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GC-MS CHARACTERIZATION OF METHANOLIC EXTRACT OF *CARICA PAPAYA* LEAVES

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ABSTRACT

Papaya (*Carica papaya* L.), one of the most studied plant and its belongs to the family *Caricaceae*. Papaya is commonly known for its nutritional and medicinal value worldwide. Many parts of papaya plant such as roots, leaves, peels, fruits, and seeds have nutritional and therapeutic significance. In ayurvedic medicine, *Carica papaya* used as medicine from long time. The Present study deals with that papaya leaves have some potential pharmacological activity. Phytochemicals, antioxidants and bioactive compounds of papaya leaves were analysed by Gas Chromatography and Mass spectrometry. Phytochemicals are non-nutrientbioactive components that are primarily responsible for scavenging toxic radicals after oxidative stress by generating antioxidants. In the methanolic leaf extract, triterpenoids, sugars, flavonoids, catechins, saponins and tannins were present. Oxidative stress is a significant player in health and disease and often is known to drive the pathogenesis of several disorders and diseases. The plant extract showed 40- 58%inhibition (in the concentration range of 10-100 µg/mL) against DPPH radical when compared to the ascorbate control (82-93%) inhibition in the concentration range of 10-100 µg/mL. GC-MS analysis of the methanolic extract was performed since it contained diverse classes of phytochemicals. The GC-MS spectrum revealed the presence of 44 compounds. The peaks were integrated and the area under the curve (AUC) was calculated in % to find the overall abundance of the phytochemicals.

Keywords: antioxidants, *Carica papaya*, DPPH, GC-MS, phytochemicals.

INTRODUCTION

Scientists have brought an interesting trend in pharmacological development since the beginning of twenty first century: return to nature as a source of potential drugs (Georgiev, 2013 and Lanzotti,2014). Various nature-origin phytochemicals, such as polyphenols, flavonoids, alkaloids, polysaccharides, stilbenoids, and essential oils, have received an increased attention due to their considerable biological benefits. In India, phytochemicals, as well as medicinal plants, have remained the most abundant source of health care and life improvement since very long. India is the richest source of traditional herbal plants with their prescriptions.

Carica papaya belongs to caricaceae family and it is commonly known as ‘papaya’. *Carica papaya* is used in ayurvedic medicines from very long time. It is used as anti-inflammatory, antioxidant, diuretic, antibacterial, abortifacient, vermifuge, hypoglycemic, antifungal activity,

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antihelmenthic and immunomodulatory etc. Papaya (*Carica papaya*) is a fruit crop that is grown both commercially as well as in the kitchen garden of many households all over India. Ripe papayais a favourite fruit all over India. It is used to make fruit salads, refreshing drinks, jam, jelly, and candies. It is rich in a number of nutrients and antioxidants and has a high medicinal value. Papain is tapped from green fruits which has industrial use.

Papaya leaf has numerous benefits. In some parts of Asia, the young leaves of the papaya are steamed and eaten like spinach. Commencing on studies of Dr. Sanath Hettige, who conducted the research on 70 dengue fever patients, said papaya leaf juice helps increase white blood cells and platelets, normalizes clotting, and repairs the liver.

General uses of Papaya:

Papaya can be used as a diuretic (the roots and leaves), anthelmintic (the Leave and seed) and to treat bilious conditions (the fruit). Parts of the plant are also used to combat dyspepsia and otherdigestive disorders (papaya contains a proteolytic enzyme which soothes the stomach and aides in digestion) and a liquid portion has been used to reduce enlarged tonsils. The juice is used for warts,cancers, tumors, corns and skin defects while the root is said to help treat tumors of the uterus. In Africaa root infusion is also used for syphilis and the leaf is smoked to relieve asthma attacks. The Javanese believes that eating papaya prevents rheumatism and in Cuba the latex is used for psoriasis,ringworm and the removal of cancerous growth.

