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Metabolic profiling through GC-HRMS analysis of medicinal and economic valuable species *Artocarpus heterophyllus* Lam. (Moraceae)

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Abstract
The global fruit intake is significantly influenced by tropical fruits. However, the majority of earlier research only found volatile and bulk components, their metabolomes have not yet been thoroughly studied. This study's objective was to use GC-HRMS to significant biopotential chemicals in *Artocarpus heterophyllus* Lam. flakes and seeds from both wild and domesticated varieties. Sixty-two phytochemical components were found in the methanolic extract of *A. heterophyllus* based on the mass fragment, retention duration and molecular weight by GC-HRMS analysis, in which fifteen are the major compounds as found out in the study. This is the first report on the flakes and seeds of genotypes of cultivated and wild jackfruit.

Keywords: wild genotype, cultivars, phytochemicals, GC-HRMS, Biological potential

Introduction

Jackfruit is belonging to the genus *Artocarpus*, the fruits is a major food item during periods of scarcity especially in the hilly areas. In March 2018, jackfruit was declared the official fruit of Kerala and International Jackfruit Day is celebrated on 4th July. In Kerala, tender fruits of about 60 days old are commonly used as vegetable. Jackfruit is cited as an example of a food prized in some areas of the world and allowed to go waste in others. Unripe (young) jackfruit can also be eaten whole after cooking. The seeds can also be eaten cooked or baked. Mature jackfruit and tender jackfruit shows different chemical components. Among the reported 7000 edible plant species, only a very small fraction is used today address food security (Bala Ravi *et al*., 2010) [1]. Despite their great potential, these crops still remain or orphans, underutilized, under-researched and neglected crops of minor importance (Haq, 2006) [2]. A few are gaining importance as health foods for urban population. These underutilised and neglected species are essential to the rural poor's ability to generate income, ensure their access to food and maintain their food culture.

Many of these phytochemical components also have varied biological functions and shield people from various ailments (Ghasemzadeh *et al*., 2015; Khan & Al-Balushi, 2021) [3, 4]. One of the reliable analytical methods for identifying the various chemical constituents in a plant sample is GC-MS, which is essential for the study of phytochemical compounds (Khan *et al*., 2019; Olivia *et al*., 2021; Ferdosi *et al*., 2019) [5, 7]. Many phytochemical investigations have employed gas chromatography and mass spectrometry as a dereplication approach to understand the likely chemicals in the extract without resorting to time-consuming separation protocols.

Material and Methods

Collection of sample

Matured pericarp and seeds of wild (N $08^0$48’31.92” E $077^0$07’19.65”) and cultivar (Koozha) (N $8^0$23’47.6” E $77^0$03’49.6”) varieties of *Artocarpus heterophyllus* were collected from Kamukincode, Thiruvananthapuram and Sankhili forest area, Kollam district in South Kerala from March to July in the 2019–2020 season. The collection numbers 95934 and 95935 were assigned to the herbarium specimens when they were placed at TBGT, JNTBGRI, Palode. The wild variant is distinguished by small-sized jackfruit, a countable number of bulbs in each fruit, and small seeds when compared to cultivars. We looked at the average yield in %, extract colour and uniformity.

Processing of the sample

The sample was cleaned, cut into manageable bits for oven drying, and then the dried parts were powdered. Healthy and disease-free edible plant part(s) were chosen. For subsequent research, the flour was kept in a small plastic container with suitable labelling at -80 ^{0}C (Fig. 1).

Phytochemical testing

Preparation of Extracts

Each sample's 5 grams of dried plant powder was utilised for extraction using a Soxhlet equipment. The powdered substance was subjected to continuous extraction using methanol (250 ml) at 40 °C for about eight syphons (Fig. 1). The extract was concentrated in a rota evaporator for around five hours at 40 °C and 30 rpm.

The formula% Yield = [(Final Weight of RB - Starting Weight of RB) / (Initial Weight of Sample)]*100 was used to determine the extract's yield.

The extracted substance from the round-bottomed flask was used for more examination.

