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Profiling of phytoconstituents in papaya varieties and wild genotypes as a omic breeding approach for Papaya Ring Spot Virus resistance (PRSV)

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Abstract

Carica papaya Linn. belongs to the family Caricaceae, which is commonly known as melon tree. Papaya Ring Spot Virus (PRSV), a major devastating disease of papaya causing a yield loss up to 100%. All the commercial varieties of papaya are susceptible to this disease. However, the wild genus *viz.*, *Vasconcella cauliflora* and *Vasconcella candamarcensis* are reported to be resistant to PRSV. Hence, this study was formulated to profile the phytoconstituents in cultivated varieties in comparison with resistant genotypes, so that the compounds contributing resistance to PRSV could be identified. The GCMS analysis revealed that a total of 114 bioactive constituents present in varieties *viz.* CO 7 (22), TNAU papaya CO 8 (20) and wild genus *viz.*, *Vasconcella cauliflora* (36) and *Vasconcella candamarcensis* (36). The wild genus possessed (22 compounds) in *V. cauliflora* and (18 compounds) in *V. candamarcensis* as extraneous compounds when compared to the cultivated varieties. Among the phytoconstituents, the compounds namely 9, 12-Octadecadienoic acid (Z, Z)-, Octadecanoic acid, Dodecanoic acid, and squalene were reported to have the properties against plant viral diseases.

Keywords: Profiling of phytoconstituents, papaya varieties, wild genotypes, papaya ring spot virus

1. Introduction

Papaya (*Carica papaya* L.) is an important fruit crop is being cultivated throughout the tropical and subtropical countries of the World. Papaya is severely infected by viral disease called Papaya Ring Spot Virus (PRSV). This disease attacks papaya plants at all stages of development and spreads rapidly, infecting the entire field in 3–7 months and resulting in catastrophic production losses of up to 100%. (Ventura *et al.*, 2004) [25]. The transmission of PRSV from plant to plant is caused by insect vector Aphids which is polyphagous in nature. PRSV-P (papaya biotype) infects papaya and cucurbits, while, PRSV-W (watermelon biotype) is restricted only to cucurbits. *Vasconcella cauliflora* and *Vasconcella candamarcensis* have been reported to be resistant to PRSV and used in conventional breeding studies against PRSV (Balamohan, *et al.*, 2008) [2]. Phytochemicals have been exploited traditionally for the cure and suppress many diseases in both plants and humans, and also have been reported to inhibit viral replication/transcription. Most of them inhibit the viruses either during the viral entry inside the host cell or during their replication. Mazid *et al.* (2011) [17] reported that the secondary metabolites including terpenes, phenolics and nitrogen (N) and (S) containing compounds defend the plant against a variety of herbivores and pathogenic microorganisms as well as various kinds of abiotic stresses. Eleazu *et al.* (2012) [6] reported that the phytochemical analysis has confirmed the presence of alkaloids, glycosides, saponins, flavonoids, phenolics, proteins, amino acids and tannin in *Carica papaya* leaf has been documented. Sam *et al.* (2013) [20] reported that the saponins are one of the most numerous and diverse groups of plant natural products and serve a range of ecological roles including plant defence against disease and herbivores and possibly as allelopathic agents in competitive interactions between plants. Leaf extracts are used to prepare anti-malarial drugs (Sharmela *et al.* 2019) [23]. For the reliable Identification of phytocompounds, GC-MS is a valuable tool, (Subramanian *et al.* (2011) [21] and Johnson *et al.* (2011) [12]. Plant derived compounds have been investigated extensively for their anti- viral principles for plant viruses. Hence, this study was attempted to study the phytoconstituents in the PRSV resistant wild papaya genotypes *viz.*, *V. cauliflora* and *V. Candamarcensis* for their anti-viral properties in comparison with the most susceptible and tolerant varieties *viz.*, CO 7 papaya and TNAU papaya CO 8, respectively.

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2. Materials and Methods

2.1. Collection and identification of plant materials

The mature leaves of the TNAU varieties CO7, CO8 (Fig.1.) and the wild genotype *V. cauliflora* were collected from the University Orchard, Department of Fruit Science, HC&RI, Tamil Nadu Agricultural University, Coimbatore. The genotype, *V. candamarcensis* leaves were collected from the Government Botanical Garden, Udhamandalam, Ooty and Horticultural Research Station, Ooty. The leaves were washed and air dried at room temperature for about 10 days and ground into fine powder using blender and sieved to give particles ≤ 1 mm size.

