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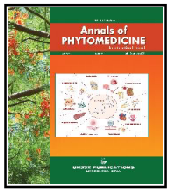

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Comparison of proximate analysis on phytochemicals and metabolite profiling of gamma-irradiated and mutagenic chemically treated Jamun leaves (*Syzygium cumini* (L.) Skeels) using GC-MS technique

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Abstract

The current study investigates the proximate analysis, phytochemical content, and metabolite profiling of gamma-irradiated and chemically mutagen-treated jamun leaves (*Syzygium cumini* (L.) Skeels) using gas chromatography-mass spectrometry (GC-MS). It included eleven different treatments from the result showed that the T_5 (Gamma irradiation at 50 Gy) achieved the highest levels of crude fat (0.68%), crude fiber (8.24%), crude protein (5.56%) and ash content (1.98%). Jamun leaves treated with gamma radiation and EMS chemical extracts were analysed using GC-MS method. A total of 15 and 16 metabolites were identified in treatments T_5 and T_{10}, respectively, based on their retention times (RT), peak areas, molecular weights, and IUPAC names. Key compounds identified in T_5 include 1,2,3-benzenetriol (RT: 8.60 min, 14.33%), 1H-cycloprop[e]azulene (RT: 9.94 min, 16.69%), and 4-((E)-((Z)-5-(4-ethoxy-3-methoxybenzylidene)-3-ethyl-4-oxothiazolidin-2-ethylidene) amino), benzoic acid (RT: 10.03 min, 15.42%). In T_{10}, significant components include ethylparaben (47.67%, RT: 10.03 min), 1,2,3-benzene-triol(13.25%, RT: 8.60 min), and 1-(4-chlorophenyl)-2-cyclopropylethanone (13.25%, RT: 8.60 min). Among the extracted principal bioactive substances belong to various groups including fatty acids, triterpenes, monoterpenoids, alcohol, heterocyclic compounds, terpenoid compounds, benzene and acetate groups.

1. Introduction

Jamun (*Syzygium cumini* (L.) Skeels), belongs to the Myrtaceae family and is classified as a perennial shrub or tree (Adnan *et al*., 2014). It is a well-known species utilized for both culinary and medicinal uses (Chellammal, 2022). This plant, grown across the globe, has been a staple in traditional medicine for ailments affecting the digestive tract and throat. It is various parts are a treasure of phytochemicals, such as flavonoids, vitamin C, and essential oils, which offer antioxidant, anti-inflammatory and antimicrobial benefits (Al-Aamri *et al.,* 2018; Sangeeta *et al.,* 2023). Additionally, alkaloid extract from jamun leaves is a 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, 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 jamun (Konda *et al.,* 2022) and it has also been documented crude extracts of various sections of the jamun leaf, stem and flower.

In perennial crops, mutation breeding has proven to be effective in creating variability at the genetic level within a short period. Mutation breeding has emerged as a key method for generating genetic diversity and providing valuable genetic resources in recent years (Sharma *et al.,* 2021). Various physical as well as chemical mutagens were used to accelerate its rate artificially (Mba *et al.,* 2010; Shu *et al.,* 2012). The effectiveness of any mutagenic agent in a breeding program depends on its ability to induce a large proportion of desirable changes as compared to undesirable ones (Atewolara-Odule and Oladosu, 2016). The choice of mutagen is influenced by several factors, including the specific tissue, desired mutation type, mutagen availability, and safety concerns. Typically, physical mutagens like gamma rays or thermal neutrons were used for mutation breeding (Bado *et al.,* 2015). Ionizing radiations, such as gamma rays and X-rays, have been more commonly used for inducing mutations in fruit plants compared to non-ionizing radiations. This is because ionizing radiations cause more widespread tissue damage, leading to a higher rate of mutations (Esnault *et al.,* 2010; Pathirana, 2011; Yoshihara *et al*., 2013). In the past four decades, gamma rays have become increasingly popular for mutagenesis due to their safety and ability to penetrate deeply into tissues (Mba *et al.,* 2010; Wani and Anis, 2015). While irradiation techniques are effective, chemical mutagens often result in more targeted and predictable mutations. Additionally, chemical mutagenesis procedures are generally simpler and do not require

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specialized or expensive equipment (Singh *et al.,* 2020). Although, many mutagenic compounds exist, only a limited number of alkylating agents are widely used in experimental mutagenesis. Ethyl methane sulphonate (EMS) is a mono-functional alkylating agent that causes genic mutations at high frequency and aberration at low frequency (Till *et al.,* 2004). EMS or alkylating compounds can covalently bounder their reactive alkyl groups to the organic macromolecules at their nucleophilic centers (Girija and Dhanavel, 2009; Pathirana, 2011).

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 limited information regarding the separated compounds or phytochemical studies by gamma radiation in jamun leaves. The study aimed to evaluate the impact of gamma irradiation and EMS on the phytochemical profile, proximate composition, and bioactive compounds in jamun leaves using GC-MS analysis.

2. Materials and Methods

2.1 Authentication of plant material

Fresh leaves of *Syzygium cumini* (L.) Skeels were collected from the nursery at the Department of Fruit Science, Horticultural College and Research Institute, Periyakulam (10.1283° N, 77.5998° E, and 411 meters above mean sea level). The plant was authenticated by the Department of Biology, Gandhigram Rural Institute, Gandhigram, Dindigul Vide GRI/BIOLOGY/Plant/Jamun/362.

The bud sticks of jamun were collected and wrapped in wet gunny bags to retain moisture. Bold, defect-free bud sticks that sank in water were selected for the experiment. These bud sticks were subjected to five distinct gamma radiation treatments at the Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Tamil Nadu, as well as to ethyl methane sulphonate (EMS), a chemical mutagen, at five different concentrations. The details of the treatments and control are provided in Table 1, as well as Figures 1 and 2.

The treated bud sticks were grafted onto rootstocks in nursery polythene bags containing a mixture of red soil, farmyard manure (FYM), and sand in a 2:1:1 ratio. Regular watering was performed to maintain adequate moisture levels. Probit analysis was used to determine the lethal dose (LD_{50}) based on germination results. A gamma radiation dose of 15 Gy was selected for GC-MS analysis since the LD_{50} value for Konkan Bahaduli jamun was calculated to be 17.56 Gy.

