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R Sri Harini
Kumaraguru College of Technology, Coimbatore, Tamilnadu, India.

A Saivikashini
Kumaraguru College of Technology, Coimbatore, Tamilnadu, India.

G Keerthana
Kumaraguru College of Technology, Coimbatore, Tamilnadu, India.

K Kumaresan
Kumaraguru College of Technology, Coimbatore, Tamilnadu, India.

S Murugesan
Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamilnadu, India.

N Senthilkumar
Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamilnadu, India.

Correspondence
N Senthilkumar
Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamilnadu, India.

Profiling metabolites of *Carica papaya* Linn. variety CO7 through GC-MS analysis

R Sri Harini, A Saivikashini, G Keerthana, K Kumaresan, S Murugesan, N Senthilkumar

Abstract
GC-MS-MS analysis was carried out for the identification of phytochemicals present in the n-hexane leaf extract of *C. papaya* variety CO7. It indicates that the leaf extract is rich in alkaloids, phenolics, fatty acids, and flavonoids. All compounds identified by GC/MS/MS screening were assessed for their anticancerous, antimicrobial, antioxidant and insecticidal property using physico-chemical property calculations according to Tice Rules. The phytochemicals such as Clausamine G, Galactitol, Ibogamine are found to have anticancer property. Garveatin D, N-Methylaspartic acid were reported as antimicrobial metabolites.

Keywords: GC-MS analysis, phytochemicals, leaf extract, *Carica papaya*, variety CO7.

1. Introduction
Carica papaya Linn. Belongs to the family Caricaceae, commonly known as papaya. The plant is native to the tropics of Southern Mexico and neighbouring Central America. Papayas came to India by way of the Caribbean and then Malaysia around 1550. A century later, explorers from Italy and China brought papaya from India's soils back to their respective countries. India is the world's largest producer of papaya which grows more papaya than the subsequent largest producing countries like Brazil, Indonesia, the Dominican Republic, and Nigeria combined together. The papaya is a tree-like plant, in which every part has its own nutritional and medicinal value. Papaya is cultivated mainly for its fruits. India grows 25 lakh tonnes of papaya every year on 1.5 lakh acres with a productivity level of 100 tonnes per acre in every two years, he said. The papaya is harvested in two years. In India, Andhra Pradesh is the largest papaya producer, growing 1.6 million tons. Gujarat is the second largest producer that produces almost 1.2 million tons. Subsequent producers are Karnataka, Maharashtra, West Bengal, Chhattisgarh, Madhya Pradesh, Assam, Tamil Nadu, and Kerala. India grows several types of papaya; each varies in their flesh colour, skin colour and size. The Coorg Honeydew, also known as Madhubindu, has yellow pulp with a dark skin colour. Others have a pulpy orange flesh, while green papayas maintain tough, pale yellow meat. The taste of papaya depends on its cultivar. Some have rust-coloured flesh that is juicier than the pale orange types. The rust red varieties also tend to have a sweeter and a sharper metallic taste than others. Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamilnadu (India) has developed hybrids viz., Arka prabhath, Arka surya, CO-1, CO-2, CO-3, CO-4, CO-5, CO-6, CO-7, CO-8, Coorg honey dew, Pant 2, Pau-selction, Pusa delicious, Pusa dwarf, Pusa nanha, Pusa giant, Red lady, Sun rise solo and Washington for its different fruit varieties. Of which, CO7 is considered as a better variety since, fruits of CO7 are very fleshy and overcome physiological and abiotic stresses. The flesh is reddish in colour and delightfully distinct taste (Fig 1 a b). Each tree can carry up to 98 fruits with 200-225t/ha. Hence, Co7 is widely cultivated in Tamil nadu, Andhra Pradesh and Karnataka. Many scientific investigations evaluated the biological activities of various parts of *C. papaya* including fruits, shoots, leaves, rind, seeds, roots or latex. *C. papaya* plants produce natural compounds in leaf, bark and twig tissues that possess both highly antitumor and pesticide properties. Papaya fruit is a rich source of nutrients such as provitamin a, carotenoids, vitamin C, vitamin B, lycopene, dietary minerals and dietary fibre. The possible health benefits of consuming papaya include a reduced risk of heart disease, diabetes, cancer, aiding in digestion, improving blood glucose control in case of diabetics, lowering blood pressure, and improving wound healing. Papaya seeds eliminate intestinal parasites, detoxify liver and kidneys and have antibacterial, antihelminthic