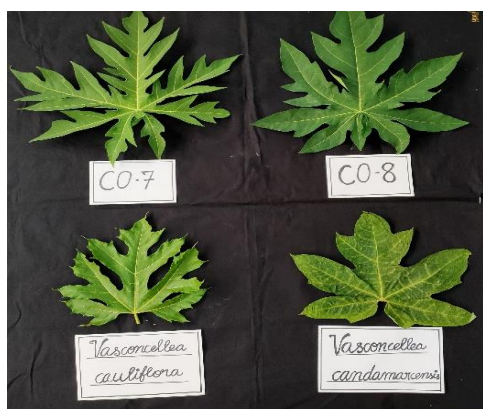


Fig 1: Plant sample used for GC-MS analysis

2.2. Plant extraction

Dried powdered leaf sample (10g) was taken in the conical flask and added with 100 ml of distilled water, kept at 37° C for overnight at shaker cum incubator. Subsequently, the extract was separated using separating funnel and the fractions were condensed by using a rotatory vacuum flask evaporator at 400 °C for 150 rpm 45 mins, (Hajji *et al.*, 2010, Gogoi *et al.* 2016) [10, 8]. After that, the fraction of samples was poured into petri plates and kept for drying. Finally, the fractions were then scraped with HPLC grade methanol and filtered through a 0.002 m syringe filter before being GCMS examined.

2.3. Gas Chromatography– Mass spectrometry (GC-MS) Analysis

GC-MS analysis of the extracts were performed using a Perkin-Elmer Clarus SQ8C system and Gas Chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an Elite-I, fused DB-5 MS capillary standard non polar column. For GC-MS detection, helium (99.999%) was used as carrier gas at a constant flow of 1 micro-litre/ min. The GC instrument vaporizes the sample and then separates and analyses the various components. Each component was ideally produced a specific spectral peak that was recorded on a paper chart electronically. The "Retention time" is the amount of time that passes between elution and injection. The Retention time was used to distinguish between various substances. The peak was measured from the base to the tip of the peak. GCMS analysis was done at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University and Coimbatore.

2.4. Identification of components

For all the four papaya samples, the resulting data in spectrum was compared with known spectrum available in NIST, PubChem and Human Metabolome Databases. The

credentials of plant materials such as molecular weight, molecular formula and molecular structures of the constituents were ascertained.

3. Result and Discussion

Report of Gas Chromatography Mass Spectrometry (GC-MS), CO 7 (Fig.2), TNAU Papaya CO 8 (Fig.3), *Vasconcellea cauliflora* (Fig.4), *Vasconcellea candamarcensis* (Fig.5). The studies on phytoconstituents in the methanolic extract of papaya leaves by GC-MS analysis clearly indicated the existence of totally 114 bioactive constituents present in varieties *viz.* Co7 (22 Nos) Table.1. TNAU papaya Co8 (20 Nos) Table. 2. and wild genus *viz.*, *Vasconcellea cauliflora* (36 Nos) Table. 3. and *Vasconcellea candamarcensis* (36 Nos) Table. 4. The wild genus possessed 40 extraneous phytoconstituents in *V. cauliflora* (22 Nos), *V. candamarcensis* (18 Nos) when compared to the cultivated varieties. This type of compounds in *Carica papaya* leaves were reported by many works [Pino *et al.*, (2003) [18], Canini *et al.*, (2007) [4], Harini *et al.*, (2016) [11], Ezekwe *et al.*, (2017) [7], Dwivedi *et al.*, (2020)] [5].

Among the two varieties *viz.*, Co.7 and TNAU Papaya Co.8 analysed, there are six compounds were found to be different in each of the above varieties, apart from the similarity of 30 number of compounds in both the varieties. This perhaps, due to the difference in their sex forms that Co.7 is gynodioecious, while Co 8 is Dioecious. Similarly there were twenty two different compounds in *V.cauliflora* (Dioecious) and eighteen compounds in *V. candamarcensis* (Gynodioecious) found to present. Here, it is important to notice that the difference in compounds may be due to the variation in the sex forms, besides varied genetic nature in climatic adaptability of these two wild relatives as *V.cauliflora* is subtropical & tropical, while *V. candamarcensis* is hilly grown genotype. The compounds exist only in the gynodioecious papaya are Dodecanoic acid, Butanoic acid and 3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7oxabicyclo [4.1.0] hept-1-yl) and dioecious papaya are Octane, 3,5-dimethyl. The compounds *viz.*, Dodecanoic acid, Octadecanoic acid, methy ester, 9,12-Octadecadienoic acid (Z, Z) and 2,4-Di-tert-butylphenol were reported to possess antiviral activity against the human viral diseases reported by Karthikeyan *et al.* (2016) [13], Nazneen *et al.* (2015), Ramya. *et al.* (2020) [19], Leila. *et al.* (2019) [15].

The GCMS analysis indicated that the three compounds 9, 12-Octadecadienoic acid, octadecenoic acid, and Squalene was found only in wild genotype *viz.*, *V. cauliflora* and *V. candamarcensis*, these compounds were not exist in varieties of papaya . The compound Dodecanoic acid is found only in the gynodioecious papaya CO 7 with (17.0 Probability %) and *V. candamarcensis* (72.8 Probability %). These four compounds have an anti-viral activity, against plant viruses as per the findings in cotton that (9,12-Octadecadienoic acid) has an anti-viral activity against cotton leaf curl virus Azmat *et al.*, 2015). Abdullah *et al.* (2019) [1] recommended that octadecenoic acid can be used as an alternative pesticide to control *Aphis gossypii*. Sujatha *et al.* (2019) [24] reported that the compound Dodecanoic acid showed the anti-viral properties and the docking studies on dodecanoic acid, protein ligand complex was identified as a suitable lead molecule for distracting the host-seeking behaviour of Mosquitoes. Sangeetha *et al.* (2020) [22] also confirmed first that the compound Squalene has an anti-viral activity against the Groundnut Bud necrosis virus in tomato. And also the six compounds having the Insectifuge activity *viz.*,

