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Phytochemicals and metabolites profiling in gamma radiated acid lime (*Citrus aurantifolia* Swingle) cv. Agamalai using GC-MS

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Abstract

Citrus fruits have one of the most well-known flavours in the world, primarily because of their high nutritious content. Beyond these nutritional benefits, citrus includes several pharmacological active compounds that assist healthy metabolic processes. Research on the relation between radiation and biological systems has produced many significant applications in pharmacology, agriculture and health. The investigation is carried out by using the radiation, especially gamma rays in citrus seeds to find the changes of bioactive components. By using GC-MS, the leaf metabolites of citrus is compared with irradiated and non-irradiated samples, to seeks a thorough understanding regarding the way that the radiation governs changes in bioactive compounds. The study concluded that the 26 compounds were found to be similar and 13 bioactive compounds which are varied in both irradiated and non-irradiated samples. Among the principal bioactive substances that were extracted belongs to various groups including fatty acids, triterpenes, monoterpenoids, alcohol, heterocyclic compounds, terpenoid compounds, benzene and acetate group.This preliminary study on citrus by using GC-MS will provide insights of various bioactive components enhanced through gamma radiation.

1. Introduction

Acid lime (*Citrus aurantifolia* Swingle), belongs to Rutaceae family and classified as a perennial shrub or tree (Adnan, 2014). It is well known species utilized for both culinary and medicinal uses (Chellammal, 2022). The crop is grown all over the world and almost every part of the plant has been utilized in traditional medicine for cold and cough, gastrointestinal problems and sore throat because there are abundant in phytochemicals, including flavonoids, vitamin C and essential oils, which possess antioxidant, anti-inflammatory and antimicrobial properties (Al-Aamri *et al*., 2018). Additionally, alkaloid extract from citrus leaves are powerful nutraceutical agent to treat neurodegenerative illnesses (Oyeniran *et al*., 2024). Plant based products have been used for a long time as treatments for many medical conditions. Modern medicine presents several obstacles, but considering the potential for serious adverse effects along with the expanding issue of drug resistance to antibiotics therefore new technologies and discoveries are required (Srivani and Mohan, 2023). The fast increase in antibiotic resistance has made it more important to investigate research on herbal remedies without adverse effects (Prasad *et al*., 2016).

Numerous metabolites and essential oils are found in the healthy leaves of citrus (Konda *et al*., 2022) and it has also been documented that crude extracts of various sections of the citrus leaf, stem, flower and root had antibacterial activity toward the clinically important bacterial strains (Mohanapriya *et al*., 2013). In India, herbal treatments reliably by Ayurveda, Unani, Siddha and Homoeopathy are legally accepted alternative therapies which have a long history of being routinely used for herbal remedies. Millions of Indians consistently utilize herbal medications not only as prescription medications in non-allopathic systems, but also as home remedies, spices, self-medication and healthy foods (Pandian and Noora 2018; Sangeeta *et al*., 2023). Until the beginning of the twentieth century, the majority of medications were made from naturally existing plant ingredients (Abdallah, 2016). With its vast assortment of natural chemicals, the kingdom of plants offers a wealth of different varieties and the plants have been extensively used to make various medicines over thousands of years. Numerous chemicals, including secondary metabolites, are produced by plants that have a wide range of qualities both in biochemical and bioactivity and are used in a number of sectors, including pharmaceuticals.

Alkaloids, coumarins, flavonoids and essential oils are just a few of the bioactive compounds found in lime leaves. Numerous volatile chemicals including limonene, linalool, citronellal and α-pinene are found in the essential oil extricate from the acid lime leaves. This chemical compound is responsible for the distinct aroma and flavor of acid lime leaves. The leaves are also used to treat digestive problems like nausea, bloating and gas. It is thought that the cooling properties of lime leaves can help reduce fever along with other inflammatory conditions and are widely used in Indonesia for the treatment of fever, coughs and colds (Arsana *et al*., 2024). Because of their chemical composition, lime leaves have potential applications in traditional medicine.