MATERIALS AND METHODS

Qualitative phytochemical analysis

The method of Brindha *et al*., (1982) was followed for analysing the phytochemical constitution of *Carica papaya* L. The reagents required for this qualitative assay were prepared freshly. All the chemicals were purchased from SRL, India. The plant extracts (test solutions) were also prepared freshly, after drying the soxhleted extracts and then reconstituting the fine pastes in the respective solvent used for extraction. Care was taken to reconfirm all the assays, in order to rule out false- positives. Based on the results for qualitative phytochemical estimation, we proceeded with soxhlation for chosen solvents, water (aqueous extract), methanol and ethanol.

DPPH radical scavenging activity

In order to determine the free radical scavenging activity of the crude plant extracts, we used a slightly modified protocol of the very popular method of Brand-Williams *et al*.,(1995), which involves the reaction of chemical compounds in the test solution with DPPH radical (2,2'-diphenyl1-picryl hydrazyl), which upon reaction with antioxidants, transfers the free radical to the electron accepting antioxidant compounds, leading to a loss of the intense pinkish purple colour of the DPPH radical. one mL of 0.1 mM DPPH radical solution was combined with 1 mL of the leaf extracts of *C. papaya* (prepared using different solvents) at varying concentrations (50-500 µg/mL). We prepared corresponding blank solutions and employed L-ascorbic acid (of equal concentrations as the test solutions, in the range of 50 500 µg/mL) as a positive control antioxidant known to rapidly scavengefree radicals. We used a mixture of 1 mL methanol with 1 mL DPPH as control. The disappearance of DPPH radical was monitored at 520 nm using a spectrophotometer, after incubation at room temperature in the dark. % inhibition was calculated using the formula given below:

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

Where, Ac corresponds to abs. of the control and as corresponds to the abs. of the sample.

GC-MS profiling of the methanolic leaf extract of *C. papaya*

The phytochemical evaluation of the methanolic leaf extract of *C. papaya* was carried out using a Gas Chromatography Mass Spectrophotometer (GC-MS) (Perkin Elmer Clarus 500, Connecticut, USA) equipped with flame ionization detector, capillary column (30 m length × 0.25 mm ID coated with 5 % phenyl 95 % dimethylpolysiloxane) with a film thickness of 0.25 µm. Helium gas (mobilephase) was the carrier gas and its flow rate was fixed at 1 mL/min. The temperature of injection port was maintained at

280°C and the volume of sample injection was 1 µL. The stationary phase (capillary column) temperature was set in the range of 60-300 °C (raising rate of 10 min). Mass spectra were programmed as scan type: full scan mode and scan range: 40-450 Daltons. The peaks of the compounds were matched with the standard peaks available in NIST (The National Institute of Standards and Technology) library.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

Phytochemicals are non-nutrient bioactive components that are primarily responsible for scavenging toxic radicals after oxidative stress by generating antioxidants, the main cause of most chronic diseases (Al-Harrasi *et al*., 2014). Fruit phytochemicals displayed high antioxidant capacities linked to lower incidence of degenerative diseases and lower mortality average in humans (Baliga *et al*., 2011; Wang *et al*., 2020). Phenolic acids, flavonoids, tannins, carotenoids, isoflavones, sterols, and lignans are some of phytochemicals of the fruits that are considered bioactive. In this study qualitative analysis (screening) showed the presence of various phytochemicals based on the extraction solvent. In the methanolic extract, triterpenoids, sugars, flavonoids, catechins, saponins and tannins were present (Table 1). While the methanolic and ethanolic extracts revealed the presence of the most diverse classes of phytochemicals, the other solvents (especially, the more nonpolar ones) were able to extract only fewer classes of phytochemicals

Table: 1 Qualitative analysis of *C. papaya* with three different solvents

S.no	Test	Aqueous	Methanol	Ethanol
1.	Sugar	+	+	+
2.	Flavonoids	+	+	+
3.	Catachin	+	+	+
4.	Saponin	+	-	-
5.	Tannin	+	+	-
6.	Aminoacid	-	-	-
7.	Steriods	-	-	-
8.	Carbohydrates	+	-	-