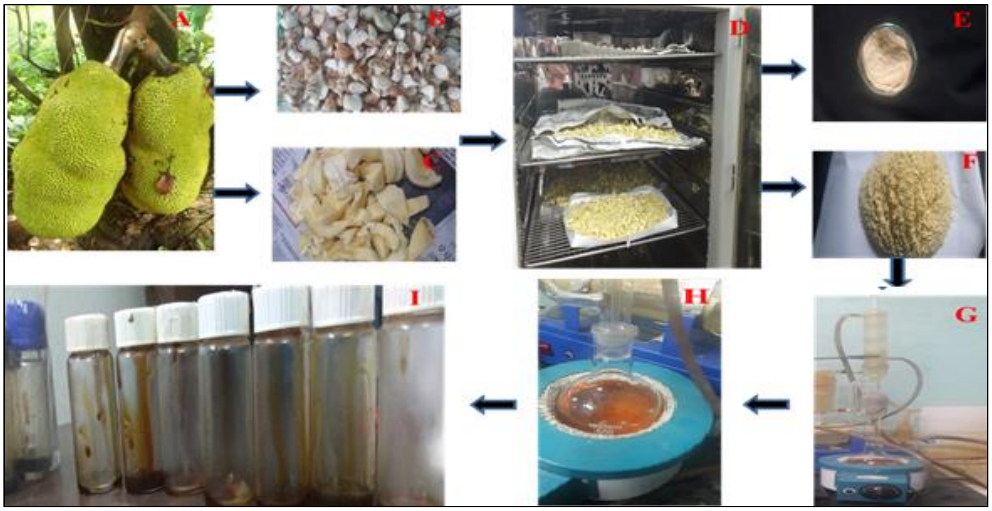

Fig 1: Various stages in the processing and extraction of phytochemicals from the flakes and seeds of jackfruit
A. sample collection, B. seeds cut in to small pieces, C. flakes cut in to small pieces, D. dried in oven, E. seed powder, F. flakes powder, G. soxhlet extraction, H. extract ready for concentrating with rotary evaporator, I. extracts stored in air tight glass bottles

GC-MS Analysis

The collected extracts of *Artocarpus heterophyllus* was subjected to GCMS at Sophisticated Analytical Instrument Facility (SAIF) IIT Bombay for the determination of volatile bioactive compounds. Calculating relative peak areas was used to quantify the components. To determine the relative peak areas, the peak area for the chemical was divided by the peak areas of all other compounds that had been discovered. The resulting percentage was then calculated. The bioactive elements of the extracts were identified using GC retention indices by comparing the mass spectra of the extracts with those kept in the National Institute of Standards and Technology (NIST) data centre. The GC-MS analysis made use of Agilent's 7890 FID detector, Head Space injector, and Combipal autosampler. The mass spectral scan range was 10–2000 amu and a mass resolution of 6000 amu. A GC-HRMS, commonly known as a gas chromatograph-mass spectrometer, is a dual-purpose analyzer that can examine compounds both qualitatively and quantitatively. The components of the extract were identified by mass spectra from the most recent library.

Result and Discussion

Phytochemical testing

Plant extract preparation: The yield percentage, extract colour, and consistency were noted (Table 1). The yield may vary depending on the fruit's maturity.

Table 1. Shows the average yield %, extract colour, and consistency for each component of wild and cultivated plants. Each value is presented as a mean with a standard deviation when the n is 3.

No.	Samples	Average % yield ± Standard Deviation	Colour of Extract	Consistency of Extract
1.	Cultivar flakes	14.925±0.803	Light yellow	Thick
2.	Cultivar Seeds	10.68±0.16	Light brown	Thick
3.	Wild flakes	20.26±0.48	yellow	Loose
4.	Wild seeds	18.8±0.4	Dark brown	Sticky

Analysis of bioactive compounds using GC-MS

Table 2 to 5 and Figures 2 to 5 list the elements identified by the GCMS analyser. GC-HRMS analysis of cultivar flakes of *Artocarpus heterophyllus* revealed the presence of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (16.63%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)-(10.87%); n-Hexadecanoic acid (8.06%); (Z)6,(Z)9-Pentadecadien-1-ol (12.6%); Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (12.5%); cis-Z-α-Bisabolene epoxide (8.06%); and Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (11.42%) (Fig. 2 & Table 2).