(Hexadecanoic acid methyl ester, 9,12,15-Octadecatrienoic acid (Z, Z, Z), 2,4, Di-tert-butylphenol, Tetradecanoic acid, Octadecanoic acid and squalene reported by [Karthikeyan., *et al.* (2016) ^[13], George *et al.* (2018) ^[9], Mahalakashmi and Thangapandian (2019)] ^[16]. From the studies reported in other crops *viz.*, cotton and tomato, the role of 9,12-

Octadecadienoic acid and Squalene may also possess the anti-viral properties against PRSV. This is the first study to analyse the anti-viral activity of resistant wild papaya genotypes for Papaya Ring Spot Virus (PRSV). Further studies is being taken up.

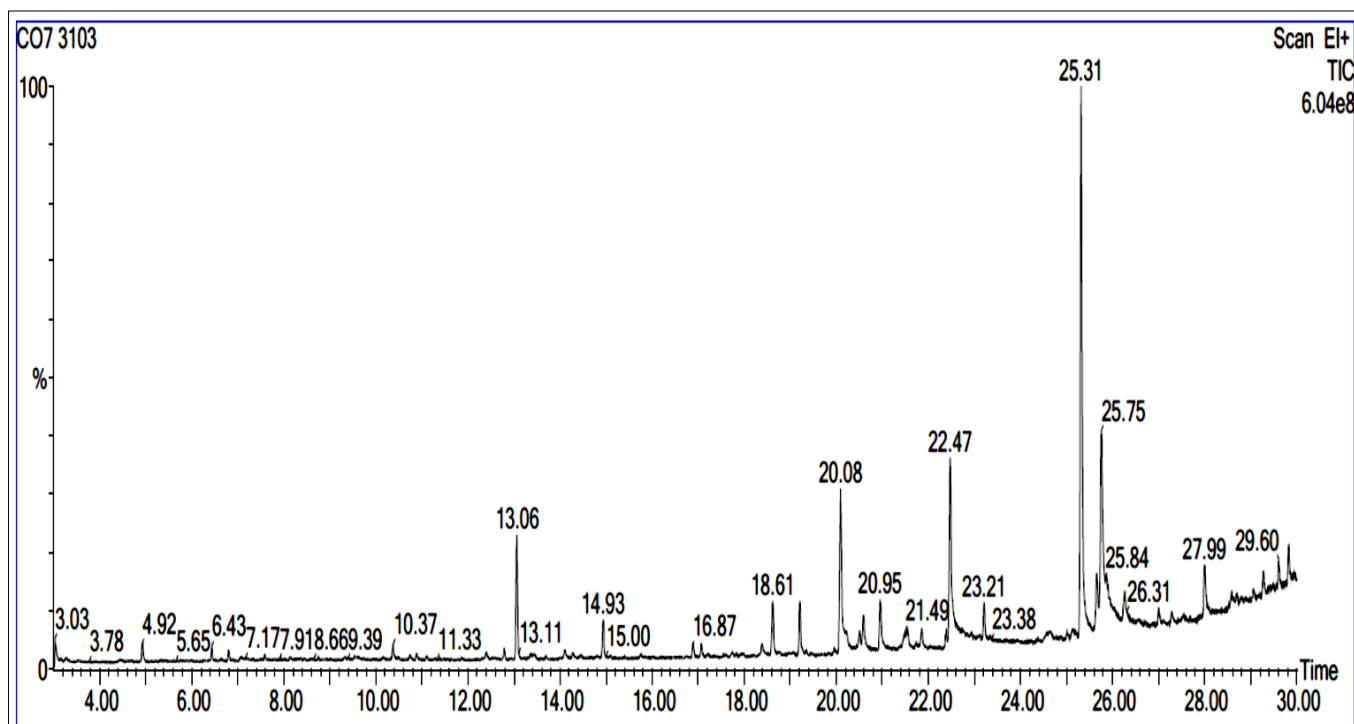


Fig 2: Report of Gas Chromatography Mass Spectrometry (GC-MS) – CO 7

Table 1: Major chemical compounds identified from the active fraction of methanol extract of CO 7 papaya leaf extract by GCMS analysis.

