



IDENTIFICATION OF NOVEL BIOACTIVE NATURAL COMPOUNDS AND THEIR BIOLOGICAL ACTIVITIES IN N-HEXANE EXTRACTS OF *CARICA PAPAYA* VARIETY OF RED LADY LEAVES WITH GAS-CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Objective: The present study was to determine of novel bioactive natural compounds and their biological activities in n-hexane extract of *Carica Papaya* variety of Red Lady Leaves.

Methods: Fresh green leaves of the C. P variety of Red Lady were used to make extract with n-hexane. Then this extract was subjected to a phyto-chemical compounds analysis utilizing an Agilent Technologies (7890-A-7000) GC-MS Triple-Quad. The Automated Mass Spectral Deconvolution and Identification System sophisticated software for GC/MS data interpretation from the National Institute of Standards and Technology, was utilized to generate a Target Component Library file.

Results: GC/MS assay used Retention Time, Peak Area, and Molecular Weight, revealing the presence of different bio-active components, an over-all of fifty-two components were detected as distinctive to be frequent in n-hexane-extract. However, when analyzing the relative abundance of phyto-components, occurring in C. P Red Lady Leaves (n-hexane extract), the phyto-components and their relative abundance were 1,2-Benzene-dicarboxylic Acid, mono 2 ethyl hexyl Ester 47.58%, 9,12,15-Octa-decatrienoic Acid, Ethyl Ester, (Z, Z, Z)- 7.29%, and Retinoic Acid, 5,6-epoxy-5,6-di hydro- 4.07%. GC/MS analysis of the n-hexane extract recognized various phyto-chemical compounds, with a mean Peak Area % of 1.94 ± 6.77 (range: 0.06–47.58) and a mean Target Component Library file similarity of 82.22 ± 8.94 (range: 46.70–94.60). One-sample *t*-test demonstrated that the mean Peak Area % was significantly <5% benchmark ($t = -3.17, p = 0.0027$), showing that most compounds were present in trace abundance.

Conclusion: The GC-MS reporting of *Carica Papaya* variety of Red Lady Leaves (n-hexane extract) unveiled the occurrence of numerous bio-active constituents as well as significant therapeutic properties. Therefore, the presence of these phyto-chemicals may exist responsible for the medicinal effects.

Keywords: *Carica Papaya*, Gas Chromatography-Mass Spectrometry, Phytocomponents, n-hexane

INTRODUCTION

C. Papaya Linn., is an orange and green fruit plant family Caricaceae, is planted as a large perennial herb known for its prompt growth, initiating from Central-America. Numerous *C. Papaya* varieties grow well world-wide, for instance Pusa Majesty, Delicious, Dwarf and Nanha, Coorg Honey Dew, Arka Surya, Solo, Red Lady, Ranchi, CO2, CO5- CO7 and Barwani, every one showing characteristics in color of flesh & skin, and size. *Carica Papaya*-Red Lady (*CPL-RL*) is a fruitful Mexican-cultivar, seen for its remarkable crop of fifty to one hundred twenty fruits/plant per season¹. However, *C. Papaya* is plentiful in different nutrients and anti-oxidants, providing it abundantly therapeutically value. By means of its pro-Vitamin A-C-B, carotenoids, lycopene, Minerals, and fibers, papaya is documented by means of a nutrient rich source. Whereas, the peel of *C. Papaya* realizes remedy in cosmetics. Furthermore, *C. Papaya* latex shows antimicrobial, antifungal, antimalarial, anti-amoebic, anthelmintic, hepatoprotective, male & female antifertility, immune modulatory properties².

The therapeutic properties of *CPL* are ascribed to their active components, comprising Alkaloids, Phenolic, Flavonoids, Saponin and other active compounds³. In Pakistan, the juice from the leaves of *C.papaya* has traditionally been employed like a home-remedy for curing Dengue-fever. Presently, *Carica Papaya* leaf (extracts) is commercially obtainable in different form. *CPL*, seeds, and latex are employed on the way to digestive issues, by papain, a protein digesting enzyme in *CP*, encouraging natural-digestion.