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Gamma radiation cause molecular level changes in the plant cells, resulting certain modifications in the gene level, this lead to increases the variation in plants, enhancing species ability to synthesis wide range of secondary metabolites which serve as an abundant source of phyotochemicals and bioactive compounds (Nielsen *et al*., 2019). Also, it is a useful technique for treating a wide range of foods to eradicate food poisoning miocrobes; however, it is unknown how the phytochemical constitution of acid lime leaves changes as a result of the irradiation. Bioactive compounds have been discovered in many natural sources to identify different compounds within a test sample for emphasizing its biological activity by an analytical method known as gas chromatography-mass spectroscopy (GC-MS). In this technique, gas chromatography and mass spectrometry are combined. The GC-MS is crucial in the evaluation of phytochemical component and chemotaxonomic research related to pharmacologically active plant components. Several studies carried out recently have confirmed the therapeutic potential of plants, which is derived from their bioactive phytochemical constituents that can influence metabolism (Sharma *et al*., 2021). This technique analyses the different types of chemicals present in a plant sample and provides detailed information about the types and concentrations of those compounds. This study investigates for the first time and the literature contains no information regarding the separated compounds or phytochemical studies by gamma radiation in acid lime leaves. The study aimed to ascertain the phytoconstituents present in the leaves and also to determine whether any bioactive phytochemical components changed after the gamma irradiation.

2. Materials and Methods

The seeds of Agamalai citrus are manually extracted from the fruits and collected in a beaker of water. The seeds that were bold and free of defects which sink in the water where chosen for the experiment. The seeds were treated with ten distinct gamma radiation treatments ranging from 5 Gy to 45 Gy with an interval of 5 Gy at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Tamil Nadu. The treated seeds were planted alongside and untreated as control in nursery polythene bags filled with red soil, farmyard manures (FYM) and sand where mixed in ratio of 2:1:1, and regular watering was done to provide adequate moisture for germination. Probit analysis was used to determine the lethal dose (LD_{50}) based on germination results. For GC-MS analysis, a treatment dose of 25 Gy has been selected since the LD_{50} value for Agamalai citrus is 26.51 Gy.

The botanical identification and authentication of the plant specimen was undertaken by Dr. Raji. R, Assistant Professor and Head of the Department, Department of Botany, St. Mary's College, Affiliated to the University of Calicut, Wayanad, Kerala and the herbarium specimen was given with Voucher Specimen Number 7703.

2.1 Preparation of leaf powder

The one year old plant leaves of were collected, cleaned and dried for five days at 65°C in a hot air oven. Once completely dried, the leaves were ground to fine powder and the bioactive compounds were extracted with 70% methanol using the Soxhlet extractor. Subsequently, the solvent was removed using a rotary vacuum evaporator to produce a viscous extract. The bioactive ingredients in the samples were examined using the GC-MS method.

2.2 GC-MS analysis

The extracts were analyzed by GC-MS using a thermal GC ultra clarus 500 apparatus. The apparatus is comprised of a mass spectrometer (GC-MS) connected to a gas chromatograph and outfitted with an Elite-I fused RMS 5 silica capillary column, which is made up entirely of dimethyl polysiloxane. An electron ionization device with a 70 eV ionizing energy was used to carry out the detection. One millilitre per minute of ultra-high purity (99.99%) helium was employed as the carrier gas. The injector temperature on the gas chromatograph was fixed between 250 and 260°C, the column was started at 110°C, as it increased by 5°C per minute up to 260°C. Mass spectra and chromatograms were managed using turbo mass software and the average peak area of each component was compared to the overall area to ascertain its percentage composition.

2.3 Determination of components

The Willey7 Library and NIST database were compared to the substance to determine its identity. A database from the National Institute of Standards and Technology was used to analyse and interpret the mass spectra from the GC-MS data. This database has details on more than 90,000 chemicals and the reference obtained was Database 1A, version 14. The gathered data is then subjected to descriptive analysis and a literature search was conducted about the applications of chemicals derived from lime leaves. The identification, composition and molecular weight of the test materials have been determined in this comparison.

2.4 Assessment of substance biological activity

GC-MS examination was utilized to detect active compounds using peak regions and retention periods in a qualitative manner. In order to investigate the biological impacts of the compounds generated was by predictions based on their structural formulas using PASS (Prediction of Activity Spectra for Biologically Active Substances). According to the PASS online database included techniques of estimating different pharmacological effects, specific toxicities, and potential modes of action associated with the drugs (Filimonov *et al*., 2014).