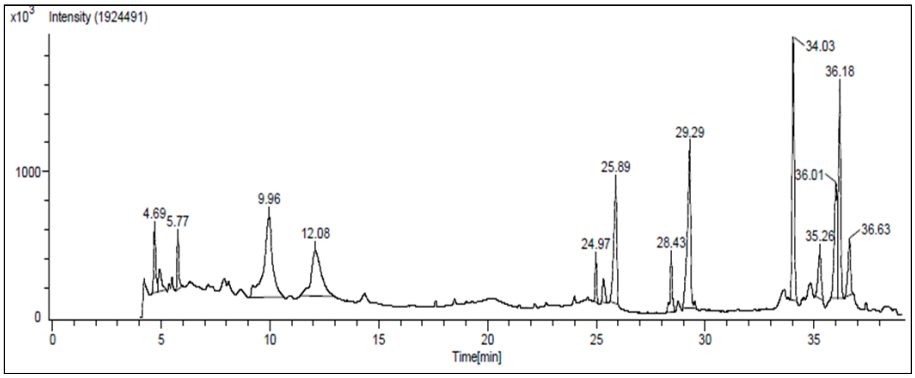

Fig 2: Chromatogram using GC-HRMS of *Artocarpus heterophyllus* Lam. cultivar flakes.

Table 2: Compound identified through HR-GCMS analysis of cultivar flakes of *Artocarpus heterophyllus* Lam.

Peak No.	Retention time	Peak area (%)	Compound name	Base m/z	Molecular formula	Molecular weight
1	4.69	3.23	γ-Butyrolactone	42.06	$C_4H_6O_2$	86
2	4.93	1.59	2-Cyclopenten-1-one, 2-hydroxy-	98.09	$C_5H_6O_2$	98
3	5.77	2.26	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	101.07	$C_6H_8O_4$	144
4	9.96	16.63	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	44.04	$C_6H_8O_4$	144
5	12.08	10.87	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	126.10	$C_6H_6O_3$	126
6	24.97	1.596	Decanoic acid, methyl ester	87.08	$C_{11}H_{22}O_2$	186
7	25.32	1.48	Undecanoic acid	73.07	$C_{11}H_{22}O_2$	186
8	25.89	8.06	n-Hexadecanoic acid	73.06	$C_{16}H_{32}O_2$	256
9	28.43	2.89	methyl ester of 9,12,15-octadecatrienoic acid, (Z,Z,Z)-	95.13	$C_{19}H_{32}O_2$	292
10	29.29	12.6	(Z)6,(Z)9-Pentadecadien-1-ol	95.13	$C_{15}H_{28}O$	224
11	34.03	12.5	2-hydroxy-1-(hydroxymethyl)ethyl ester of hexadecanoic acid	98.11	$C_{19}H_{38}O_4$	330
12	35.26	3.44	22-Stigmasten-3-one	109.17	$C_{29}H_{48}O$	412
13	36.01	8.06	cis-Z-α-Bisabolene epoxide	95.13	$C_{15}H_{24}O$	220
14	36.18	11.42	2-hydroxy-1-(hydroxymethyl)ethyl ester of octadecanoic acid	98.11	$C_{21}H_{42}O_4$	358
15	36.63	3.38	4,14-dimethyl-, acetate, (3β,4α,5α)- 9,19-Cycloergost-24(28)-en-3-ol	95.13	$C_{32}H_{52}O_2$	468

Cultivar seeds included the following compounds: Octadecanoic acid, 2,3-dihydroxypropyl ester (15.98%), 9,19-Cyclolanost-24-en-3-ol, acetate, (3)- (11.08%), Cycloartenol (23.03%), and Cycloartenyl acetate (22.96%). (Fig. 3 and Table 3).

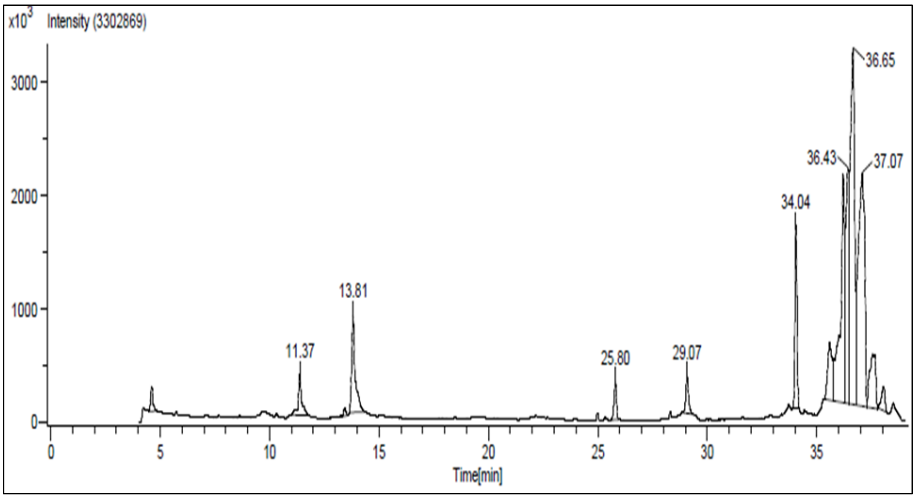

Fig 3: Chromatogram using GC-HRMS of *Artocarpus heterophyllus* Lam. cultivar seeds.