And antiamoebic properties. Juice from papaya roots is used in some countries of Asia to ease urinary troubles. The milky sap called latex, of an unripe papaya contains papain and chymopapain. The latex cures whooping cough, used as an anti-helminthic, relives dyspepsia, cures diarrhea, stops bleeding hemorrhoids etc. Papaya peel is often used in cosmetics. The papaya peel can also be used in many home remedies as a sunscreen, a soothing slave and also to fight dandruff [1]. The papaya leaves contain active compounds like carpaine, pseudoscorpion and dehydrocarpaine I and II, choline, carposide, vitamin C and E which have health benefits. Both leaf and fruit of the *C. papaya* possess carotenoids namely β- carotene, lycopene, anthraquinones, glycosides as compared to matured leaves and hence possess medicinal properties like antiinflammatory, hypoglycemic, antifertility, abortifacient, hepatoprotective, wound healing, recently its antihypertensive and antitumor activities. The presence of carpaine in leaves of papaya explains the reason why it is being effectively used as an antimalaria agent. The high level of natural self-defence compounds in papaya leaves make it highly resistant to insects and diseases [2]. Papaya leaf extracts are available in the market as capsules, tea leaves and in the powder form for consumption. Many studies have shown that choosing fermented papaya extracts or papaya supplements are more beneficial due to their enhanced antioxidant and immune-boosting effects. Therefore, the present study aims at profiling metabolites of *C. papaya* variety CO7 leaf extract to understand their biological activity.

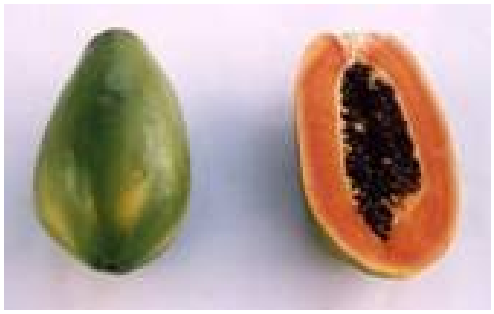

Fig 1: (a) CO7 fruit

(b) CO7 leaves and fruits

2. Materials and methods

2.1 Collection of plant material

Fresh leaves of *C. papaya* variety CO7 were collected from the trial established by Department of Fruit Crops, Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu (India) located between 11.0183° N, latitudes and 76.9725° E longitudes. It is situated at an altitude of 411 metres above sea level. The collected plant material was authenticated by a taxonomist at IFGTB, Coimbatore.

2.2 Preparation of plant extracts

The collected plant materials were air dried and ground into uniform powder. Dry powder of plant sample was extracted with n-hexane using soxhlet apparatus for 6 hours. The extract was filtered over anhydrous sodium sulphate followed by concentrated using rotary evaporator. The concentrated extract was subjected to freeze drying in a lyophilizer till dry powder was obtained. Finally the extracted powder was resuspended with the n-hexane at the concentration of 100mg/ml (w/v) followed by filtration through Varian Bond Elut C_{18} solid phase extraction to remove impurities. 1µl of this solution was employed for GC-MS-MS analysis.

2.3 GC-MS-MS analysis

The GC-MS-MS analysis was carried out using Varian 4000 Ion trap GC/MS/MS with Fused silica 15m x 0.2 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 ºC, at the rate of an increase of 5 ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250 ºC and Helium flow rate as 1 ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS-MS compounds present in the plants sample were identified.