Therefore, this research tries to find of novel bio-active natural components and their biological properties *CPL-RL* extracts by using Gas-Chromatography-Mass-Spectrophotometry. The analytical technique of Gas-Chromatography-Mass-Spectrophotometry permits for a comprehensive examination of the chemical components existent in the n-hexane extract, assisting a better understanding of their potential properties and uses. However, this study purposes to provide important data that may augment our knowledge of the therapeutic and nutritional properties related with the phyto-chemicals originate in *CPL-RL*.

METHOD

Study Design: This pre-clinical experimental study was accomplished in the Department of pharmacology and Therapeutics at Baqai Medical College & Karachi University duration of 6months.

Plant Collecting and Identification: Freshly gathered samples of *CPL-RL* were acquired from the Karachi University (KU) and authenticated via an ethnobotanist. The plant received an official identification (G.H#97627).

Plant Extraction: Plant material extraction was carried out at the International Center for Chemical & Biological science (ICCBS), KU, Pakistan. 5kg of green fresh *CPL-RL* were gathered, sorted, cleaned with tap water, and rinsed by distilled water. The cleaned material was air dried, chopped, and blended. However, extraction was accomplished by using solvent (n-hexane) in a Soxhlet-Extractor. Crushed samples were placed in the Soxhlet-Thimble, with solvent heated in the Bottom-Flask, Vaporized, condensed in the condenser, and dripped back into the flask. This cyclic method repeated until solvent of n-hexane-dried sample were extracted. A total of 10liters of n-hexane were used over 72hours. Post-extraction, the concentrate was evaporated using a Buchi-Rotavapor R-200 at 60°C, dried, and stored in a refrigerator for further analysis⁴.

Analysis and Identification of Phytochemicals Using GC-MS: The assessment exploited the National Institute of Standards and Technology (NIST) database with the Automated Mass Spectral Deconvolution and Identification Software (AMDIS) for detailed analysis. Furthermore, spectra from unknown compounds were cross-referenced with known compounds at ICCBS, KU. The identification process involved comparing molecular ions, base ions, fragment ions, and peak intensities with reference standards in the database. The analysis employed an Agilent-Technologies 7890A/7000 GC/MS Triple-Quad system with Mass-Hunter software. Each sample was analyzed over a 90.714-minute cycle using helium as the carrier gas at 1ml/min. The GC oven temperature profile started at 50°C (5 minutes), ramped at 7°C/min to 200°C (20 minutes), and further ramped at 7°C/min to 300°C (30 minutes). MS detection in Electron Impact mode at 70eV covered a full scan range from m/z 40 to 700amu. AMDIS software facilitated mass spectral deconvolution, peak picking, and identification, producing the final report.

Statistical Analysis

Peak area percentage (PA%) and NIST spectral similarity values were subjected to descriptive statistical analysis (mean $\pm$ standard deviation, range). For the purpose of distinguishing major and minor phytoconstituents, a cut-off of 5% PA was chosen, which is consistent with the thresholds reported in GC-MS analyses of phytochemicals. One-sample t-tests were used to determine whether mean PA% values differed significantly from this reference value. A p-value of <0.05 was regarded as statistically significant. All analyses were performed using IBM SPSS Statistics, Version 26.0.

RESULTS

The GC-MS Report revealed investigations into phytoconstituents within the n-hexane extracts of the *CPL-RL*. The retention time of each phytocomponent was determined by its boiling point, and variations in retention time can arise from interactions with the stationary phase, represented by the column in the gas chromatography system. The resulting chromatogram was created based on the total number of ions generated for each chemical compound in a sample. The peak produced in the chromatogram is proportional to the % of a compound in the sample.

The presentation of phytocomponents in *CPL*, including their retention time, formula, weight, relative abundance% peak area, similarity%, compound nature, and pharmacological activity, is detailed in the figures and tables below.