S. No.	Compound name	Prob %	RT	Peak area %	Molecular formula	Molecular weight
1.	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	86.7	18.614	1.606	C ₁₁ H ₁₆ O ₃	196.24
2.	n-Hexadecanoic acid	66.7	22.471	8.280	C ₁₆ H ₃₂ O ₂	256.42
3.	2,4-Di-tert-butylphenol	53.2	13.062	2.981	C ₁₄ H ₂₂ O	206.32
4.	Phytol	52.5	25.312	16.661	C ₂₀ H ₄₀ O	296.5
5.	Neophytadiene	49.5	20.085	6.212	C ₂₀ H ₃₈	278.5
6.	Benzyl isocyanate	45.3	6.795	0.336	C ₈ H ₇ NO	133.15
7.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	38.5	20.945	1.928	C ₂₀ H ₄₀ O	296.5
8.	Benzene, (isothiocyanatomethyl)-	38.1	10.371	0.420	C ₈ H ₇ NS	149.21
9.	Hexadecanoic acid, methyl ester	36.5	21.851	0.633	C ₁₇ H ₃₄ O ₂	270.5
10.	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-	36.4	16.884	0.366	C ₁₃ H ₂₀ O ₃	224.30
11.	Hexanedioic acid, bis(2-ethylhexyl) ester	33.9	29.824	1.014	C ₂₂ H ₄₂ O ₄	370.56
12.	Pentadecanoic acid, 14-methyl-, methyl ester	28.7	21.851	0.633	C ₁₇ H ₃₄ O ₂	270.5
13.	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	22.7	21.546	0.725	C ₂₂ H ₃₂ O ₃	344.5
14.	1,3-Dioxolane-4-methanol, 2-ethyl-	20.6	3.028	0.866	C ₆ H ₁₂ O ₃	132.16
15.	5H-Cyclopropanal	19.2	28.693	0.411	C ₂₈ H ₃₆ O ₁₁	548.6
16.	Tetradecanoic acid	18.9	18.379	0.568	C ₁₄ H ₂₈ O ₂	228.37
17.	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	18.5	25.752	12.142	C ₁₉ H ₃₂ O ₂	292.5
18.	Dodecanoic acid	17	14.108	0.350	C ₁₂ H ₂₄ O ₂	200.32
19.	Oleic Acid	17	26.257	1.293	C ₁₈ H ₃₄ O ₂	282.5
20.	Decane	15.8	4.929	0.541	C ₁₀ H ₂₂	142.28
21.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	15.7	28.583	0.470	C ₂₆ H ₅₄	366.7
22.	Phenol,2,6,-bis(1,1-dimethylethyl)-	15.4	13.062	2.981	C ₁₅ H ₂₄ O	220.35

RT: Retention time

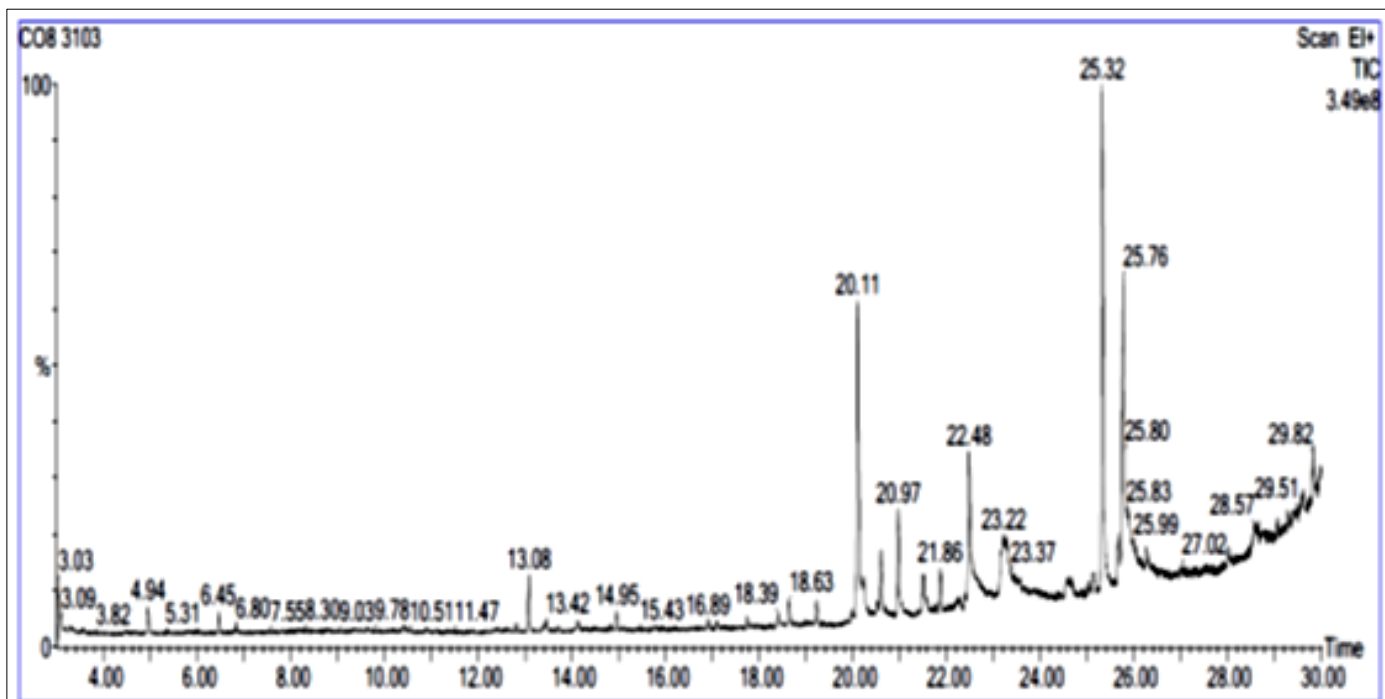


Fig 3: Report of Gas Chromatography Mass Spectrometry (GC-MS) – TNAU Papaya CO 8

Table 2: Major chemical compound identified from the active fraction of methanol extract of TNAU Papaya CO 8 leaf extract by GCMS analysis.