3. Results

Chromatogram data indicate that there were approximately 60 compounds in both irradiated and non-irradiated samples (Table 1 and 2), along with their chemical formula, peak region (%) and retention time (RT) and also important information about the chemical makeup both before and after radiation exposure. For comparison with the chromatograms of the irradiated samples, the chromatogram of the nonirradiated sample acts as a baseline. This was consistent with the findings of the GC-MS study, which demonstrate that exposure to radiation modifies the chemical composition.

According to retention time, the relative concentrations of different chemicals that elute were displayed on the gas chromatogram. Acid lime components with proportional concentrations were indicated by the peak's heights (Figure 1). To determine the nature and structure of the molecules, the mass spectrometer examines the substances that elute at different times. The data library was used to identify the molecule based on its mass spectra, which serve as its fingerprint. Studies on phytochemicals have demonstrated their diuretic, antifungal, allergenic, anaesthetic, antipyretic, analgesic, antioxidant, immunostimulant, anthelmintic, antiseptic, antibacterial, antispasmodic, laxative, nephrotoxic, anti-inflammatory, chemopreventive properties.

The fourteen major phytocompounds present in untreated plant are such as benzofuran, 2,3-dihydro (1.72%), caryophyllene (1.90%), 1, 4 benzodioxan-6-amine (1.16%), benzoic acid, 4-ethoxy-, ethyl ester (2.51%), ethyl alpha-d-glucopyranoside (6.39%), 2H-1-benzopyran-2-one, 7-methoxy (1.80%), lactose (3.81%), 7H-furo[3,2-g][1]benzopyran-7-one 4-methoxy (8.91%), methoxsalen (9.00%), phytol (1.76%), cyclolanost (3.62%), hexadecanoic acid (1.29%), 12-oleanen-3-yl acetate (3.18%), urs-12-en-3-ol, acetate (21.97%) and 44 different minor compounds.

Similarly, gamma irradiated samples recorded 38 minor compounds and 19 major compounds, *viz.,* benzofuran, 2,3-dihydro (1.53%), oxalic acid (1.35%), caryophyllene (4.26%), 2(3H)-benzothiazolone (2.35%), benzoic acid, 4-ethoxy-, ethyl ester (4.46%), ethyl alpha -d-glucopyranoside (10.63%), 6-methoxy-3-methyl-2-benzofurancar boxylic acid (1.30%), 2-methylindene (2.08%), 2H-1-benzopyran-2-one, 7-methoxy (2.54%), methyl beta-d-galactopyranoside (3.96%), H-Furo [3,2-g][1]benzopyran-7-one (2.07%), n-hexadecanoic acid (1.38%), 2H-1-benzopyran-2-one, 5,7-dimetho (1.01%), methoxsalen (12.89%), 7H-Furo[3,2-g][1]benzopyran-7-one 4-methoxy (12.33%), phytol (3.95%), neoisolongifolene (1.72%), octadecanoic acid (1.03%)and dl-alpha -tocopherol (1.90%).

In the current study, 26 compounds were found to be similar in both irradiated and non-irradiated samples. The growing trend of antimicrobial resistance emphasizes the necessity to investigate herbal therapies with low side effects. The chemical compositions of 13 bioactive compounds which are varied between the irradiated and non-irradiated samples were identified through GC-MS analysis even though they all are associated with various biological activities (Table 3). The findings displayed in Table 4 suggest that the primary bioactive substances that were extracted using various solvents were members including fatty acids, alkaloids, triterpenes, monoterpeniod alchohol, organoheterocyclic compounds, terpenoid compounds, benzene, aromatic acid and acetate group.