This study disclosed the existence of 52 phytocomponents in RL during a 90.14 minute cycle, and the detection of constituents were conducted through an evaluation of GC-MS spectra by the mass library. The n-hexane extract from the *CP-RL* were examined for the relative abundance of phytocomponents. The analysis revealed the following composition: 1,2-Benzene-dicarboxylic acid, mono2-ethylhexyl ester(47.58%), 9,12,15-Octadecatrienoic acid, ethyl ester,(Z,Z,Z)-(7.29%), Retinoic Acid, 5,6-epoxy-5,6-dihydro-(4.07%), Hentriacontane(2.89%), 13,27-Cycloursan-3-one(2.75%), 9,12,15-Octadecatrienoic Acid, methyl-Ester,(Z,Z,Z)- (2.21%), and various other compounds in descending order of abundance.

The highest peak area, at a retention time of 52.8 minutes, was observed for 1,2-Benzene dicarboxylic Acid, mono2-ethylhexyl Ester, while the second highest, with a retention time of 33.2 minutes, was recorded for 9,12,15-Octadecatrienoic Acid, Ethyl Ester,(Z,Z,Z). The retention times for Retinoic Acid, 5,6 epoxy 5,6-dihydro, Hentriacontane, 13,27-Cycloursan-3-one, & 9,12,15-Octadecatrienoic Acid, Methyl Ester,(Z,Z,Z) were 69.8, 5.3, 63.5, and 55.7 minutes, respectively table1&2. The mean PA% of the compounds from n-hexane extract was 1.94 ± 6.77 (range: 0.06–47.58) while that of NIST spectral similarity was 82.22 ± 8.94 (46.70–94.60). From the one-sample t-test analysis, it was found that the mean PA% was significantly lower than the reference benchmark set at 5% $t = -3.17$, $p = 0.0027$ with the conclusion that most of the compounds extracted were present at trace to low abundance levels.

Table 1:

Pk#	RT	Phytocomponents/MF/MW	PA%	Similarity%
1.	14.4	Trichloroacetic acid, pentadecyl ester(C ₁₇ H ₃₁ Cl ₃ O ₂)(372)	0.1	78.9
2.	14.6	2-Isopropyl-5-methyl-1-heptanol(C ₁₁ H ₂₄ O)(172)	0.11	80.4
4.	20.1	Hexadecane(C ₁₆ H ₃₄)(226)	0.06	87.6
5.	20.2	1-Hexadecene(C ₁₆ H ₃₂)(224)	0.16	89.9
6.	20.6	Phenol,2,4 bis (1,1-di-methylethyl)(C ₁₄ H ₂₂ O)(206)	0.13	88.5
7.	21.7	Tetra-decane,2,6,10-tri-methyl(C ₁₇ H ₃₆)(240)	0.08	79.8
	21.7	Heptadecane(C ₁₇ H ₃₆)(240)		78.9
8.	23.4	E-15-Heptadecenal(C ₁₇ H ₃₂ O)(252)	1.64	92.1
9.	24.1	Phytol(C ₂₀ H ₄₀ O)(296)	0.32	81.8
10.	26.3	Hexa-decanoic Acid, Methyl Ester(C ₁₇ H ₃₄ O ₂)(270)	0.15	91.9
11.	26.4	5-Eicosene,(E)- (C ₂₀ H ₄₀) (280)	0.77	90.9
12.	27.3	Hexa-decanoic Acid, Ethyl Ester(C ₁₈ H ₃₆ O ₂)(284)	1.44	92.3
13.	27.5	n-Hexadecanoic acid(C ₁₆ H ₃₂ O ₂)(256)	0.83	93.0
14.	29.2	1,2-Benzenedicarboxylic acid,butyl decyl ester(C ₂₂ H ₃₄ O ₄)(362)	0.87	89.8
	29.9	9,12-Octa-decadienoic Acid (Z,Z),Methyl Ester(C ₁₉ H ₃₄ O ₂)(294)		84.6
15.	30	1-Docosene(C ₂₂ H ₄₄)(308)	0.07	84.8
16.	30	1-Nonadecene(C ₁₉ H ₃₈)(266)	0.65	85.5
17.	30.5	9,12,15Octa-decatrienoic Acid,Methyl Ester,(Z,Z,Z)(C ₁₉ H ₃₂ O ₂)(292)	2.21	92.9
18.	31.6	Octa-decanoic Acid,Ethyl Ester(C ₂₀ H ₄₀ O ₂)(312)	1.1	85.1
19.	32.2	9,12-Octadecadienoic acid (Z,Z)- (C ₁₈ H ₃₂ O ₂)(280)	1.22	83.6
20.	33.2	9,12,15Octa-decatrienoic Acid, Ethyl Ester,(Z,Z,Z)(C ₂₀ H ₃₄ O ₂)(306)	7.29	81.1
	33.1	9,12,15-Octa-decatrienoic Acid,(Z,Z,Z)-(C ₁₈ H ₃₀ O ₂)(278)		89.9
21.	36.8	Behenic alcohol(C ₂₂ H ₄₆ O)(326)	0.67	88.1
22.	44.1	Butyl9,12,15-octadecatrienoate(C ₂₂ H ₃₈ O ₂)(334)	1.92	84.5
25.	51	Di-n-octyl phthalate(C ₂₄ H ₃₈ O ₄)(390)	0.25	73.9
26.	52.8	1,2 Benzene-di-carboxylic Acid, mono2 ethylhexyl Ester(C ₁₆ H ₂₂ O ₄)(278)	47.58	94.6
27.	52.9	Octadecanoic acid, phenylmethyl ester(C ₂₅ H ₄₂ O ₂)(374)	0.26	76.4
30.	54.2	1-Octacosanol(C ₂₈ H ₅₈ O)(410)	0.37	84.2
31.	54.7	Oxalic acid, dodecyl2-phenylethyl ester(C ₂₂ H ₃₄ O ₄)(362)	1.23	88.3
32.	55.3	Ethyl tetracosanoate(C ₂₆ H ₅₂ O ₂)(396)	0.11	79.1
33.	55.7	Octacosane(C ₂₈ H ₅₈)(394)	0.65	86.3
34.	55.7	Squalene(C ₃₀ H ₅₀)(410)	0.21	91.1
36.	57	1-Hexadecanol,2-methyl-(C ₁₇ H ₃₆ O)(256)	0.34	78.6
37.	57.2	17-Pentatriacontene(C ₃₅ H ₇₀)(490)	0.26	82.0
39.	57.7	Oxalic Acid,hexa-decyl2phenyl-ethyl Ester(C ₂₆ H ₄₂ O ₄)(418)	0.23	83.1
40.	57.8	Benzene,[1-(1-cyclohexen-1-yl)ethyl]- (C ₁₄ H ₁₈)(186)	0.13	63.8
41.	58.1	Bi-cyclo [3.1.1] hept-2-ene-2-ethanol,6,6-di-methyl(C ₁₁ H ₁₈ O)(166)	1.69	71.1
42.	58.3	Hentriacontane(C ₃₁ H ₆₄)(436)	2.89	87.0
43.	58.5	Phen1,4diol,2,3di-methyl-5-tri-fluoro-methyl(C ₉ H ₉ F ₃ O ₂)(206)	0.33	66.8
47.	60.8	VitaminE(C ₂₉ H ₅₀ O ₂)(430)	1.12	86.9
48.	61.1	9,12,15Octa-decatrienoic Acid,2,3di-hydroxypropyl Ester,(Z,Z,Z)(C ₂₁ H ₃₆ O ₄)(352)	0.53	75.3
50.	62.7	Stigmasterol(C ₂₉ H ₄₈ O)(412)	0.34	84.1
51.	63.6	β-Sitosterol(C ₂₉ H ₅₀ O)(414)	0.6	88.6
52.	64.2	3,7,11,15 Tetra-Methyl 2hexa-decen-1-ol(C ₂₀ H ₄₀ O)(296)	2.4	78.6