S. No.	Compound name	Prob %	RT	Peak area %	Molecular formula	Molecular weight
1.	Hexadecanoic acid	62.5	22.477	6.663	C ₁₆ H ₃₂ O ₂	256.42
2.	Hexadecanoic acid, methyl ester	59.0	21.861	0.746	C ₁₇ H ₃₄ O ₂	270.5
3.	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	57.4	18.630	0.541	C ₁₁ H ₁₆ O ₃	196.24
4.	Phytol	53.6	25.323	11.151	C ₂₀ H ₄₀ O	296.5
5.	Neophytadiene	52.7	20.106	7.104	C ₂₀ H ₃₈	278.5
6.	Undecane	37.5	6.450	0.331	C ₁₁ H ₂₄	156.31
7.	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	35.6	25.763	13.039	C ₁₉ H ₃₂ O ₂	292.5
8.	2,4-Di-tert-butylphenol	34.2	13.078	0.871	C ₁₄ H ₂₂ O	206.32
9.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	33.7	20.966	2.397	C ₂₀ H ₄₀ O	296.5
10.	N-2,4-Dnp-L-arginine	26.2	23.652	0.348	C ₁₂ H ₁₆ N ₆ O ₆	340.29
11.	Phenol, 2,6-bis(1,1-dimethylethyl)-	23.5	13.078	0.871	C ₁₅ H ₂₄ O	220.35
12.	Hexanedioic acid, mono(2-ethylhexyl) ester	22.0	29.830	1.158	C ₁₄ H ₂₆ O ₄	258.35
13.	2-Dimethylsilyloxytridecane	21.9	3.028	1.697	C ₁₅ H ₃₃ OSi	257.51
14.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	20.2	28.654	0.465	C ₃₅ H ₆₈ O ₅	568.9
15.	Phenol, 2,5-bis(1,1-dimethylethyl)-	18.9	13.078	0.871	C ₁₄ H ₂₂ O	206.32
16.	Hexanedioic acid, bis(2-ethylhexyl) ester	17.7	29.830	1.158	C ₂₂ H ₄₂ O ₄	370.56
17.	Pentadecanoic acid, 14-methyl-, methyl ester	17.7	21.861	0.746	C ₁₇ H ₃₄ O ₂	270.5
18.	Propanoic acid	17.6	24.638	0.358	C ₂₀ H ₃₄ O ₇	386.5
19.	Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methyloxime)	17.5	26.163	0.644	C ₂₃ H ₃₆ N ₂ O ₄	404.5
20.	1,3-Dioxolane-4-methanol, 2-ethyl-	15.5	3.028	1.697	C ₆ H ₁₂ O ₃	132.16