Table 1: Major phytochemicals identified in acid lime before gamma radiation

Bioactive compounds	Time of retention (min)	Peak area (%)	Molecular formula
Methanamine, N-methoxy	4.565	0.34	C_2H_7NO
Trifluoromethyl t-butyl disulfide	4.864	0.27	$C_5H_9F_3S_2$
1,2-Benzenediol	6.942	0.24	$C_6H_6O_2$
Benzofuran, 2,3-dihydro-	7.220	1.72	C_8H_8O
2,6-Octadienal, 3,7-dimethyl	7.753	0.23	$C_{10}H_{16}O$
2-Methoxy-4-vinylphenol	8.197	0.48	$C_9H_{10}O_2$
Cyclohexane, 1-ethenyl-1-methyl-2	8.953	0.17	$C_{15}H_{24}$
Benzoic acid, 2-(methylamino)	9.120	0.34	$C_9H_{11}NO_2$
Caryophyllene	9.297	1.90	$C_{15}H_{24}$
1,4 Benzodioxan-6-amine	9.642	1.16	$C_8H_9NO_2$
2,4-Difluorobenzoic acid	9.775	0.33	$F_2C_6H_3CO_2H$
Alpha – Farnesene	9.864	0.23	$C_{15}H_{24}$
Cyclohexene, 1-methyl-4-(5-methyl)	9.942	0.19	$C_{15}H_{26}$
Benzoic acid, 4-ethoxy-, ethyl ester	10.053	2.51	$C_{11}H_{14}O_3$
2,3,5,6-Tetrafluoroanisole	10.342	0.16	$C_7H_4F_4O$
Beta-D-Glucopyranoside, methyl	10.453	0.28	$C_7H_{14}O_6$
4-Dimethoxy-3-methylpropiophe	10.497	0.19	$(CH_3O)_2CHCH_2CO_2CH_3$
Cevane -3,4,14,15,16,20-hexol,	10.819	0.41	$C_{29}H_{45}NO_8$
Ethyl alpha-d-glucopyranoside	11.008	6.39	$C_8H_{16}O_6$
Ethyl-(4-n-butylamino) benzoate	11.075	0.44	$C_{18}H_{16}N_2O_4$
2-Methylindene	11.230	0.96	$C_{10}H_{10}$
Heneicosane	11.386	0.20	$C_{21}H_{44}$
2H-1-Benzopyran-2-one, 7-methoxy-	11.741	1.80	$C_{10}H_8O_3$
Lactose	11.875	3.81	C, H, O
1(2H)-Naphthalenone, 3,4-dihydro-	11.964	0.21	$C_{10}H_{10}O$

Alpha-D-Galactopyranoside	12.008	0.53	$C_7H_{14}O_6$
D-Glucose, 4-O-alpha-D-glucopyra	12.064	0.52	$C_{12}H_{22}O_{11}$
2H-Furo[2,3-H]-1-benzopyran-2-one	12.530	0.95	$C_{12}H_8O_4$
7,9-Di-tert-butyl-1-oxaspiro (4,5) d	12.930	0.20	$C_{17}H_{24}O_3$
Hexadecanoic acid, methyl ester	12.986	0.15	$C_{17}H_{34}O_2$
n-Hexadecanoic acid	13.197	0.79	$C_{16}H_{32}O_2$
5-(4-Isopropoxy-phenyl)-2,4-dihydr o-pyrazol-3-one	13.352	0.44	$C_{12}H_{14}N_2O_2$
2H-1-Benzopyran-2-one, 5,7-dimetho	13.497	0.92	$C_{11}H_{10}O_4$
Methoxsalen	13.863	9.00	$C_{12}H_8O_4$
7H-Furo[3,2-g][1] benzopyran-7-one 4-methoxy-	14.030	8.91	$C_{12}H_8O_4$
Phytol	14.197	1.76	$C_{20}H_{40}O$
Acetamide, N-(3-methylphenyl)- 2,2,2-trifluoro-	14.241	1.69	$C_9H_8F_3NO$
6-Octadecenoic acid	14.341	0.25	$C_{18}H_{34}O_2$
Octadecanoic acid	14.474	0.57	$C_{18}H_{36}O_2$
Pentacosane	14.519	0.40	$C_{25}H_{52}$
1,3-Butanedione, 4,4,4-trifluoro-1	14.830	0.16	$C_6H_5COCH_2COCF_3$
Alpha -Amyrin	15.163	0.64	$C_{30}H_{50}O$
Vanadium, Vanadium, (η7-cycloheptatrienylium) (η5-2,4-cyclopentadien-1-yl)	15.297	0.19	$C_{12}H_{12}V$
4,4,6a,6b,8a,11,11,14b-Octamethyl-			
1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12	15.630	0.31	$C_{30}H_{48}O$
1-Bromo-11-iodoundecane	15.685	0.37	$C_{11}H_{22}Brl$
9, 19-Cyclolanost-24-en-3-ol	16.152	3.62	$C_{32}H_{52}O_2$
Prangenin	16.252	0.53	$C_{16}H_{14}O_5$
Hexadecanoic acid	16.496	1.29	$C_{16}H_{32}O_2$
3-Methyl-2,3-dihydro-benzo	16.607	0.22	$C_{14}H_{14}O_2$
12-Oleanen-3-yl acetate	17.163	3.18	$C_{32}H_{52}O_2$
Trimethyl [4-(2-methyl-4-oxo-2-pentyl) phenoxy] silane	17.585	0.16	$C_{15}H_{24}O_2Si$
Urs-12-en-3-ol, acetate	18.063	21.97	$C_{32}H_{52}O_2$
1,2-Bis(trimethylsilyl)benzene	19.863	0.61	$C_{12}H_{22}Si_2$
Trimethyl [4-(1,1,3,3-tetramethylbutyl) phenoxy] silane	20.307	0.28	$C_{17}H_{30}OSi$
dl-alpha-Tocopherol	20.707	0.44	$C_{29}H_{50}O_2$
Silicic acid	21.962	0.17	H_4O_4Si
Cyclotrisiloxane	22.273	0.52	$C_6H_{18}O_3Si_3$
Tetrasiloxane	22.629	0.15	$H_{10}O_3Si_4$