Table 1: Bud sticks were treated with different mutagens

Mutagen details	Treatments	Dosages
Control	T_0	-
Gamma-rays	T_1	10 Gy
	T_2	20 Gy
	T_3	30 Gy
	T_4	40 Gy
	T_5	50 Gy
EMS (ethyl methane sulphonate)	T_6	0.2%
	T_7	0.4%
	T_8	0.6%
	T_9	0.8%
	T_{10}	1.0%

Figure 1: Gamma irradiation treated plant.

Figure 2: EMS treated plant.

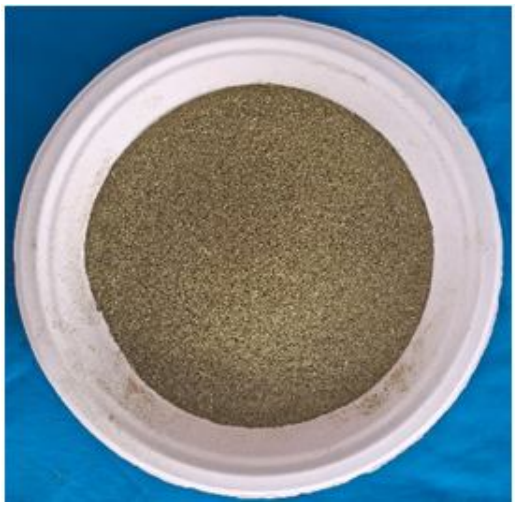

Figure 3: Jamun leaf powder.

Figure 4: Jamun leaf powder extract prepared for GC-MS analysis.

2.2 Preparation of leaf extract from its powder

The leaves of one-year-old plants (Figures 1 and 2) were collected, cleaned, and oven-dried at 65°C for five days. The dried leaves were ground into a fine powder (Figure 3) and extracted with 70% ethanol using a Soxhlet extractor, as described by Pandidurai *et al.* (2022). The solvent was evaporated to obtain a viscous extract (Figure 4), which was then analyzed for bioactive compounds using GC-MS.

2.3 Gas chromatography mass spectrometry (GC-MS) analysis

The extracts were analyzed using a GC-MS system, specifically a clarus 500 apparatus. This system consisted of a gas chromatograph coupled with a mass spectrometer and a dimethylpolysiloxane-based elite-I fused silica capillary column. For detection, an electron ionization device with a 70-eV ionizing energy was employed. Helium gas (99.99% purity) was used as the carrier gas at a flow rate of 1 ml/min. The GC injector temperature was maintained between 250 and 260°C, while the column temperature was programmed to start at 110°C and increase at a rate of 5°C/min up to 260°C (Dutta *et al*., 2020). Mass spectra and chromatograms were processed using turbo mass software. The percentage composition of each component was determined by comparing its average peak area to the total area.

2.3.1 Determination of component

The substance was identified by comparing it to entries in the Willey 7 Library and the NIST database. The NIST database, containing information on over 90,000 chemicals, was used to analyze the mass spectra obtained from GC-MS. The identified compounds were then researched for their potential applications, particularly about lime leaves. This comparative analysis allowed for the determination of the identity, composition, and molecular weight of the test substances.

2.3.2 Assessment of substance biological activity

GC-MS analysis was employed to qualitatively identify active compounds based on their peak regions and retention times. To predict the potential biological effects of these compounds, their structural formulas were input into the PASS (prediction of activity spectra for substances) online database. This database uses computational techniques to estimate various pharmacological activities, toxicities, and potential mechanisms of action associated with the compounds (Filimonov *et al.,* 2014).

2.4 Proximate analysis

The proximate composition of jamun leaves, including ash, crude fiber, crude fat, and crude protein, was determined in triplicate following the methods outlined by the Association of Official Analytical Chemists (AOAC, 1999).

3. Results

3.1 Proximate analysis

3.1.1 Ash content (%)

The data about the ash content showed significant differences among the treatments and the mean values are presented in Figures 5 and 6. The mean value of ash content is ranged between 1.12 % and 1.98 %. In terms of different irradiation, T_5 (gamma radiation 50 Gy) recorded the highest ash content (1.98 %), followed by T_{10} - EMS 1.0 % (1.96 %), while the lowest ash content (1.12 %) was recorded in T_2 (gamma radiation 10 Gy) shown in Table 2.

3.1.2 Crude fat content (%)

The data about the changes in crude fat content in jamun leaves are presented in the Figures 5 and 6, with mean values ranging from 0.01 per cent to 0.68 per cent. Among the different treatments of radiations with chemical mutagen, T_5 (Gamma radiation 50 Gy) exhibited the highest crude fat content of 0.68 per cent, followed by T_{10} (EMS at 1.0%) with 0.58 per cent. Alternatively, T_0 (Control) displayed the lowest crude fat concentration of 0.15 per cent shown in Table 2.

3.1.3 Crude fibre content (%)

Observations on the crude fiber content are presented in Figures 5 and 6. The mean value of crude fiber content varied between 7.07 and 8.24 % among different treatments. In terms of physical mutation, (T_5 gamma radiation 50 Gy) noted the highest crude fiber content (8.24 %), followed by T_4 (gamma radiation 40 Gy) at 8.12 %, while the lowest crude fiber content was recorded in T_0 (control) with the value of 7.07 % shown in Table 2.

3.1.4 Crude protein content (%)

The observations recorded on the crude protein content of jamun leaves are depicted in Figures 5 and 6. Within the gamma radiation, T_5 (50 Gy) reported the highest crude protein content at 5.56 per cent, followed by T_{10} (EMS at 1.0%) with 5.48 per cent. Whereas, T_0 (Control) exhibited the lowest crude protein concentration at 4.42 per cent shown in Table 2.