RT: Retention time

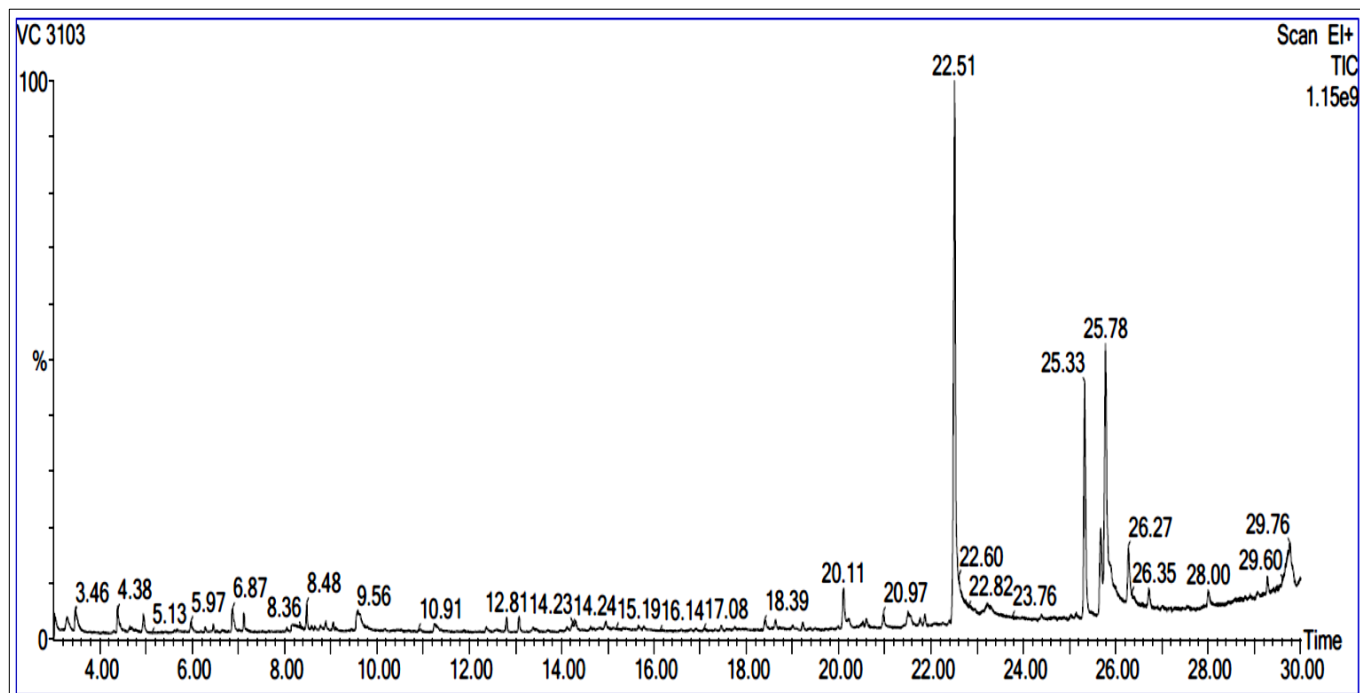


Fig 4: Report of Gas Chromatography Mass Spectrometry (GC-MS)- *V. cauliflora*

Table 3: Major chemical compound identified from the active fraction of methanol extract of *Vasconcellea cauliflora* leaf extract by GCMS analysis.

S. No.	Compound name	Prob %	RT	Peak area %	Molecular formula	Molecular weight
1.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	85.7	7.115	0.452	C ₆ H ₈ O ₄	144.12
2.	n-Hexadecanoic acid	78.7	22.506	23.981	C ₁₆ H ₃₂ O ₂	256.42
3.	dl-Glyceraldehyde dimer	66.3	3.474	1.848	C ₆ H ₁₂ O ₆	180.16
4.	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	51.5	18.630	0.312	C ₁₁ H ₁₆ O ₂	180.24
5.	Neophytadiene	48.1	20.105	1.415	C ₂₀ H ₃₈	278.5
6.	Phytol	47.2	25.327	6.872	C ₂₀ H ₄₀ O	296.5
7.	Octadecanoic acid	46.1	26.273	2.359	C ₁₈ H ₃₆ O ₂	284.5
8.	2H-1-Benzopyran	39.5	14.228	0.291	C ₁₅ H ₁₄ O ₃	242.27
9.	Hexadecanoic acid, methyl ester	38.6	21.866	0.307	C ₁₇ H ₃₄ O ₂	270.5
10.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	34.6	25.777	13.990	C ₁₉ H ₃₂ O ₂	292.5
11.	Thymine	34.1	5.975	0.379	C ₅ H ₆ N ₂ O ₂	126.11
12.	4-Hydroxy-2-methylacetophenone	33.4	9.576	1.460	C ₉ H ₁₀ O ₂	150.17
13.	Phenol, 2,6-bis(1,1-dimethylethyl)-	32.5	13.077	0.402	C ₁₅ H ₂₄ O	220.35
14.	2-Methoxy-4-vinylphenol	30.8	9.576	1.460	C ₉ H ₁₀ O ₂	150.17
15.	Decane	29.1	4.939	0.582	C ₁₀ H ₂₂	142.28
16.	(2-Aminothiazol-4-yl) acetic acid	28.5	12.807	0.362	C ₅ H ₇ ClN ₂ O ₂ S	194.64
17.	Niclosamide	28.1	14.303	0.350	C ₁₃ H ₈ Cl ₂ N ₂ O ₄	327.12
18.	Tetradecanoic acid	27.9	18.414	0.377	C ₁₄ H ₂₈ O ₂	228.37
19.	5,5-Dimethylimidazolidine-2,4-dione	26.9	21.501	0.979	C ₅ H ₆ BrClN ₂ O ₂	241.47
20.	Tramadol	26.9	21.501	0.979	C ₁₃ H ₁₈ ClN ₃	251.75
21.	Squalene	26.8	29.774	4.363	C ₃₀ H ₅₀	410.7
22.	Propane-1,1-diol diacetate	26.7	3.018	1.021	C ₇ H ₁₂ O ₄	160.17
23.	2,4-Di-tert-butylphenol	26.2	13.077	0.402	C ₁₄ H ₂₂ O	206.32
24.	O, O-Diethyl phosphate	21.5	14.303	0.350	C ₄ H ₁₁ O ₄ P	154.10
25.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	20.8	20.970	0.516	C ₂₀ H ₄₀ O	296.5
26.	9,12-Octadecadienoic acid (Z, Z)-	20.2	25.667	2.748	C ₁₈ H ₃₂ O ₂	280.4
27.	Butanamide, 2-hydroxy-N,2,3,3-tetramethyl-	18.9	3.018	1.021	C ₈ H ₁₇ NO ₂	159.23
28.	Octane, 3,5-dimethyl-	17.6	4.939	0.582	C ₁₀ H ₂₂	142.28
29.	1,2-Benzenedimethanol	17.2	8.175	0.285	C ₈ H ₁₀ O ₂	138.16
30.	Butylaldehyde, 4-benzyloxy-4-[2,2,-dimethyl-4-dioxolanyl]-	17.2	23.296	1.502	C ₁₆ H ₂₂ O ₄	278.34
31.	2,4-Dimethyl-5-methylthiopent-4-en-2-ol	16.6	3.283	1.965	C ₇ H ₁₄ O	114.19
32.	DL-Arabinose	16.1	6.875	0.808	C ₅ H ₁₀ O ₅	150.13
33.	Phenol, 2,5-bis(1,1-dimethylethyl)-	15.9	13.077	0.402	C ₁₄ H ₂₂ O	206.32
34.	3-O-Methyl-d-glucose	15.6	11.252	0.261	C ₇ H ₁₄ O ₆	194.18
35.	1,2-Dihydroindeno[1,2,3-cd] pyrene	15.2	23.296	1.502	C ₂₂ H ₁₄	278.3
36.	Sulforaphane	6.0	3.474	1.848	C ₆ H ₁₁ NOS ₂	177.3