2.4 Identification of phytocompounds

Interpretation on mass-spectrum GC-MS-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Results and discussion

The GC/MS/MS analysis of n-hexane extract of leaf of *C. papaya* variety CO7 gave us nineteen major compounds (Fig 1). All compounds identified by GC/MS/MS screening were assessed for their biological property using physico-chemical property calculations according to Tice Rules. As per Tice rule compounds are more likely to have properties of antimicrobial, anticancerous antioxidants and anti-insect if molecular weight is within ≥ 150 and ≤ 500; theoretical logarithm of the noctanol/water partition coefficient (log P), is less than or equal to 5.0; hydrogen bond acceptor is within 1-8; hydrogen bond donar is less than or equal to 2 and the number of rotatable bond is less than or equal to 12 (Table 1). The compounds those are strictly following the Tice rules are considered as antimicrobial, anticancerous, antioxidant and andti-insect potential compounds for new or novel drugs [3]. Almost all the compounds detected in GC-MS analysis follow Tice rule. The phytochemicals such as Clausamine G, Galactitol, Ibogamine are found to have anticancer property [4]. In our earlier study on anticancer activity of n-hexane extract of leaf of *C. papaya* variety CO7 on the human breast adenocarcinoma cell line (MCF7) was found to have mild cytotoxic activity of 6-14% cell daeth (unpublished).