Table 2: Effect of different mutagenic treatments on proximate composition of the jamun leaves

S. No.	Treatment details	Ash (%)	Crude fat (%)	Crude fibre (%)	Crude protein (%)
1.	T_0 - Control	1.15	0.15	7.07	4.42
2.	T_1	1.12	0.21	7.55	4.58
3.	T_2	1.45	0.39	7.78	4.79
4.	T_3	1.61	0.417	7.96	4.99
5.	T_4	1.84	0.55	8.12	5.23
6.	T_5	1.98	0.683	8.24	5.56
7.	T_6	1.18	0.19	7.17	4.64
8.	T_7	1.33	0.26	7.35	4.923
9.	T_8	1.58	0.33	7.58	5.117
10.	T_9	1.72	0.42	7.69	5.293
11.	T_{10}	1.96	0.583	7.84	5.48
	SE (d)	**0.03**	**0.01**	**0.14**	**0.20**
	CD (p<0.05)	**0.07**	**0.03**	**0.29**	**0.05**

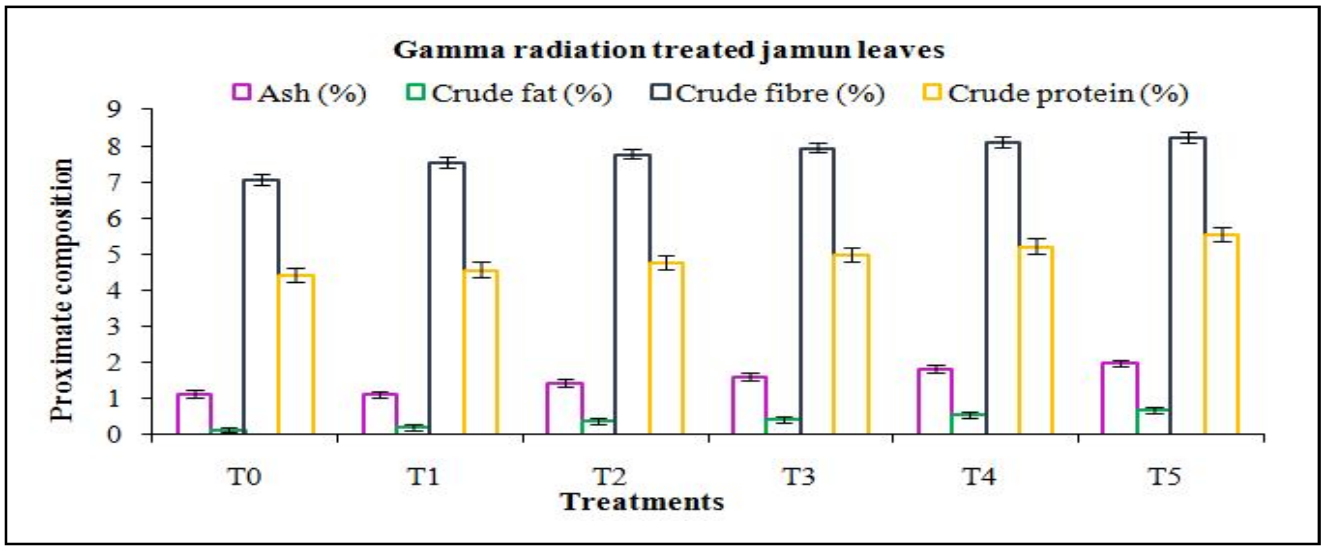

Figure 5: Effect of gamma irradiation on per cent ash, crude fat, crude fibre, and crude protein content of jamun leaves.

3.2 Metabolite profiling using GC-MS analysis

GC-MS analysis was used to identify the bioactive compounds present in jamun leaves. The chromatogram and mass spectrum (Figure 7) revealed the presence of various 15 components in jamun leaves from the T_5 treatment (Gamma radiation at 50 Gy). Table 3 lists the identified compounds, including their retention time (RT), peak area (%) and molecular formula. A total of 15 compounds were detected in T_5 jamun leaves. Significant compounds included beta-Pinene (6.44%), 1,2,3-benzenetriol (14.33%), 1H-cycloprop[e]azulene (16.69%), 4-((E)-((Z)-5-(4-ethoxy-3-methoxy benzylidene)-3-ethyl-4-oxothiazolidin-2-ethylidene)amino) benzoic acid (15.42%), 1,6,10-dodecatrien-3-ol (6.42%), d-nerolidol (6.42%), 13,5-trimethyl-2-cyclohexylbenzene (15.05%), 2H-2-chromenone (15.05%), pentane (17.17%), n-butyric acid 2-ethylhexyl ester (17.17%), octyl thioglycolate (17.17%), 2,6-bis (1,1-dimethyl ethyl)-4-[5-(methylsulfonyl)-1,3,4-thiadiazol-2-yl]-phenol (8.46%), ethylparaben (8.46%), 3,5-di-tert-butyl-4-hydroxybenzaldehyde (8.46%) and ethyl 3-amino-4-pyrazolecarboxylate (15.05%).

Various components present in jamun leaves treated with chemical mutagens identified by chromatogram and mass spectrum (Figure 8) indicate that the jamun leaves from the T_{10} (1.0 %). The identified compound's retention time (RT), peak area (%), and molecular formula are presented in Table 5. Notably, 16 components were found in jamun leaves from the T_{10} treatment with significant compounds

such as 1,2,3-benzene-triol (13.25%), -3,4-dihydro-2H-1,5-benzodiazepine-3-ol (13.25%), 2',4'-dihydroxy-3'-methylpropiophenone (8.26%), 1,4-benzodioxan-6-amine (8.26%), 2-phosphonopropionic acid (8.26%), benzoic acid, 4-ethoxy-, ethyl ester (5.82%), ethylparaben (47.67%), 1-(4-chlorophenyl)-2-cyclopropylethanone (13.25%), 1-hydroxycyclopropane-1-carboxamide (2.00%), L-trans-pinocarveol (6.42%), 2-bromopropionic acid (2.00%), diphenyl sulfone (12.01%), benzene, 1,1'-[1,2-ethenediyl bis (sulfonyl)] bis-, (z)- (12.01%), 1-allylcyclopropanecarboxylic acid (16.82%), 2-cyclopenten-1-one, 2,3,4-trimethyl- (16.82%), 2-n-heptylfuran (16.82%).