Table 2: Major Phytochemicals identified in acid lime after gamma radiation

Bioactive compounds	Time of retention (min)	Peak area (%)	Molecular formula
Methanamine, N-methoxy	4.576	0.37	C_2H_7NO
Butane, 2,2,3,3-tetramethyl	4.864	0.82	C_8H_{18}
Limonene	5.353	0.59	$C_{10}H_{16}$
1,2-Benzenediol	6.942	0.37	$C_6H_6O_2$
Benzofuran, 2,3-dihydro	7.220	1.53	C_8H_8O
Ethanol, 2-phenoxy	7.297	0.36	$C_8H_{10}O_2$
2,6-Octadienal, 3,7-dimethyl	7.753	0.50	$C_{10}H_{16}O$
2-Methoxy-4-vinylphenol	8.197	0.77	$C_9H_{10}O_2$
2,6-Octadien-1-ol, 3,7-dimethyl acetate	8.731	0.58	$C_{10}H_{16}O$
Cyclohexane, 1-ethenyl-1-methyl-2	8.953	0.39	$C_{15}H_{24}$
Benzoic acid, 2-(methylamino)	9.119	0.44	$C_9H_{11}NO_2$
Oxalic acid	9.242	1.35	$C_2H_2O_4$
Caryophyllene	9.297	4.26	$C_{15}H_{24}$
2(3H)-Benzothiazolone	9.642	2.35	C_7H_5NOS
Trimethyl-2-thienylsilane	9.775	0.73	$C_7H_{12}SSi$
Alpha -Farnesene	9.864	0.55	$C_{15}H_{24}$
Cyclohexene, 1-methyl-4-(5-methyl)	9.942	0.45	$C_{15}H_{26}$
Benzoic acid, 4-ethoxy-, ethyl ester	10.053	4.46	$C_{11}H_{14}O_3$
4-Methyl-2,5-dimethoxybenzaldehyde	10.342	0.47	$C_{10}H_{12}O_3$
3H-Indazol-3-one,	10.497	0.46	$C_7H_6N_2O$
Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)	10.675	0.53	$C_{12} H_{20}$
n-Decanoic acid	10.753	0.48	$C_{10}H_{20}O_2$
Ethyl alpha-d-glucopyranoside	10.975	10.63	$C_8H_{16}O_6$
6-Methoxy-3-methyl-2-benzofurancar boxylic acid	11.108	1.30	$C_{11}H_{10}O_4$
2-Methylindene	11.219	2.08	$C_{10}H_{10}$
Benzene, 1-methoxy-2-(1-methyl-2-m ethylenecyclopentyl)	11.341	0.29	$C_{14}H_{18}O$
Heneicosane	11.386	0.49	$C_{21}H_{44}$
2H-1-Benzopyran-2-one, 7-methoxy	11.741	2.54	$C_{10}H_8O_3$
Methyl beta-d-galactopyranoside	11.875	3.96	$C_7H_{14}O_6$
1(2H)-Naphthalenone, 3,4-dihydro-	11.964	0.40	$C_{10}H_{10}O$
alpha-D-Galactopyranoside	12.008	0.50	$C_7H_{14}O_6$
3,4-Altrosan	12.064	0.67	$C_6H_{10}O_5$
H-Furo[3,2-g][1] benzopyran-7-one	12.530	2.07	$C_{12}H_8O_4$
7,9-Di-tert-butyl-1- oxaspiro(4,5)d	12.930	0.38	$C_{17}H_{24}O_3$
Pentadecanoic acid	12.986	0.34	$CH_3(CH_2)_{13}COOH$
n-Hexadecanoic acid	13.197	1.38	$C_{16}H_{32}O_2$
1,4,5,7-Tetramethyl-furo	13.352	0.71	$C_{10}H_{12}N_2O$
2H-1-Benzopyran-2-one, 5,7-dimetho	13.497	1.01	$C_9H_6O_5$
Methoxsalen	13.863	12.89	$C_{12}H_8O_4$