Table 3. Compound identified through HR-GCMS analysis of cultivar seeds of *Artocarpus heterophyllus* Lam.

Peak No.	Retention time	Peak area (%)	Compound name	Base m/z	Molecular formula	Molecular weight
1	4.59	0.91	Butyrolactone	42.06	$C_4H_6O_2$	86
2	11.37	2.09	1,2-Ethanediamine, N,N-dimethyl-	58.09	$C_4H_{12}N_2$	88
3	13.43	0.24	1-Cyclopropyl-1-methyl-ethylamine	84.12	$C_6H_{13}N$	99
4	13.81	5.2	N-(1,1-Dimethylpropyl)-2,2,3-trimethylaziridine-1-carboxamide	85.12	$C_{11}H_{22}N_2O$	198
5	25.80	1.34	n-Hexadecanoic acid	73.07	$C_{16}H_{32}O_2$	256
6	29.07	1.5	13-Tetradece-11-yn-1-ol	95.13	$C_{14}H_{24}O$	208
7	34.04	5.5	2-hydroxy-1-(hydroxymethyl)ethyl ester of hexadecanoic acid	98.11	$C_{19}H_{38}O_4$	330
8	35.58	3.97	22-Stigmasten-3-one	411.53	$C_{29}H_{48}O$	412
9	36.21	15.98	Octadecanoic acid, 2,3-dihydroxypropyl ester	98.11	$C_{21}H_{42}O_4$	358
10	36.43	11.08	acetate, (3β)-, 9,19-Cyclolanost-24-en-3-ol	183.24	$C_{32}H_{52}O_2$	468
11	36.65	23.03	Cycloartenol	95.12	$C_{30}H_{50}O$	426
12	37.07	22.96	Cycloartenyl acetate	183.24	$C_{32}H_{52}O_2$	468
13	37.55	4.82	9,19-Cyclolanostan-3-ol, acetate, (3β)-	109.16	$C_{32}H_{54}O_2$	470
14	38.05	1.35	Kauran-18-al, 17-(acetyloxy)-, (4β)-	109.17	$C_{22}H_{34}O_3$	346

In wild flakes, the following compounds were found: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (14.7%); 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (18.4%); Octadecanoic acid, 2,3-dihydroxypropyl ester (8.02%); and Dodecanoic acid, 1,2,3-propanetriyl ester (11.6%) (Fig Wild seeds have the following chemical compositions: 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (7.3%), 3-Buten-1-amine,N,N-dimethyl(15.8%),N-(1,1-Dimethylpropyl)-2,2,3-trimethylaziridine-1-carboxamide (22.4%), and n-Hexadecanoic acid (9.4%) (Fig. 5 & Table 5).

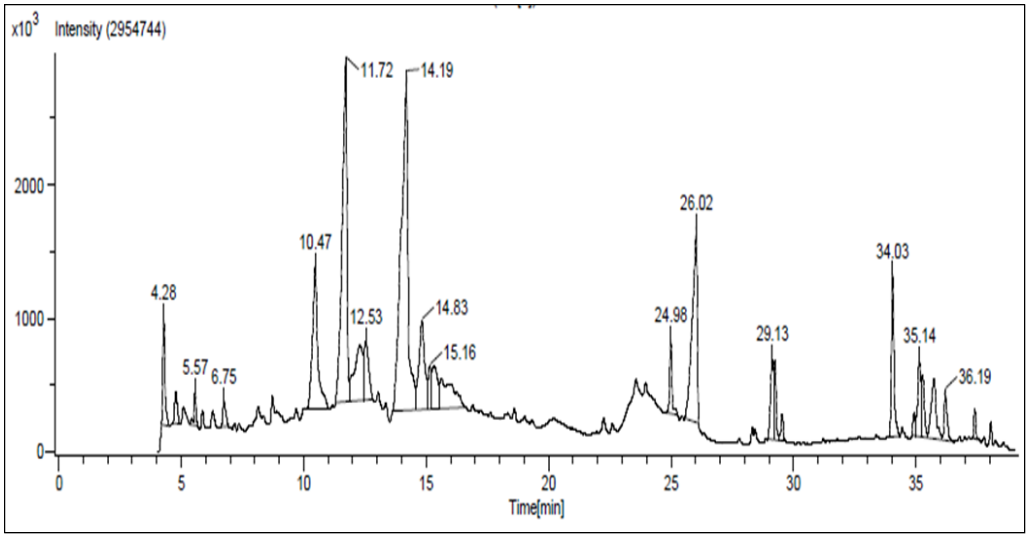

Fig 4: Chromatogram using GC-HRMS of *Artocarpus heterophyllus* Lam. wild seeds.