Therefore, the jamun leaf extract treated with gamma irradiation and EMS was analyzed using GC-MS for comparison. The GC-MS chromatogram (Figures 7 and 8) illustrates the diverse phytochemical compounds found in jamun leaves under physical and chemical treatment. Tables 4 and 6 provide a detailed list of these bioactive compounds, including their molecular weights and potential therapeutic properties. EMS-treated jamun has 16 various bioactive components recorded by chromatogram and mass spectrum analysis, which was the first time reported. Ethylparaben reaches the highest peak time (47.47%) in chemical mutagen compared to the physical mutation.

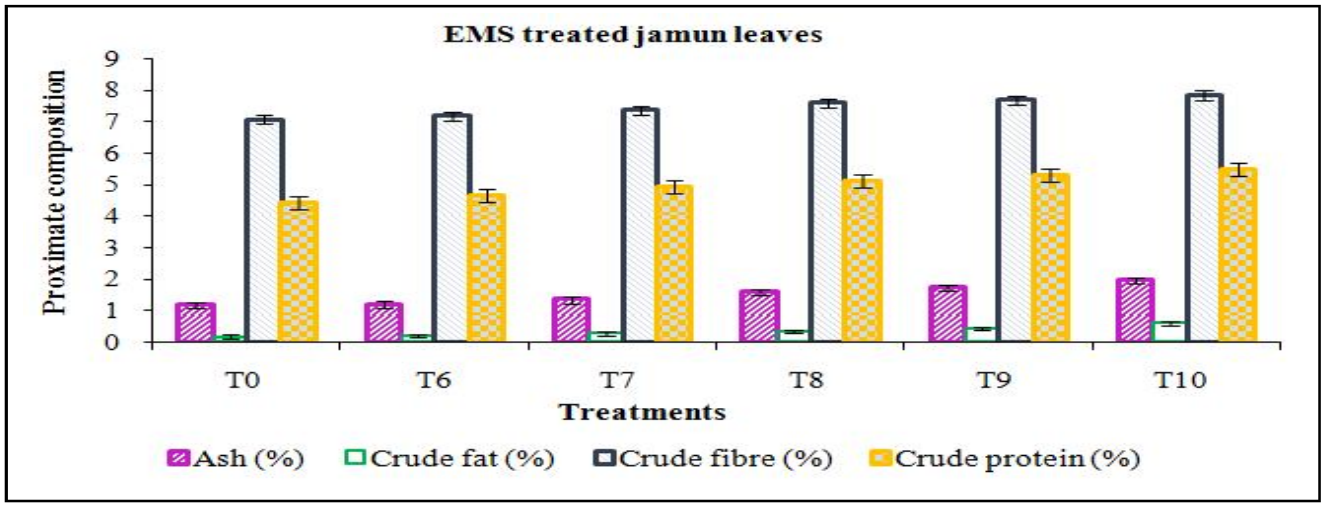

Figure 6: Effect of EMS on per cent ash, crude fat, crude fibre, and crude protein content of jamun leaves.

Table 3: Different bioactive compounds present in the leaves of jamun treated with gamma irradiation and their IUPAC name, molecular weight, retention time and peak area by using GC-MS analysis

S. No.	Compound name	IUPAC name	Molecular weight (g/mol)	Peak area (%)	Retention time (RT) (min)
1.	Beta.- pinene	6,6-dimethyl-2-methylidenebicyclo [3.1.1] heptane	136.23	6.44	4.79
2.	1,2,3-benzenetriol	Benzene-1,2,3-triol	126.11	14.33	8.60
3.	1H- cycloprop[e] azulene, 1a,2,3,4,4a, 5,6,7b-octahydro-1, 1,4,7-tetramethyl-, [1aR-(1a.alpha., 4alpha.,4a.beta.,7b. alpha.)]-	1,1,4,7-tetramethyl-1a,2,3,4,4a,5,6, 7b-octahydrocyclopropa[e]azulene	204.35	16.69	9.94
4.	4-((e)-((z)-5-(4-eth-oxy-3-methoxy ben-zylidene)-3-ethyl-4-oxothiazolidin-2-ethylidene) amino) benzoic acid	4-[[(5Z)-5-[(4-ethoxy-3-methoxyphenyl) methylidene]-3-ethyl-4-oxo-1,3-thiazolidin -2-ylidene] amino] benzoic acid	426.5	15.42	10.03
5.	1,6,10-dodecatrien-3 -ol, 3,7,11-trimethyl-, (6e)-	(6E)-3,7,11-trimethyldodeca-1,6,10-trien -3-ol	222.37	6.42	10.30
6.	D-nerolidol	(3s,6z)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol	222.37	6.42	10.30
7.	13,5-trimethyl-2-cyclohexylbenzene	2-cyclohexyl-1,3,5-trimethylbenzene	202.33	15.05	10.55

8.	2H-2-chromenone, 7-[(2-propenyl)oxy]	7-prop-2-enoxychromen-2-one	202.21	15.05	10.55
9.	Pentane, 1-propoxy	1-propoxypentane	130.229	17.17	10.76
10.	N-butyric acid 2-ethylhexyl ester	2-ethylhexyl butanoate	200.32	17.17	10.76
11.	Octyl thioglycolate	Octyl 2-sulfanyl acetate	204.33	17.17	10.76
12.	2,6-bis (1,1-dimethyl ethyl)-4-[5-(methyl sulfonyl)-1,3,4-thia-diazol-2-yl]-phenol	2,6-ditert-butyl-4-(5-methylsulfonyl-1,3, 4-thiadiazol-2-yl) phenol	368.5	8.46	11.23
13.	Ethylparaben	Ethyl 4-hydroxybenzoate	166.17	8.46	11.23
14.	3,5-di-tert-butyl-4-hydroxybenzaldehyde	3,5-ditert-butyl-4-hydroxybenzaldehyde	234.33	8.46	11.23
15.	Ethyl 3-amino-4-pyrazolecarboxylate	Ethyl 5-amino-1h-pyrazole-4-carboxylate	155.15	15.05	10.55

Table 4: Using GC-MS analysis determined the molecular formula of these components in jamun leaves treated with gamma irradiation and analyzed their potential pharmaceutical properties