7H-Furo[3,2-g][1] benzopyran-7-one 4-methoxy	14.030	12.33	$C_{12}H_8O_4$
Phytol	14.197	3.95	$C_{20}H_{40}O$
Neoisolongifolene	14.241	1.72	$C_{15}H_{22}$
Bacchotricuneatin	14.341	0.45	$C_{20}H_{22}O_5$
Octadecanoic acid	14.474	1.03	$C_{18}H_{36}O_2$
Heptacosane	14.519	0.58	$C_{27}H_{56}$
1,3-Butanedione, 4,4,4-trifluoro-1	14.619	0.54	$C_6H_5COCH_2COCF_3$
6H-Cyclobuta[jk]phenanthrene	14.830	0.35	$C_{15}H_{10}$
2H-2-Chromenone,7-[(2-propenyl) oxy	16.263	0.45	$C_{24}H_{18}0_3$
7H-Furo(3,2-g) (1) benzopyran-7-one	16.419	0.68	$C_{12}H_8O_4$
Hexadecanoic acid	16.496	0.73	$C_{17}H_{34}O_2$
Alpha-Amyrin	15.163	0.64	$C_{30}H_{50}O$
Cyclotrisiloxane	18.029	0.34	$H_6O_3Si_3$
1,4-Phthalazinedione, 2,3-dihydro	18.141	0.32	$C_{21}H_{22}N_4O_3$
Tetrasiloxane	20.307	0.51	$H_{10}O_3Si_4$
dl-alpha-Tocopherol	20.707	1.90	$C_{29}H_{50}O_2$
Silicic acid	22.273	0.59	H_4O_4Si
1H-Indole, 1-methyl-2-phenyl	22.618	0.43	$C_{15}H_{13}N$

Table 3: Various bioactive substances and pharmacological activities found in gamma treated acid lime cv. Agamalai citrus

Bioactive compounds	Molecular weight (g/mol)	Role in pharmacological activity	Reference
Limonene	136.23	Antibacterial, neuroprotective anticancer, analgesic, immunological modulation, antioxidant and anti-inflammatory qualities	Chen *et al*., 2024
2(3H)- Benzothiazolone	151.19	Diuretic, anti-inflammatory antitumour, antiasthmatic, antidiabetic, antimicrobial, antitubercular, antiviral, anticonvulsant, antioxidant, antimalarial and anthelmintic	Donia *et al*., 2023
4-Methyl-2,5-dimethoxy-benzaldehyde	180.20	Antimicrobial activity	Setyati *et al*., 2024
3H-Indazol-3-one,		Antitubercular, antidiabetic, anti-inflammatory and ant androgenic	Hussain *et al*., 2011
n-Decanoic acid	172.26	Seizure control	Morah *et al.,* 2023
Heneicosane	296.5	Antimicrobic activity	Vanitha *et al*., 2020
Methyl beta-d-galactopyranoside	194.18	Antimicrobial and anticancer	Amin *et al*., 2021
3,4-Altrosan	162.14	Bacteriostatic and fungicidal	Makeen *et al*., 2020
Pentadecanoic acid	242.40	Antibacterial	Keawsa-Ard., 2016
Neoisolongifolene	218.33	Antimicrobial activity	Tajick *et al*., 2014
Bacchotricuneatin	342.4	Anticancer	Ikpa and Chidozie -Ikpa., 2024
Heptacosane	380.7	Anti-inflammatory	Atewolara-Odule and Oladosu, 2016
1H-Indole, 1-methyl-2-phenyl	207.27	Antifungal activity	Susilawati *et al*., 2023

Table 4: Other pharmacological activity of GC-MS identified compounds in acid lime