Table 4. Compound identified through HR-GCMS analysis of wild seeds of *Artocarpus heterophyllus* Lam.

Peak No.	Retention time	Peak area (%)	Compound name	Base m/z	Molecular formula	Molecular weight
1	4.28	2.4	2-Cyclopentene-1,4-dione	96.05	$C_5H_4O_2$	96
2	4.78	0.79	Ethanamine, N-ethyl-N-[(1-methylethoxy)methyl]-	55.05	$C_8H_{19}NO$	145
3	5.57	0.77	2-Furancarboxaldehyde, 5-methyl-	110.07	$C_6H_6O_2$	110
4	6.75	0.74	Cyclohexanone, 3-hydroxy-	68.05	$C_6H_{10}O_2$	114
5	10.47	7.3	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	44.04	$C_6H_8O_4$	144
6	11.72	15.8	N,N-dimethyl- 3-buten-1-amine	58.08	$C_6H_{13}N$	99
7	12.29	4.7	Oxirane, (2-methylpropyl)-	85.06	$C_6H_{12}O$	100
8	12.53	2.7	5 hydroxy-2,3-dimethyl-2-cyclopenten-1-one	110.07	$C_7H_{10}O_2$	126
9	14.19	22.4	N-(1,1-Dimethylpropyl)-2,2,3-trimethylaziridine-1-carboxamide	85.11	$C_{11}H_{22}N_2O$	198
10	14.83	5.04	1,4-Butanediamine, N,N,N',N'-tetramethyl-	144.15	$C_8H_{20}N_2$	144
11	15.16	1.35	N-[3-Methylaminopropyl]aziridine	58.08	$C_6H_{14}N_2$	114
12	15.31	2.5	N-Allyl-N,N-dimethylamine	58.08	$C_5H_{11}N$	85
13	15.63	3.8	1-Cyclopropyl-1-methyl-ethylamine	84.09	$C_6H_{13}N$	99
14	24.98	1.7	14-methyl pentadecanoic acid, methyl ester	87.08	$C_{17}H_{34}O_2$	270
15	26.02	9.4	n-Hexadecanoic acid	43.06	$C_{16}H_{32}O_2$	256
16	29.13	2.4	13-Tetradece-11-yn-1-ol	95.13	$C_{14}H_{24}O$	208
17	29.23	1.7	10-Undecenal	81.11	$C_{11}H_{20}O$	168
18	29.53	0.57	Octadecanoic acid	73.07	$C_{18}H_{36}O_2$	284
19	34.03	4.07	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	98.11	$C_{19}H_{38}O_4$	330
20	34.92	0.46	Decanoic acid, 2-propenyl ester	57.10	$C_{13}H_{24}O_2$	212
21	35.14	2.4	1,2-15,16-Diepoxyhexadecane	207.26	$C_{16}H_{30}O_2$	254
22	35.25	1.8	Dodecanoic acid, ethenyl ester	183.25	$C_{14}H_{26}O_2$	226
23	35.74	3.02	4-Nitrophenyl laurate	183.24	$C_{18}H_{27}NO_4$	321
24	36.19	1.52	2-hydroxy-1-(hydroxymethyl)ethyl ester of pentadecanoic acid	98.12	$C_{18}H_{36}O_4$	316
25	37.40	0.55	Squalene	81.11	$C_{30}H_{50}$	410

Table 5: Compound identified through HR-GCMS analysis of wild flakes of *Artocarpus heterophyllus* Lam.