S. No.	Compound name	Molecular formula	Pharmaceutical properties
1.	Beta.- pinene	$C_{10}H_{16}$	An isomer of pinene having an exocyclic double bond is called beta-pinene. It is a part of many plant's essential oils. It functions as a metabolite of plants.
2.	1,2,3-benzenetriol	$C_6H_6O_3$	A benzene-triol with hydroxy groups at positions 1, 2, and 3 is called pyrogallol. It functions as a metabolite of plants. It is a benzene-triol and a phenolic donor.
3.	1H- cycloprop[e]azu-lene, 1a,2,3,4,4a,5,6, 7b-octahydro-1,1,4,7 -tetramethyl-, [1aR-(1a.alpha.,4alpha.,4a. beta.,7b.alpha.)]-	$C_{15}H_{24}$	The compound is a stereoisomeric organic compound. Its pharmaceutical activity would depend on its structural and stereochemical properties.
4.	4-((E)-((Z)-5-(4-ethoxy-3-methoxy benzylidene)-3-ethyl-4-oxothiazolidin-2-ylidene) amino) benzoic acid	$C_{22}H_{22}N_2O_5S$	The compound is a derivative with a thiazolidinone core structure. Such compounds are often studied for their diverse pharmacological activities due to the presence of thiazolidinone, benzylidene, and carboxylic acid functional groups, which provide multiple points for biological interaction.
5.	1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl -, (6E)	$C_{15}H_{26}O$	It is a naturally occurring substance with a floral scent in various flowers and plants. Trans and cis are its two geometric isomers in terms of chemistry. It serves various purposes as a flavouring agent, pheromone, neuroprotective, antifungal, anti-inflammatory, antihypertensive, antioxidant, volatile oil component, insect attractant, and herbicide. It is a volatile, tertiary allylic alcohol, and farnesane sesquiterpenoid.
6.	D-nerolidol	$C_{15}H_{26}O$	A (6Z)-nerolidol with the hydroxy group at position 3 taking on an S-configuration is called (3S,6Z)-nerolidol. It is a (3R,6Z)-nerolidol enantiomer.
7.	13,5-trimethyl-2-cyclohexylbenzene	$C_{15}H_{22}$	The compound appears to be a hydrocarbon with an aromatic benzene ring substituted with three methyl groups and a cyclohexyl group. While such compounds are less commonly discussed in direct pharmaceutical contexts compared to heterocyclic or functionalized aromatic compounds, their structural properties suggest potential indirect biological or pharmaceutical relevance.
8.	2H-2-chromenone,7-[(2-propenyl)oxy]	$C_{12}H_{10}O_3$	A class of benzopyranone compounds known for their diverse pharmacological activities. The 7-[(2-propenyl) oxy] substitution adds an allyloxy group to the coumarin core, which could influence its bioactivity.
9.	Pentane, 1-propoxy	$C_8H_{18}O$	While this specific compound is commonly associated with direct pharmaceuti-cal activity, its properties make it potentially useful in certain applications.
10.	N-butyric acid 2 ethylhexyl ester	$C_{12}H_{24}O_2$	It is derived from n-butyric acid and 2-ethylhexanol. While this compound is -primarily known for industrial and cosmetic uses (*e.g.,* as a solvent or flavoring agent), its pharmaceutical relevance can be considered functional.

11.	Octyl thioglycolate	$C_{10}H_{20}O_2S$	This compound combines the properties of the thiol group (-SH) and the ester functionality, potentially contributing to its pharmaceutical or cosmetic relevance. It is more commonly known for its applications in cosmetics, but its structural components suggest potential pharmaceutical utility.
12.	2,6-bis (1,1-dimethyl ethyl)-4-[5-(methyl-sulfonyl)-1,3,4-thiadi-azol-2-yl]-phenol	$C_{17}H_{24}N_2O_3S_2$	The compound is a complex aromatic molecule containing a phenol group, bulky tert-butyl substituents, and a thiadiazole ring with a methylsulfonyl group. Such structural elements suggest the potential for a range of pharmaceutical activities based on known bioactive functionalities.
13.	Ethylparaben	$C_9H_{10}O_3$	Ethylparaben is a member of the parabens class, widely recognized for its use as a preservative in pharmaceuticals, cosmetics, and food products. It is derived from p-hydroxybenzoic acid and is primarily valued for its antimicrobial properties. However, it may also have other potential pharmaceutical applications.
14.	3,5-di-tert-butyl-4-hydro-xybenzaldehyde	$C_{15}H_{22}O_2$	The phenolic group is known to scavenge free radicals and prevent oxidative damage in biological systems. The presence of bulky tert-butyl groups enhances the stability of the phenoxyl radical formed during antioxidant activity, improving its efficacy. Compounds with phenolic and aldehyde functionalities often exhibit antibacterial and antifungal properties.
15.	Ethyl 3-amino-4-pyrazo-lecarboxylate	$C_6H_9N_3O_2$	Pyrazole derivatives are known for their diverse biological activities and can play important roles in plant and pharmacological studies. Here's an overview of the potential phytochemical activities of Ethyl 3-amino-4-pyrazolecarboxylate

Table 5: Different bioactive compounds present in the leaves of jamun treated with EMS mutagens and their IUPAC name, molecular weight, retention time and peak area by using GC-MS analysis

S. No.	Compound name	IUPAC name	Molecular weight (g/mol)	Peak area (%)	Retention time (RT) (min)
1.	1,2,3-benzenetriol	Enzene-1,2,3-triol	126.11	13.25	8.60
2.	-3,4-dihydro-2h-1,5-benzodiazepine-3-ol	3-methyl-2,4-dihydro-1,5-benzodiazepine-3-ol	176.24	13.25	8.60
3.	2',4'-dihydroxy-3'-methylpropiophenone	1-(2,4-dihydroxy-3-methyl phenyl) propane-1-one	180.2	8.26	9.61
4.	1,4-benzodioxan-6-amine	2,3-dihydro-1,4-benzo-diox in-6-amine	151.16	8.26	9.61
5.	2-phosphonopropionic acid	2-phosphonopropanoic acid	154.06	8.26	9.61
6.	Benzoic acid, 4-ethoxy-, ethyl ester	Ethyl 4-ethoxy benzoate	194.23	5.82	6.79
7.	Ethylparaben	Ethyl 4-hydroxybenzoate	166.17	47.67	10.03
8.	1-(4-chlorophenyl)-2-cyclopropylethanone	1-(4-chlorophenyl)-2-cyclo propylethanone	194.66	13.25	8.60
9.	1-hydroxycyclopropane-1-carboxamide	1-hydroxy cyclopropane-1-carboxamide	101.1	2.00	12.36
10.	L-trans-pinocarveol	(1S,3R,5S)-6,6-dimethyl-2-methylidenebicyclo [3.1.1] heptane-3-ol	152.23	6.42	10.30
11.	2-bromopropionic acid	2-bromopropanoic acid	152.97	2.00	12.36
12.	Diphenyl sulfone	Benzene sulfonyl benzene	218.27	12.01	13.13
13.	Benzene, 1,1'-[1,2-ethenediyl bis (sulfonyl)] bis-, (z)-	[(E)-2- (benzene sulfonyl) ethenyl] sulfonyl benzene	308.4	12.01	13.13
14.	1-allylcyclopropanecarboxylic acid	1-prop-2-enyl cyclopropane-1-carboxylic acid	126.15	16.82	13.17
15.	2-cyclopenten-1-one, 2,3,4-trimethyl-	2,3,4-trimethyl cyclo pent-2 -en-1-one	124.18	16.82	13.17
16.	2-n-heptylfuran	2-heptylfuran	166.26	16.82	13.17