RT: Retention time

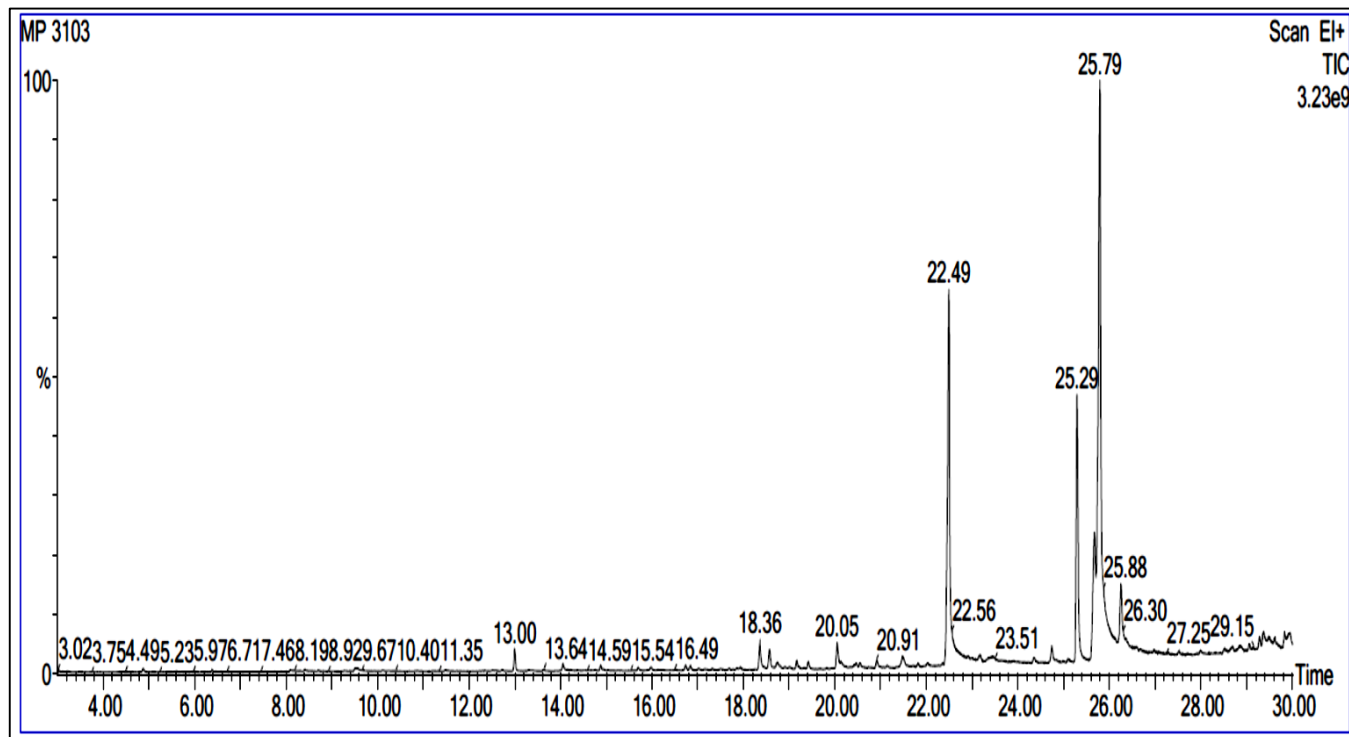


Fig 5: Report of Gas Chromatography Mass Spectrometry (GC-MS) – *V. candamarcensis*

Table 4: Major chemical compound identified from the active fraction of methanol extract of *Vasconcellea candamarcensis* leaf extract by GCMS analysis.