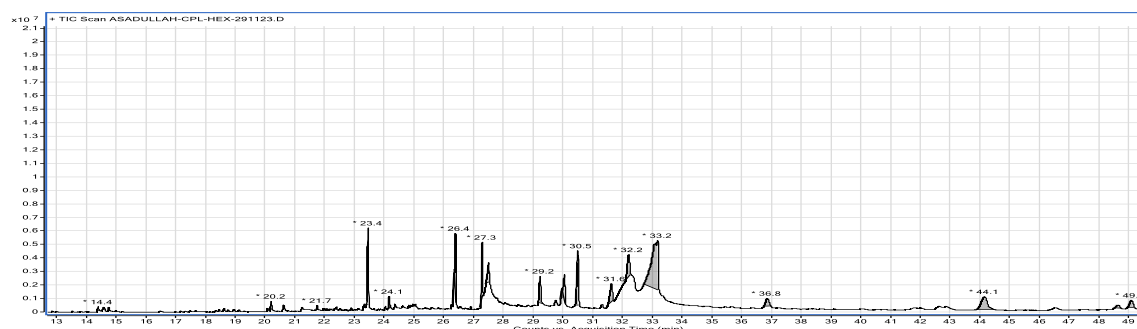
53.	64.5	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octa-deca-hydro-2 H-picen-3-one(C ₃₀ H ₄₈ O)(424)	0.94	86.3
54.	65	13,27-Cycloursan-3-one(C ₃₀ H ₄₈ O)(424)	2.75	80.0
55.	66.1	1-Heptatriacotanol(C ₃₇ H ₇₆ O)(536)	0.75	78.0
56.	66.8	Retinoic-Acid,5,6 epoxy5,6-di-hydro(C ₂₀ H ₂₈ O ₃)(316)	4.07	46.7
57.	67.7	E,E,Z1,3,12 Nonadecatriene-5,14 diol(C ₁₉ H ₃₄ O ₂)(294)	0.61	76.0
58.	68.1	i-Propyl9,12-octadecenadienoate(C ₂₁ H ₃₈ O ₂)(322)	0.59	78.0
59.	68.9	i-Propyl9,12,15-octadecatrienoate(C ₂₁ H ₃₆ O ₂)(320)	1.27	77.3
60.	69.8	Palmitic acid,9-hexadecenyl ester, (Z)-(C ₃₂ H ₆₂ O ₂)(478)	0.6	64.4

Table2:

<i>Phytocomponents</i>	<i>Nature</i>	<i>Activity</i>
Trichloroacetic acid, pentadecyl ester	Ester	Cosmetic
2-Isopropyl-5-methyl-1-heptanol	Alcoholic	Ant-imicrobial
Hexadecane	Hydrocarbons	Anti-microbial, Anti-oxidant ⁶
1-Hexadecene	Alkene	Hypo-cholesterolaemic, Hypo-tensive, Anti -Cancer, Anti-Inflammatory ⁷
Phenol,2,4bis (1,1-di-methyl-ethyl)-	Phenol	Anti-bacterial ⁶
Tetradecane,2,6,10-trimethyl-	Hydrocarbons	NAR
Heptadecane	Hydrocarbon	Anti-microbial ⁶
E-15-Heptadecenal		Anti-oxidant, Anti-bacterial ⁶
Phytol	Alcohol	Anti-oxidant, Anti-microbial, Anti-cancer, Anti-Inflammatory, Diuretic ⁸
Hexa-decanoic Acid,Methyl Ester	Esters	Anti-oxidant, Anti-microbial, Anti-Inflammatory ⁸
5-Eicosene,(E)-	Alkane	Anti-fungal, Anti-tumor, Anti-bacterial, Larvicidal ⁹
Hexadecanoic acid, ethyl ester	Palmitic Acid	Anti-oxidant & -androgenic Hypo-cholesterolemic, Nematicide, ¹⁰
n-Hexadecanoic acid	Folic acid	Mosquito Larvicide, Anti-bacterial, Anti-fungal, Anti-Inflammatory ⁸
1,2Benzene-di-carboxylic Acid, butyl decyl ester	Esters	Antibiotic, Anti-fouling ¹¹
9,12Octa-decadienoic acid (Z,Z), Methyl Ester	Esters	Anti-inflammatory, Anticancer, Antimicrobial, Insectifuge ⁸
1-Docosene	Folic acid	Anti-microbial, Strong Radical Scavengers, Anti-oxidant ⁷
1-Nonadecene	Hydrocarbons	Anti-fungal, Anti-cancer ¹²
9,12,15Octa-decatrienoic Acid, Methyl Ester,(Z,Z,Z)	Methyl Ester	Anti-oxidant, Antifungal, -Inflammatory &-Melanogenic ¹³
Octa-decanoic Acid, Ethyl Ester	Esters	Anti-microbial ¹⁴
9,12 Octa-decadienoic Acid (Z, Z)	Folic acid	Hepato-protective, Anti-arthritis, Anti-Inflammatory, Anti-Histaminic ⁸
9,12,15Octa-decatrienoic Acid, Ethyl Ester,(Z,Z,Z)	Folic acid	Anti-Cancer, Antiarthritic, Antihistaminic, Insectifuge ¹⁰
9,12,15 Octa-decatrienoic Acid,(Z,Z,Z)	Linolenic Acid	Anti-inflammatory, Anticancer, Hepato-protective, Insectifuge ¹⁵
Behenic alcohol	Alcohol	Anti-microbial ⁷
Butyl 9,12,15-octadecatrienoate		NAR
Di-n-octyl phthalate	Plasticizer	Anti-microbial, Anti-fouling ¹⁶

1,2Benzene di-carboxylic Acid, mono2 ethylhexyl Ester	Phthalic Ester	Cytotoxicity, Anti-oxidant, inflammatory, -microbial, & viral ¹⁷
Octadecanoic acid, phenylmethyl ester	Esters	Hypo-cholesterolemic, Nematicide ¹⁶
1-Octacosanol	fatty alcohol	Anti-fatigue ¹⁷
Oxalic acid, dodecyl 2-phenylethyl ester		NAR
Ethyl tetracosanoate	Folic acid	Anti-hypercholesterolemic ¹⁸
Octacosane	Alkane	Antitumor ¹⁹
Squalene	Triterpenoid	Antibacterial, Antioxidant, Antitumor, Immuno-stimulant, Pesticide ¹⁶
1-Hexadecanol,2-methyl-	Alcoholic	Anti-microbial, Antioxidant, Anticancer & Antiinflammatory ²⁰
17-Pentatriacontene		Anti-inflammatory, Anticancer, Antibacterial & Antiarthritic ²¹
Oxalic acid, hexadecyl2-phenylethyl ester		NAR
Benzene, [1-(1-cyclohexen-1-yl)ethyl]-		NAR
Bi-cyclo [3.1.1] hept-2-ene-2-ethanol,6,6-di-methyl	Alkane	NAR
Hentriacontane	Alkane	Anti-bacterial ⁶
Phen1,4 diol,2,3-di-methyl-5-tri-fluoro-methyl		Anti-microbial ²²
Vitamin E	Phenols	Anti-oxidant, Anticoagulant, Antiviral & Anti proliferative, Immune-Modulatory ¹⁵
9,12,15Octa-decatrienoic Acid,2,3di-hydroxy propyl Ester,(Z,Z,Z)		Anti-oxidant, Antimicrobial, Antiinflammatory & Insectifuge ²³
Stigmasterol	Sterol	Anti-oxidant, Antimicrobial, Anticancer, Antiasthama & AntiInflammatory, ¹⁰
β-Sitosterol	Sterol	Anti-lipidic, Anti-cancer ¹⁵
3,7,11,15-Tetra-methyl 2 hexa-decen-1-ol	Alcohol	Anti-microbial & AntiInflammatory
4,4,6a,6b,8a,11,11,14b Octamethyl 1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octa-decahydro 2H picen-3-one		Anti-microbial, Anticancer, Antioxidant & Antifungal ²¹
13,27-Cycloursan-3-one	Steroid	Anti-oxidant, Anti-bacterial, Anti-diabetic ²⁴
1-Heptatriacotanol	Alcoholic	Anti-oxidant, Anti-cancer, Anti-Inflammatory ²⁵
Retinoic Acid,5,6 epoxy5,6-di-hydro	Retinoic acid	Anti-diabetic
E, E,Z1,3,12 Nonadecatriene-5,14 diol		Antimicrobial ²⁵
i-Propyl 9,12-octadecenadienoate		NAR
i-Propyl 9,12,15-octa-decatrienoate		NAR
Palmitic acid,9-hexadecenyl ester,(Z)-	Folic acid	NAR