Table 6: Using GC-MS analysis the molecular formula of these components in jamun leaves treated with EMS mutagens and analyzed their potential pharmaceutical properties

S. No.	Compound name	Molecular formula	Pharmaceutical properties
1.	1,2,3-benzenetriol	$C_6H_6O_3$	A benzene-triol with hydroxy groups at positions 1, 2, and 3 is called pyrogallol. It functions as a metabolite of plants. It is a benzene-triol and a phenolic donor.
2.	3-methyl-3,4-dihydro-2H-1,5-benzodiazepine-3-ol	$C_{10}H_{12}O_3$	Pyrogallol's antioxidant properties make it useful in certain medical formulations. Historically, it was used in dermatology for treating skin conditions like psoriasis, though its use has declined due to toxicity concerns.
3.	2',4'-dihydroxy-3'-methylpropiophenone	$C_{10}H_{12}O_3$	A compound structure suggests potential bioactive properties, but its specific pharmaceutical activities depend on its interaction with biological systems. While it may not be a widely known drug or active pharmaceutical ingredient, compounds with similar structural features (*e.g.*, phenolic groups and ketones)
4.	1,4-benzodioxan-6-amine	$C_8H_9NO_2$	The compound contains a benzodioxin core structure with an amine ($-NH_2$) framework suggests potential applications in medicinal chemistry, but its specific pharmaceutical activity depends on detailed biological and pharmacological evaluation. The combination of an aromatic core and functional groups like amines is often explored for antibacterial or antifungal activity.
5.	2-phosphonopropionic acid	$C_3H_7O_5P$	It is structurally related to phosphonoalkanoic acids and can exhibit various biochemical activities due to the presence of a phosphonic acid group ($-PO_3H_2$), which is known for its role in biological interactions. Its structure suggests potential biological significance, especially in plant and microbial systems.
6.	Benzoic acid, 4-ethoxy-, ethyl ester	$C_{11}H_{14}O_3$	It is an aromatic ester compound derived from benzoic acid. Its structure includes a benzene ring, an ethoxy group ($-OCH_2CH_3$) at the para position, and an ethyl ester functional group ($-COOCH_2CH_3$). This combination of features can confer various biological activities relevant to phytochemistry and pharmacology.
7.	Ethylparaben	$C_9H_{10}O_3$	Ethylparaben is a chemical compound formed by combining 4-hydroxybenzoic acid and ethanol. It is commonly used as a preservative in food and cosmetics to prevent the growth of bacteria and fungi. Additionally, it has been identified as a plant metabolite and a potential phytoestrogen.
8.	1-(4-chlorophenyl)-2-cyclopropylethanone	$C_{11}H_{11}ClO$	It is an organic compound with a unique structure that combines a chlorinated aromatic ring, a cyclopropyl group, and a ketone functional group. Its uses depend on its chemical reactivity and biological activity, which can be relevant in various fields such as medicinal chemistry, agrochemistry, and materials science.
9.	1-hydroxycyclopropane-1-carboxamide	$C_4H_7NO_2$	A hydroxy group (-OH) and a carboxamide group ($-CONH_2$) are attached to a cyclopropane ring. Its small, strained cyclic structure combined with functional groups makes it chemically interesting and potentially useful in several fields, including pharmaceuticals, agrochemicals, and materials science.
10.	L-trans-pinocarveol	$C_{10}H_{16}O$	L-trans-pinocarveol exhibits antibacterial and antifungal properties and its ability to disrupt microbial cell membranes. The hydroxyl group in its structure can scavenge free radicals, contributing to antioxidant properties that protect plant tissues from oxidative damage. Monoterpenoids like L-trans-Pinocarveol are known to inhibit pro-inflammatory pathways.
11.	2-bromopropionic acid	$C_3H_5BrO_2$	Halogenated compounds, including brominated acids, are often effective as antimicrobial agents. 2-Bromopropionic acid may inhibit microbial growth by interfering with cell wall synthesis or enzyme activity in bacteria and fungi. The bromine atom and carboxylic acid group may contribute to herbicidal properties, disrupting plant metabolism or enzyme systems.
12.	Diphenyl sulfone	$C_{12}H_{10}O_2S$	Diphenyl sulfone is a chemical compound characterized by two phenyl groups attached to a sulfur atom. It has been identified in certain plants, such as *Gnidia glauca* and *Dioscorea bulbifera*, and is considered a plant metabolite.
13.	Benzene, 1,1'-[1,2-ethenediyl bis (sulfonyl)] bis-, (z)-	$C_{14}H_{12}O_4S_2$	Compounds with sulfonyl groups are frequently explored for their potential role as agents due to their ability to disrupt microbial enzyme systems. Sulfonyl derivatives may interfere with tumor cell metabolism or signaling pathways. Sulfonyl functionality plays a role in modulating inflammatory mediators.

