituents including 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) which contribute the activities like reduced inflammation, antibacterial, antiviral, anticancer, antioxidant, etc. Hence, the presence of phytochemicals responsible for their antioxidant and therapeutic potentials. Further, detailed investigations are required for possible development of novel drugs using some of the bioactive compounds present in *C. papaya* leaves.

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