S. No.	Compound name	Prob %	RT	Peak area %	Molecular formula	Molecular weight
1.	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	90	18.565	0.765	C ₁₁ H ₁₆ O ₃	196.24
2.	n-Hexadecanoic acid	85.3	22.486	20.779	C ₁₆ H ₃₂ O ₂	256.42
3.	Tetradecanoic acid	74.4	18.359	1.089	C ₁₄ H ₂₈ O ₂	228.37
4.	Dodecanoic acid	72.8	14.053	0.312	C ₁₂ H ₂₄ O ₂	200.32
5.	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept 1-yl)	64.1	16.734	0.203	C ₁₃ H ₂₀ O ₃	224.30
6.	Neophytadiene	57.3	20.045	1.086	C ₂₀ H ₃₈	278.5
7.	Phytol	54.7	25.292	11.174	C ₂₀ H ₄₀ O	296.5
8.	2,4-Di-tert-butylphenol	52.8	12.997	0.732	C ₁₄ H ₂₂ O	206.32
9.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	48.8	25.792	30.799	C ₁₉ H ₃₂ O ₂	292.5
10.	9,12-Octadecadienoic acid (Z,Z)-	41.5	25.667	6.228	C ₁₈ H ₃₂ O ₂	280.4
11.	Oleoyl 3-carbacyclic phosphatidic acid	39.4	9.561	0.214	C ₂₂ H ₄₁ O ₅ P	416.5
12.	Octadecanoic acid	35.6	26.253	3.048	C ₁₈ H ₃₆ O ₂	284.5
13.	Dasycarpidan-1-methanol, acetate (ester)	34.3	22.906	0.344	C ₂₀ H ₂₆ N ₂ O ₂	326.4
14.	Hexanedioic acid, mono(2-ethylhexyl) ester	33.4	29.829	0.264	C ₁₄ H ₂₆	258.354
15.	7-Hydroxy-6-methoxyisoflavone	32.8	24.742	0.761	C ₁₆ H ₁₂ O ₄	268.26
16.	2,3,3-Trimethyl-2-(4-methylpentanoyl)-cyclopentanone	31.0	19.415	0.307	C ₁₄ H ₂₄ O ₂	224.34
17.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	28.7	20.910	0.392	C ₂₀ H ₄₀ O	296.5
18.	4H-Cyclopropa [5',6'] benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one	28.5	28.869	0.522	C ₂₆ H ₃₄ O ₁₁	522.5
19.	6-Hydroxy-7-methoxy-4-phenylcoumarin	28.3	21.476	0.946	C ₁₆ H ₁₂ O ₄	268.26
20.	10-Methyl-8-tetradecen-1-ol acetate	27.4	19.415	0.307	C ₁₇ H ₃₂ O ₂	268.4
21.	4,8,12,16-Tetramethylheptadecan-4-olide	27.2	29.284	0.532	C ₂₁ H ₄₀ O ₂	324.5
22.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	24.6	23.447	0.727	C ₃₅ H ₆₈ O ₅	568.91
23.	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	24.4	26.253	3.048	C ₂₆ H ₅₂ O ₆	460.7
24.	2-Pentadecanone, 6,10,14-trimethyl-	21.6	20.130	0.435	C ₁₈ H ₃₆ O	268.5
25.	Octadecanal, 2-bromo-	20.3	29.049	0.285	C ₁₈ H ₃₅ BrO	347.4
26.	12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	20.2	28.514	0.244	C ₁₄ H ₂₄ O ₃	240.34
27.	Hexanedioic acid, dioctyl ester	20.2	29.829	0.264	C ₂₂ H ₄₂ O ₄	370.6
28.	Glycerol 1-palmitate	20.8	23.447	0.727	C ₁₉ H ₃₈ O ₄	330.5
29.	Clocortolone pivalate	19.9	28.689	0.508	C ₂₇ H ₃₆ ClFO ₅	495.0
30.	3-Methoxyflavone	18.9	24.742	0.761	C ₁₆ H ₁₂ O ₃	252.26
31.	Hexanedioic acid, bis(2-ethylhexyl) ester	17.1	29.829	0.264	C ₂₂ H ₄₂ O ₄	370.6
32.	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	16.3	21.476	0.946	C ₉ H ₁₂ N ₂ O ₄	212.2
33.	Methyl 4-hydroxy-3,5 dimethoxybenzoate	16.3	21.476	0.946	C ₁₀ H ₁₂ O ₅	212.20
34.	Benzeneethanol, ã,ã-dimethyl-	16.1	3.213	0.200	C ₁₂ H ₁₈ O	178.27
35.	Butanoic acid	16.0	29.479	1.083	C ₃₆ H ₅₈ O ₆	586.8
36.	Phenol, 2,5-bis(1,1dimethylethyl)-	15.8	12.997	0.732	C ₁₄ H ₂₂ O	206.32

4. Conclusion

The methanol extract of cultivated papaya varieties in comparison with wild genotypes indicated the presence of bioactive compounds *viz.*, Octadecadienoic acid, octadecenoic acid, Squalene and Dodecanoic acid in wild genotypes might be responsible for the anti-viral activities against the Papaya Ring Spot Virus (PRSV), since these compounds are reported to possess anti-viral principles in crops like Tomato and Cotton. However, further study is necessary for the confirmation of anti-viral activity of the compounds against PRSV and their role of defence mechanism against Papaya Ring Spot Virus.

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