14.	1-allylcyclopropane-carboxylic acid	$C_7H_{10}O_2$	It is a small organic compound containing a cyclopropane ring with an allyl group and a carboxylic acid functionality. Cyclopropane derivatives are often investigated for their potential as intermediates in the synthesis of bioactive molecules. They may exhibit antimicrobial, anti-inflammatory, or antiviral properties depending on structural modifications.
15.	2-cyclopenten-1-one, 2,3,4-trimethyl-	$C_8H_{12}O$	It is a cyclic ketone often explored for its potential as a synthetic intermediate rather than direct pharmaceutical applications. Its derivatives may exhibit antimicrobial, anti-inflammatory or antioxidant properties. Specific activities depend on modifications and interactions within a biological system.
16.	2-n-heptylfuran	$C_{11}H_{18}O$	2-heptyl furan is a member of the furan class in which a heptyl group replaces the hydrogen at position 2. It is an effective inhibitor of chemical-induced carcinogenesis. It has a role as an antineoplastic agent, a plant growth regulator, a fungal metabolite, a mammalian metabolite, a Maillard reaction product, a plant metabolite and a flavoring agent.

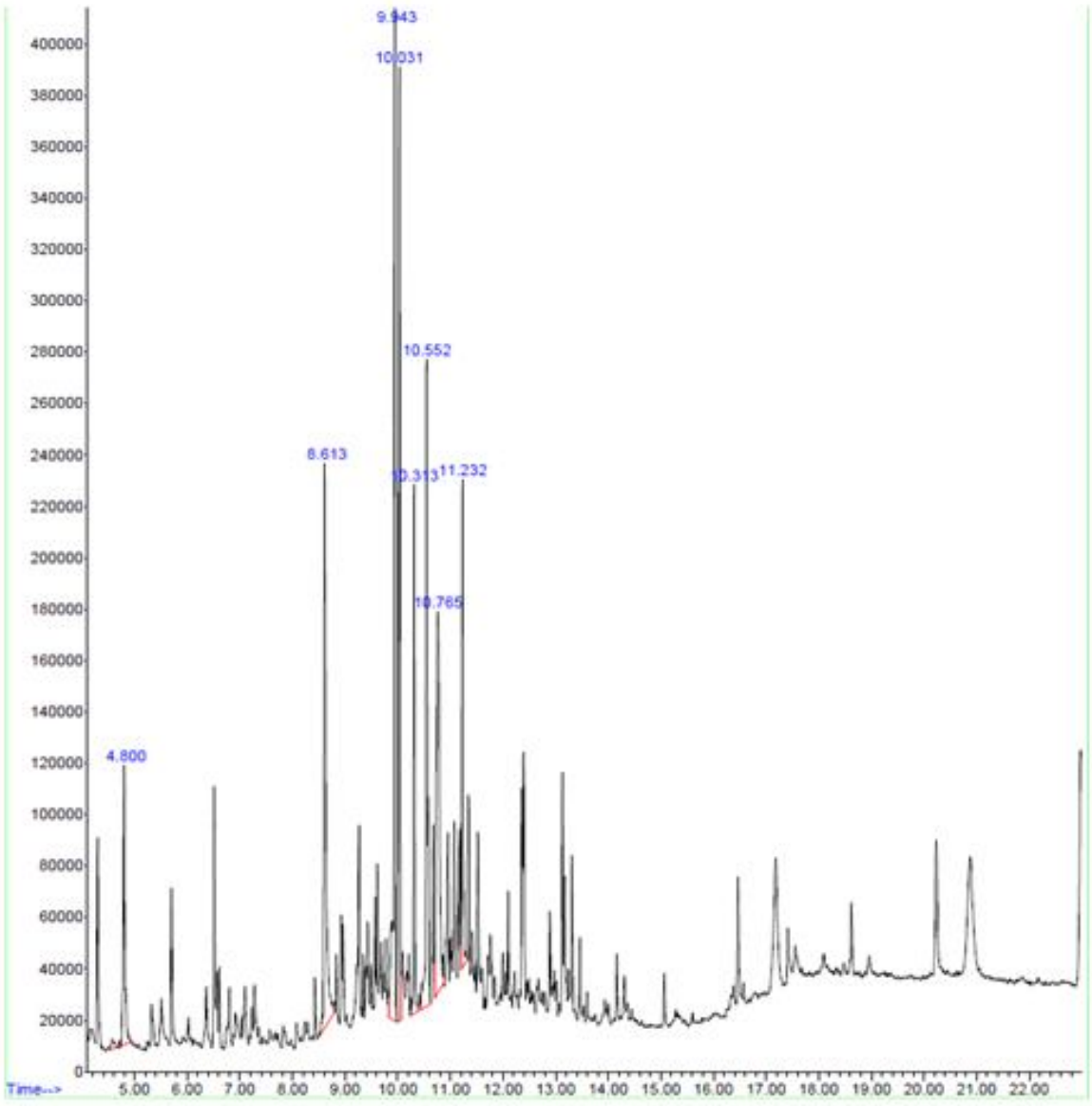

Figure 7: Chromatogram of ethanolic extract of jamun leaves treated with gamma radiation by GC-MS.