Name of the compound	Phytochemical group	Therapeutic activity	Reference
1,2-Benzenediol	Phenols	Antioxidant properties	Karaket and Pansuksanm, 2024
Benzofuran, 2,3-dihydro-	Organoheterocyclic compounds	Antimicrobial activity	Ferdosi *et al*., 2023
2,6-Octadienal, 3,7-dimethyl	Monoterpeniod alchohol	Anti-inflammatory, antiviral, anticonvulsant, antimicrobial, antioxidant, antidiabetic activity and also improves functions of endocrine system	Oganezi *et al*., 2024
2,6-Octadien-1-ol, 3,7-dimethyl acetate	Terpenoid compounds	Anticancer	Swantara *et al*., 2022
Cyclohexane, 1-ethenyl-1-methyl-2	Benzene	Strong scent, flavour and antimicrobial	Sintya *et al*., 2023
Caryophyllene	Sesquiterpene	Anti-inflammatory activity, analgesic, anticonvulsant, sedative, antidepressant properties	Sintya *et al*., 2023
Benzoic acid, 4-ethoxy-, ethyl ester	Aromatic acid	Antimicrobial Preservative	Daffodil *et al*., 2012
n-Hexadecanoic acid	Ester groups	Antimicrobial, antioxidant and anti-inflammatory activities	Oganezi *et al*., 2024
Phytol	Triterpene	Detoxifier, Anticancer and antioxidant	Cheng *et al*., 2024; Prabhadevi *et al*., 2012
Hexadecanoic acid	Fatty acid ethyl ester	Antimicrobial	Bharathi *et al*., 2023
Octadecanoic acid	Steroids	Wound healing	Uttu *et al*., 2023
Alpha-Amyrin	Acetate group	Antihepatoxic, anti-inflammatory, antioxidant and	Nnamonu *et al*., 2016
Cyclotrisiloxane	organosilicon compound	Antibacterial	Lingfa *et al*., 2024
2H-1-Benzopyran-2-one, 7-methoxy	organic compounds	Anti-inflammatory, anticonvulsant, antihypertensive, antiadipogenic and neuroprotective	Venugopala *et al*., 2013
2H-1-Benzopyran-2-one, 5,7-dimetho	organic compounds	Antifungal agents	Umaiyambigai *et al*., 2017
2-Methoxy-4-vinylphenol	Phenols	Anti-inflammatory	Kim *et al*., 2019