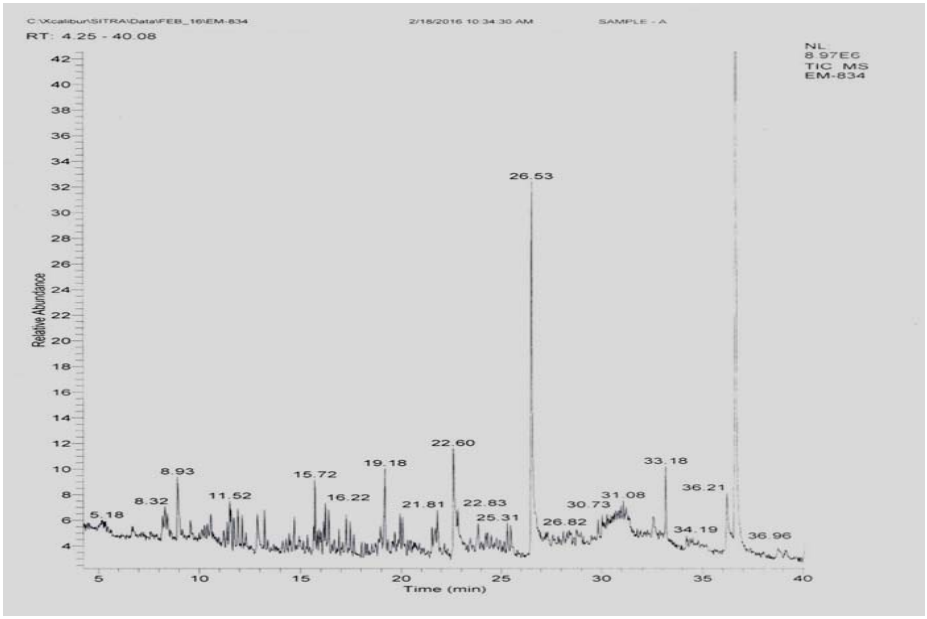

Fig 2: GC-MS chromatogram of hexane extract of leaf of *C. papaya* variety CO7

Table 1: GC-MS analysis of hexane leaf extract of *C. papaya* variety CO7

S. No.	Constituent	Rt	%	MF	MW (Da)	LogP	HD	HA	RB	Biological properties
1.	1-(3'-Chloropropyl)-7-methoxymethyloxy-8,9-dihydro-4a,7-methano-4aH-benzocycloheptene-4,6(5H,7H)-dione	7.42	26.27	$C_{17}H_{21}ClO_4$	324.79	4.2	0	4	8	Unknown
2.	1,5-Dichloro-9,10dihydroxyanthracene	11.09	97.68	$C_{26}H_{18}Cl_2O_2$	433.32	6.8	2	2	2	Anticancer
3.	4-Cyano-7-(2,4,6-trimethylphenyl)-5H-furo[2,3-c]]thiopyran	16.37	63.33	$C_{17}H_{15}NOS$	281.00	3.9	1	2	0	Insecticide
4.	Eicosane, 3-methyl- (CAS)	17.39	16.46	$C_{21}H_{44}$	296.00	11.3	0	0	17	Anti-bacterial
5.	Dimethyl-flubendazole	14.69	80.28	$C_{18}H_{16}FN_3O_3$	341.00					Antihelminthic
6.	4(S)-[à-Methoxy-à-(trifluoromethyl)phenylacetoxy]-2,3-(isopropylidenedioxy) heptaan-1-ol isomer	20.16	78.36	$C_{20}H_{27}F_3O_6$	420.42	4.04	0	6	8	Unknown
7.	Clausamine G	21.26	57.36	$C_{20}H_{21}NO_5$	355.38	4.63	2	6	6	Anticancer
8.	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane	21.77	77.85	$C_{12}H_{38}O_5Si_6$	430.94	11.26	0	5	10	Anti-bacterial
9.	1-(2'-Amino-5'-hydroxy-4'-methoxyphenyl)-5,6,7-trim ethyloxy-3,4dihydroisoquinoline	23.99	88.45	$C_{19}H_{22}N_2O_5$	358.38	1.8	4	5	8	Cytotoxic
10.	1-Piperazinepropanamide, N-(4-fluorophenyl)-4-methyl-	24.66	5.75	$C_{14}H_{20}FN_3O$	265.32	0.38	1	4	4	Antimicrobial
11.	Ethyl (6Z,8Z)-9-(4-methoxy-2,3,6-trimethylphenyl-3,7-di methylnona-2,4,6,8-tetraenoate	26.53	82.86	$C_{23}H_{30}O_3$	354.48	6.8	0	3	8	Vitamin D analog
12.	Garveatin D	25.29	12.57	$C_{21}H_{22}O_5$	354.39	4.61	2	5	1	Active form of Vitamin D
13.	N-Methylaspartic acid	26.86	14.55	$C_5H_9NO_4$	147.12	-0.44	3	5	4	Agonist of the glutamate receptor Neurotransmission
14.	6-Bromohexanoic acid, heptadecyl ester	29.96	8.33	$C_{23}H_{45}BrO_2$	433.50	10.66	0	2	22	Anti-cancer
15.	Cis-Vaccenic acid	30.83	2.65	$C_{18}H_{34}O_2$	282.46	7.70	1	2	15	Nod factor
16.	D-Mannitol, hexaacetate (CAS)	31.61	0.56	$C_{18}H_{26}O_{12}$	434.39	0.12	0	12	17	Immunomodulator
17.	Galactitol, hexaacetate, D- (CAS)	31.61	4.22	$C_{18}H_{26}O_{12}$	434.39	-0.2	0	12	17	Cytotoxic, antiinflammatory, antiviral
18.	Ibogamine (CAS)	37.01	3.43	$C_{19}H_{24}N_2$	280.40	3.9	1	1	1	Anticancer
19.	Methyl [2,4-Di-t-butyl-6-(phenyloxymethylphenyl]phosphin ate	35.72	93.39	$C_{22}H_{31}O_3P$	374.45	6.21	0	3	10	Antimicrobial activity, sedatives and anesthetics

Compound namely Dimethyl-flubendazole (80.28%) was reported to have antihelminthic activity [5]. Clausamine G (57.36%), 1-(2'-Amino-5'-hydroxy-4'-methoxyphenyl)-5,6,7-trimethyloxy-,4dihydroisoquinoline (88.45%), 6-Bromohexanoic acid, heptadecyl ester (8.33%) and Ibogamine (3.43%) were reported as anticancerous metabolites [4, 6]. Garveatin D (12.57), N-Methylaspartic acid (14.55%) were reported as antibiotics [7]. The compound Cis-Vaccenic acid (2.65%) was reported as nod factor for nodulation in microsymbiont plants (Cardenas *et al*., 1995). Hence, isoaltion and bioassay studies with respect to aforementioned compounds will be an insight into develop novel drugs for application in pharmacology.

4. Conclusion

C. papaya is one of the tree like plants found to possess many medicinal values. The present study therefore has provided some biochemical basis for the medicinal values of extracts from *C. papaya* in the treatment of various ailments. As a rich source of phytochemicals, minerals and vitamins *C. papaya* leaves could be a potential source of useful drugs. GC-MS analysis of leaf extract of *C. papaya* revealed the presence of secondary metabolites of anticancerous, antimicrobial, antihelminthic, immunomodulatory, antioxidants and insecticidal properties and hence it could be used in pharmacology industry as an efficient novel drug.

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