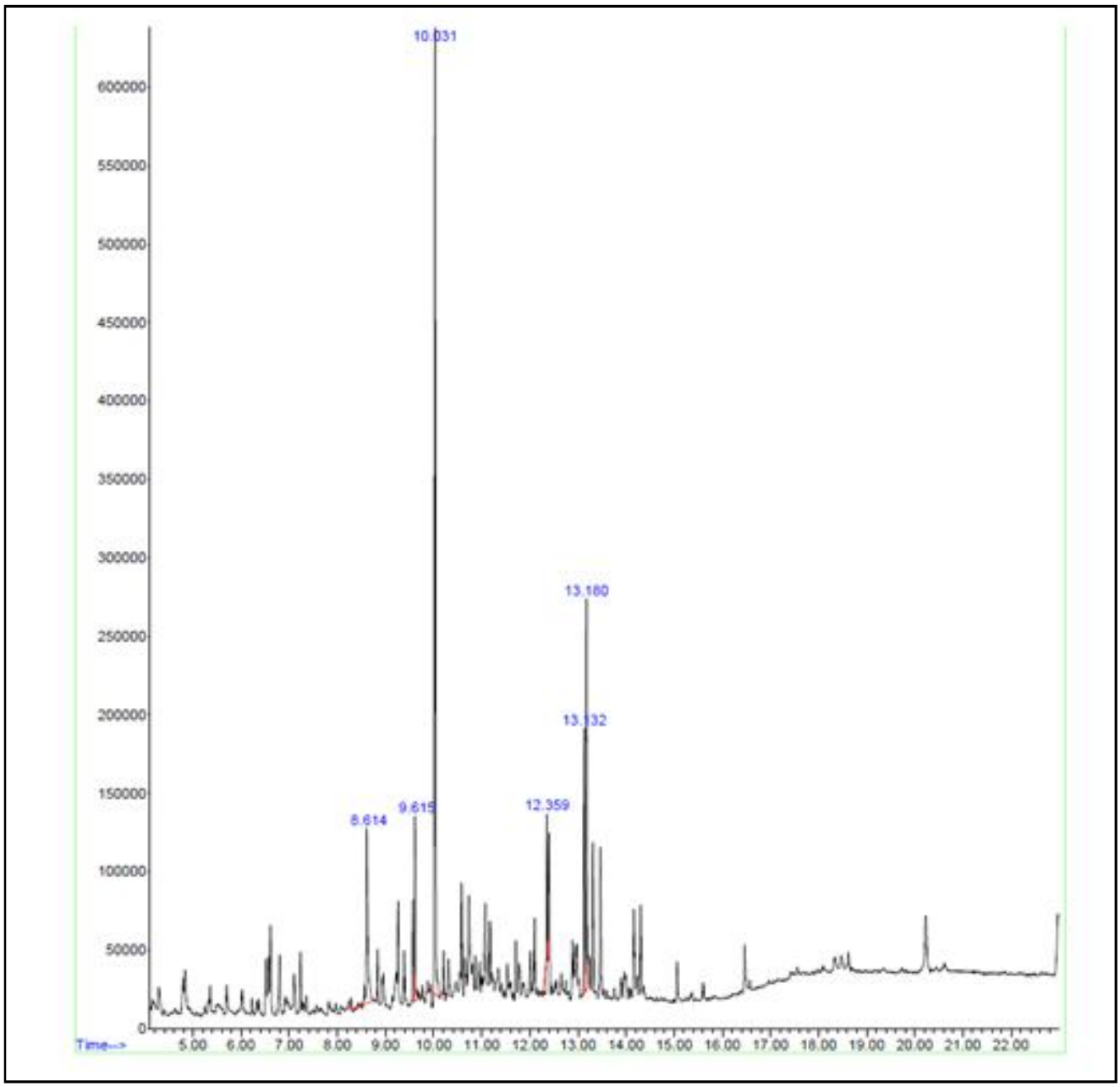

Figure 8: Chromatogram of ethanolic extract of jamun leaves treated with EMS chemical mutagen by GC-MS.

4. Discussion

The high-energy gamma rays likely affected cells undergoing meiotic division in the bud region (Deshpande *et al.,* 2010). The use of physical agents to induce mutations can generate novel genetic variations in agriculturally important crops that are not naturally occurring (Perez-Jimenez *et al.,* 2020). Selvi *et al.,* (2008) reported the highest protein content (3.41%) in four-month-old jack rootstocks during October, while two-month-old rootstocks had the lowest protein content (2.62%). Free radical formation can induce structural and metabolic changes in plants (Wi *et al.,* 2005). For instance, studies on the effects of gamma radiation on chloroplasts have shown their sensitivity to high radiation levels, with mutations occurring after 50 Gy (Borzouei *et al.,* 2010). High doses of gamma radiation (GR) can disrupt various biological processes, including protein synthesis, hormone levels, enzyme activity, and gas exchange (Borzouei *et al.,* 2010). Gamma rays are commonly used for inducing mutations in fruit crops due to their deep and even penetration into plant tissue (Jain *et al.,* 2013). Scientists are actively exploring the use of GR to create new crop varieties with improved nutritional value, resilience, and yield. Previous research has shown that GR can alter protein profiles in plants, leading to the appearance or disappearance of specific protein bands (Rashed *et al.,* 1994). Kawamura *et al.* (1996) examined the impact of gamma radiation on the DNA of corn, soybeans, and wheat. They discovered that low radiation doses fragmented large DNA strands into smaller pieces,

while higher doses caused fragmentation of both large and small DNA strands. Artýk and Peksen (2006) observed a similar trend in faba beans, noting decreased seed yield and harvest index in certain varieties exposed to low doses of 25 and 50 Gy of gamma radiation. Compared to physical mutagens, chemical mutagens are generally more effective in inducing genetic mutations (Bhat *et al.,* 2005). Ethyl methanesulfonate (EMS), a chemical mutagen from the alkaline sulfonate group, is widely used in plant breeding due to its effectiveness in inducing genetic mutations. EMS can alter the DNA nucleotides, leading to point mutations that can improve traits like yield, fruit quality, and stress tolerance in plants (McCallum *et al.,* 2000). 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). Induced mutation studies in fruit crops were mainly undertaken particularly in citrus, apple, grape, papaya, cherry, *etc*., though little efforts have been made in guava.

The ethanolic leaf extract of *S. cumini* contains various minor phytocomponents. These components were exposed to gamma radiation and EMS treatment. The levels of 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (6E)-, D-nerolidol, and 1-hydroxycyclopropane-1-carboxamide were the lowest among all the photo components, both the treatments, as shown in Tables 3 and 5, similar to the research of Khan and Ahmad (2021) in quince leaves.

5. Conclusion

The study concludes that the quality attributes of jamun leaves were significantly improved through the use of two different induced mutations. The impact of physical mutation T_5 (Gamma radiation at 50 Gy), chemical mutagens T_{10}(EMS -1.0%) on jamun leaves and its 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 the final analysis, the investigation revealed that 15 bioactive compounds in gamma-irradiated samples and EMS-treated samples 16 compounds that were determined to be ethylparaben had the highest peak time (47.67%). This study indicates that jamun 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, bioactivity, isolate and identify the secondary metabolites responsible for the biological activity. To investigate the active principles in jamun leaves, this kind of GC-MS study serves as a first step and these findings will open up novel possibilities for a crucial raw material for drug development, production and research in features.

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Conflict of interest

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

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