Source by Dr. Duke's: Phyto-chemical and Ethno-botanical data-bases. NAR-No activity reported



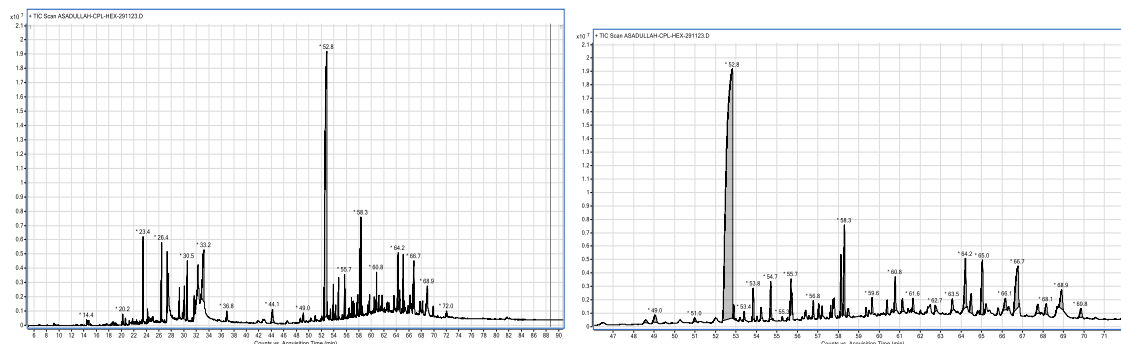


Figure 1: Total Ion Chromatograms (TICs) of n-Hexane Extracts from CPL-RL

TICs of the n-hexane extracts of *CPL-RL* output from a GC-MS. Chromatogram illustrating the elution order of the total 54-investigated compounds. Chromatogram, which shows peaks: the size of a peak indicates the amount of each component reaching the detector. The number of peaks shows different compounds present in the sample. X-axis of the gas-chromatogram shows the amount of time taken for the analytes to pass through the column and reach the mass spectrometer detector. Typically, the y-axis, or the area of the peak, is a reflection of the amount of a specific analyte that present.

- A) TIC scan mix17 compounds in n-hexane extracts, Retention Time/Data.
- B) TIC scan mix15 compounds in n-hexane extracts, Retention Time/Data.
- C) TIC scan mix22 compounds in n-hexane extracts, Retention Time/Data

DISCUSSION

Study uncovered that n-hexane extract from the papaya variety contain active compounds like ester, alcoholic, hydrocarbons, alkene, phenol, diterpene alcohol, Fatty Acid, unsaturated Fatty Acid, aliphatic hydrocarbons, methyl ester, linolenic acid, plasticizer, phthalic ester, triterpenoid, alkane, methylated phenols, sterol, terpene alcohol, and retinoic acid.