DPPH radical scavenging activity

Oxidative stress is a significant player in health and disease and often is known to drive the pathogenesis of several disorders and diseases (Elfawy,H.A and Das.B, 2019). Thegeneration of free radicals (and oxidants) such as superoxide (O2.-), hydroxyl radical (OH.), hydroperoxyl radical (COOH), hydrogen peroxide (H_2O_2) and singlet oxygen has been demonstrate to be much higher during oxidative stress (Pardini.R.S, 1995). *C. papaya* possesses prominent antioxidant and anti-inflammatory properties. The methanolic extract ofthe plant had shown satisfactory scavenging/inhibitory activity against ROS and RNS. Theplant extract showed 40-58% inhibition (in the concentration range of 10-100 µg/mL) against DPPH radical when compared to the ascorbate control (82- 93% inhibition in the concentration range of 10-100 µg/mL). Using two-way ANOVA, the results were found to be significant when the data points were compared between each ascorbate control point (same concentration of ascorbate as the test); Bonferroni post-tests revealed that the P-valuefor all the four assays (between the control and test points in all the cases) were significant (*** at $P < 0.001$); however, in isolated instances, the level of significance was ** (at $P < 0.01$).

Table: 2 Antioxidant activity (DPPH assay) of *C. papaya*

S. No	Concentration of aqueous extract µg/mL	%of inhibition
1.	10	37.27±1.4
2.	20	38.17±0.9
3.	40	56.12±1.2
4.	60	55.25±1.1
5.	80	58.32±1.2
6.	100	60.15±0.8

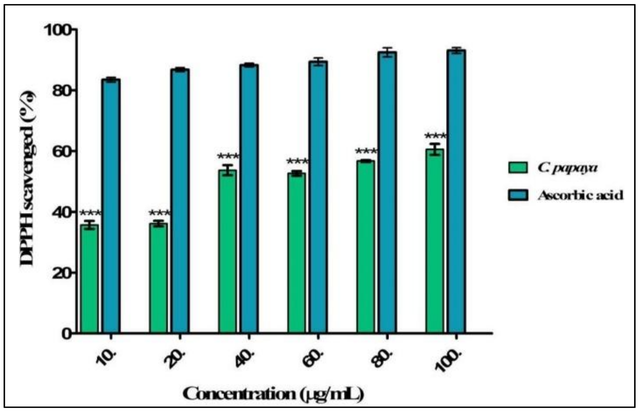

Figure:1 DPPH radical scavenging activity of methanolic leaf extract of *C. papaya*. The values indicate mean ± standard deviation (n=3). The mean difference is significant at the levels of *p<0.05, **P<0.01 and ***P<0.001 vs standard. ns - non significant.

GC-MS profiling of the methanolic leaf extract of *C. papaya*

GC-MS analysis of the methanolic extract was performed since it contained diverse classes of phytochemicals. The GC-MS spectrum (Figure 2 & Table 3) revealed the presence of over44 compounds. The peaks were integrated and the area under the curve (AUC) was calculated (in %) to find the overall abundance of the phytochemicals. After peak identification using a compound library, each of the compounds was searched for earlierreports of bioactivity using Google Scholar as well as PubChem. The uses of the compounds (identified from literature survey) are mentioned in Table 3.For compounds with no significant literature reports, the description has been given as "nosignificant report". These may be novel compounds and bioinformatics explorations may pave the way to identify their potential biochemical targets. These compounds can be isolated and further in vitro assays will lead to the discovery of their mechanisms of action as well as their specific macromolecular targets. Big data analytics for specific protein interactions will be more advantageous in deciphering the pivotal roles of the plants and molecular dissection of the same can lead to formulation of applications to take plant-based breakthroughs from the bench to the bedside.