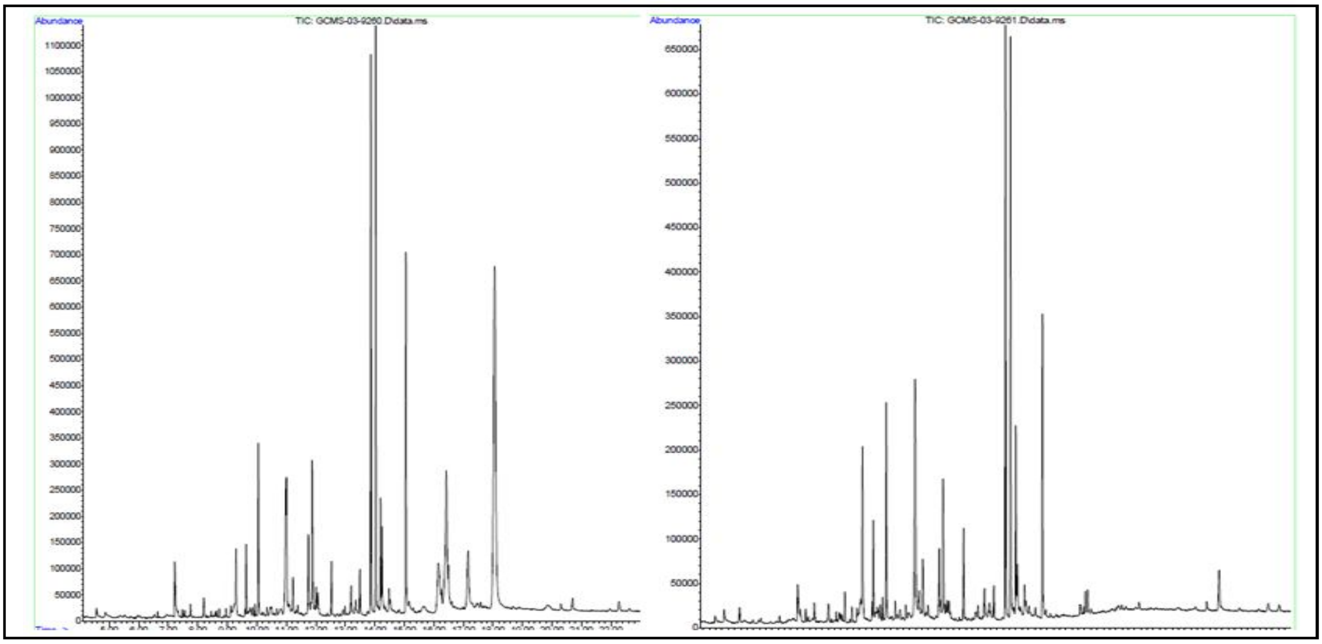

Figure 1: GC-MS assessment of bioactive compounds. (a) Control and (b) Gamma irradiated.

4. Discussion

The application of nuclear techniques in agriculture has increased during the last few decades. According to research findings, morphology and photochemistry are greatly impacted by nuclear technologies, which increase productivity in agriculture and enhance quality and its effects on plants can be seen at the morphological, biochemical and physiological levels. The degree of the change that results is largely determined by exposure level, soil, farming practices and other environmental factors. However, only a small percentage of these studies have used high intensities to precisely measure changes in the main components of the gamma treated plants. This gap emphasizes the necessity of conducting more thorough research in order to fully understand the impacts. A few numbers of plants that may have therapeutic value have seen a sharp increase in standardization over recent years. Citrus has a wide range of medical applications and a very low toxicity. Pharmacognostic studies continue to be a more trustworthy method of identifying plant medicines, even in light of current technology advancements.

In the present study, the radiation exposure can alter the concentrations of phytochemicals, and the effect is dependent upon the radiation dosage. The geographic locations of the plant, surrounding vegetation and the kind of solvent utilized throughout the extraction process have an influence on determining the concentration of the compounds. The high frequency, along with high energy photons and a short wavelength of gamma radiation which makes it a significant form of ionizing radiation for the environment since it harms macromolecules including DNA, proteins and lipids (Golz and Bradshaw, 2019). The application of ionising radiation is to alter components and enhance their physicochemical and biological characteristics (Song *et al.,* 2022). Increased bioactive substances and antioxidant activity were observed in acid lime when exposed to gamma rays these comparable outcomes were also documented by Gajbar *et al.*, 2022.

The current investigation using gas chromatography-mass spectrometry showed that acid lime leaves contain a variety of bioactive chemicals that are effective in therapeutic activity. Of the 26 compounds identified by GCMS analysis, 13 components are known to be bioactive and have good antimicrobial, antioxidant anticonvulsant, anti-inflammatory, antimalarial, anthelmintic anti-diabetic and anticancer activities even though no activity has been documented for several of the compounds like silicic acid tetrasiloxane, 1,4-phthalazinedione, 2,3-dihydro, cyclotrisiloxane, 1,4,5,7-tetramethyl-furo, alpha-farnesene and 2H-2-chromenone,7-[(2-propenyl)oxy, similar to the research of (Khan and Ahmad 2021) in quince leaves. In evaluating the effects of radiation, it is important to take into account the sample states (solid or liquid, fresh or dried, moisture content), irradiation processes (dose rates, sample temperature during irradiation), and experimental procedures (extraction techniques, solvent exposure times, storage conditions, temperature, light, *etc.* (Thongphasuk and Thongphasuk, 2012).

Understanding these bioactive compounds mechanisms of action will be crucial for preparing them for clinical investigations, which could lead to new avenues for research. It is necessary to isolate and purify these substances to comprehend their mechanisms and obtain a better understanding of their effects.

5. Conclusion

The impact of gamma irradiation on acid lime leaves chemical composition was investigated and the findings revealed that the irradiation process significantly altered the composition and the presence of existing substances has pharmacological characteristics. In final analysis, the investigation revealed that, 13 bioactive compounds that differ in both irradiated and non-irradiated samples and the 26 compounds that were determined to be identical. This study indicates that acid lime leaves are an excellent source of bioactive compounds with notable therapeutic potential. However, more studies are needed to enhance our understanding of the chemical classification of these compounds. Therefore, it is necessary to use solvents with different polarities to obtain extracts, analyze their composition and bioactivity and also isolate and identify the secondary metabolites responsible for the biological activity. In order to investigate the active principles in citrus leaves, this kind of GC-MS study serves as a first step and these findings will open up novel possibilities for research in future.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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