The mean PA% of compounds in the extract of n-hexane were far below 5%, an indication that most of the detected phytoconstituents are in trace amounts. This correlates with earlier GC-MS-based phytochemical studies wherein most compounds generally account for a minute fraction of the overall chromatographic profile but may still play important synergistic or bioactive roles. Because of small amounts of existing compounds, it may be suggested that the biological activity of the extract in n-hexane would probably be due to collective influence of several low-abundance compounds and not a single predominant metabolite. Such patterns have been found in several plant extracts, indicating the importance of consideration about qualitative and quantitative aspects of phytochemical composition when conclusions are drawn on the pharmacological potential.

Phytol, serves as a precursor for synthesizing Vitamin E and K. In 2020, Momoh JO et al. revealed that the *CP* (methanolic leaf extract) was 10.16%⁸. In the current study, phytol was identified in n-hexane extract of *RL Cp*, constituting 0.32%. The vitamin E family encompasses 8 naturally occurring constituents, including 4tocopherols (α - β - γ - δ) and 4tocotrienols (α - β - γ - δ). In 2013, Upgrade A et al. showed that the *C. Papaya* (methanolic-leaf extract) contained the same compounds, with a peak area of 2.70% as determined by GC-MS¹⁵. In the present study, vitamin E was identified as a phytocomponent in *CPL-RL* extract 1.12% in n-hexane.

Squalene, a natural lipid essential for cholesterol biosynthesis, is commonly used in drug formulations to treat illnesses. The study revealed squalene 0.21% as a phytocomponent in the *CPL-RL* extracts (n-hexane) due to its relative abundance. Al-Seadi HL et al. 2021 reported the detection of the local variety and *CPL-RL* (ethanolic) using GC/MS, with 3.04% and 1.29%, respectively²⁴.

In the *CPL-RL*, the n-hexane extract includes 3,7,11,15Tetra-methyl-2-hexa-decen1-ol, at concentrations of 2.4%. Al-Seadi HL et al. in 2021 reported the occurrence of the same constituents appearing in the *CPL-RL* (ethanolic), determined by GC-MS was 4.42%²⁴.

N-Hexa-decanoic acid (0.83%),9,12Octa--decadienoic Acid(Z,Z)1.22%, and 9,12Octa-decadienoic acid(Z,Z) Methyl Ester are prominent phytochemical compounds. These compounds unveil a diverse

array of biological activities, by way of described in Table 2⁸. Al-Seadi HL et al. 2021 exhibited the presence of n-Hexa-decanoic Acid in the ethanolic leaf extract of C.P (local variety and RL) by GC-MS, with 5.24% and 4.41%, respectively²⁴. 9,12,15 Octa-decatrienoic Acid, Ethyl Ester, (Z,Z,Z) at 7.29%. The predominant constituent of the *CPL-RL* (n-hexane) is Retinoic Acid, 5,6 epoxy5,6-dihydro (4.07%), which is thought to be derived from retinoic acid.

In 2021, Al-Seadi HL et al. emphasized the compound 13,27-Cycloursan-3-one. Recent research has exposed that 13,27-Cycloursan-3-one is present at a concentration of 2.75% in the *CPL-RL* extracts (n-hexane). However, Octacosane 0.65% in n-hexane extract from *CPL-RL* exhibits antibacterial properties and protects against grafted subcutaneous melanoma, while displaying potent cytotoxicity against mouse melanoma B16F10-Nex2 cells¹⁹.

CONCLUSION

Its phytochemical-rich leaves, especially from the *CPL-RL*, show promise in drug development. GC-MS analysis revealed secondary metabolites with anticancer, antimicrobial, antihelminthic, immunomodulatory, antioxidant, and insecticidal properties.

Recommendations

The leaves of this plant species offer noteworthy potential for discovering new compounds to treat various illness. Further phytochemical and biological studies are essential, indicating *Cp* promise as a novel and effective drug candidate in the pharmacological industry.

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Authors' Contribution

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