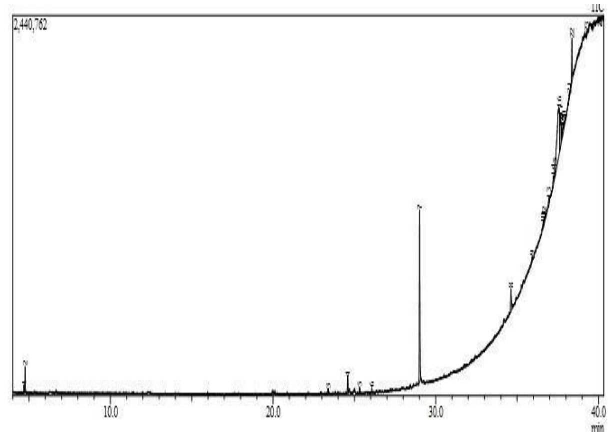

Figure:2 GC-MS chromatogram of *C. papaya* methanolic leaf extract

Table: 3 GC-MS analysis of *C. papaya* leaves

Peak No.	RT	Area (%)	Height (%)	Compound name	Molecular weight g/mol	Molecular Formula
1	4.685	1.18	1.64	1-Butanol,2-amino-3-methyl-,(.+/-)-	103.16	$C_5H_{13}NO$
2	4.76	2.32	5.4	4,4-dimethoxy-2-Methyl-2-butanol	214.06	C_8H_8BrNO
3	23.386	0.59	1.04	2(4h)-benzofuranone,5,6,7,7a-tetrahydro-6-hydroxy- 4,4,7a-trimethyl-,(6s-cis)-	196.24	$C_{11}H_{16}O_3$
4	24.587	2.34	3.35	Neophytadiene	278.5	$C_{20}H_{38}$
5	25.319	0.83	1.22	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	296.5	$C_{20}H_{40}O$
6	26.07	0.44	1	Hexadecanoic acid,methyl ester	270.5	$C_{17}H_{34}O_2$
7	29.008	23.54	36.03	2-hexadecen-1-ol,3,7,11,15-tetramethyl-,[r-[r*,r*-(e)]]-	280.5	$C_{20}H_{40}$
8	34.621	4.3	4.74	Hexadecanoic acid,2-hydroxyl-1-(hydroxymethyl)ethyl ester	274.40	$C_{15}H_{30}O_4$

9	35.927	0.62	0.99	Tetracosamethyl - cyclododecasiloxan	889.8	$C_{24}H_{72}O_{12}S_{112}$
10	36.59	0.72	1.1	1,3,3,3-tetramethyl disiloxanyl tris(trimethylsilyl)orthosIlicate#	248.66	$C_9H_{28}Si_4$
11	36.63	0.41	1.2	3-carbazolyl methyl ketone Thiosemicarbazone	145.25	$C_5H_{11}N_3S$
12	36.95	1.46	1.42	9,12,15-Octadecanoic acid,2,3-dihydroxypropyl ester	306.5	$C_{20}H_{34}O_2$
13	37.22	1.85	1.96	Octadecanoic acid,2,3-dihydroxypropyl ester	498.8	$C_{31}H_{62}O_4$
14	37.32	1.02	1.82	3-ethoxy-1,1,1,5,5,5-Hexamethyl-3-(trimethysiloxy) trisiloxane	142.075	F_6Si_2
15	37.815	1.97	1.69	Cyclotrisiloxane,heXamethyl-	222.46	$C_6H_{18}O_3Si_3$
16	37.89	1.31	1.18	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-Decanmethyl-	354.77	$C_{10}H_{30}O_4Si_5$
17	38.205	1.28	1.05	1h-Benzoimidazole,2-benzyl-1-isobutyl	264.162	$C_{18}H_{20}N_2$
18	38.372	5.62	8.91	Squalene	410.7	$C_{30}H_{50}$

CONCLUSION

Papaya plant is mostly used as the food ingredient throughout the world because of its fruits and its nutritive values.It is used as anti-inflammatory, antioxidant, diuretic, antibacterial, abortifacient, vermifuge, hypoglycemic, antifungal activity, antihelmenthic and immunomodulatory etc. Scientific evidences suggest their versatile biological function that supports its traditional use in different diseases.

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