Peak No.	Retention time	Peak area (%)	Compound name	Base m/z	Molecular formula	Molecular weight
1	4.37	2.1	2-Cyclopentene-1,4-dione	96.05	$C_5H_4O_2$	96
2	4.89	1.7	Butyrolactone	42.06	$C_4H_6O_2$	86
3	5.12	2.3	6-Oxa-bicyclo[3.1.0]hexan-3-one	98.08	$C_5H_6O_2$	98
4	5.63	2.2	2-Furancarboxaldehyde, 5-methyl-	110.07	$C_6H_6O_2$	110
5	5.91	2.6	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	101.06	$C_6H_8O_4$	144
6	7.59	4.6	1,3-Butanediol	45.05	$C_4H_{10}O_2$	90
7	8.22	3.6	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	43.03	$C_6H_8O_3$	128
8	10.62	14.7	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	43.03	$C_6H_8O_4$	144
9	12.74	18.4	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	126.08	$C_6H_6O_3$	126
10	15.01	1.7	1H-Azepine, 1-acetylhexahydro-	85.13	$C_8H_{15}NO$	141
11	18.59	0.79	Undecanoic acid	73.07	$C_{11}H_{22}O_2$	186
12	25.84	2.4	n-Hexadecanoic acid	73.06	$C_{16}H_{32}O_2$	256
13	28.40	0.8	Octanal	43.06	$C_8H_{16}O$	128
14	29.15	5.4	13-Tetradece-11-yn-1-ol	95.13	$C_{14}H_{24}O$	208
15	30.51	1.002	6,7-Dibromo-Z-11-tetradecene-1-ol acetate	43.05	$C_{16}H_{28}Br_2O_2$	410
16	34.06	6.9	2-hydroxy-1-(hydroxymethyl)ethyl ester of hexadecanoic acid	98.11	$C_{19}H_{38}O_4$	330
17	34.57	0.5	Hexadecanoic acid, (3-bromoprop-2-ynyl) ester	43.05	$C_{19}H_{33}BrO_2$	372

18	35.93	2.3	E-2-Tetradecen-1-ol	67.09	$C_{14}H_{28}O$	212
19	36.21	8.02	Octadecanoic acid, 2,3-dihydroxypropyl ester	98.11	$C_{21}H_{42}O_4$	358
20	36.85	11.16	Dodecanoic acid, 1,2,3-propanetriyl ester	183.24	$C_{39}H_{74}O_6$	638
21	37.04	5.01	1-(hydroxymethyl)-1,2-ethanediyl ester of dodecanoic acid	183.24	$C_{27}H_{52}O_5$	456
22	37.41	1.8	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	81.11	$C_{14}H_{24}O$	208

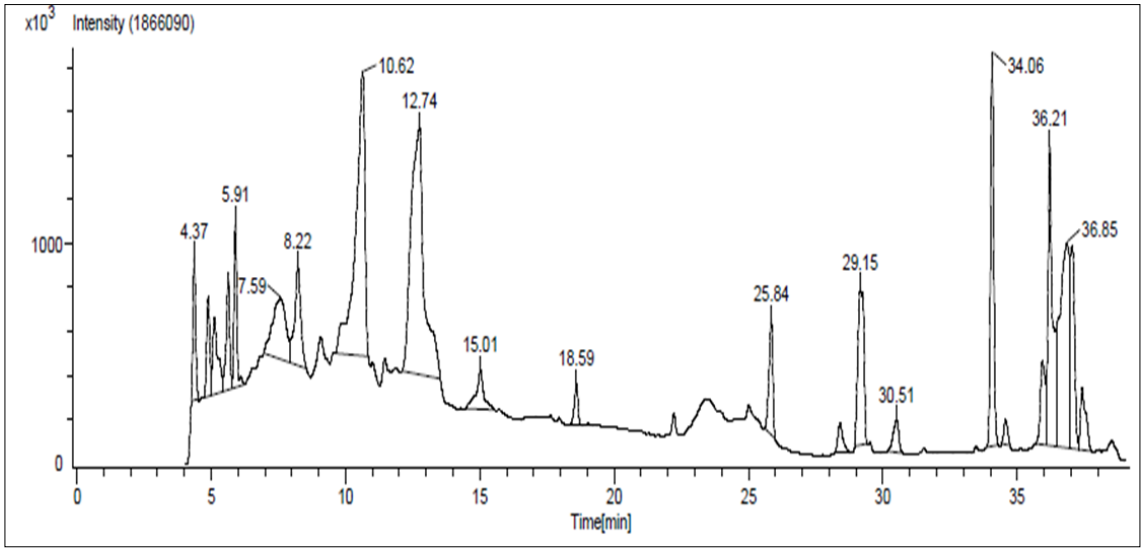

Fig 5: Chromatogram using GC-HRMS of Artocarpus heterophyllus Lam. wild flakes.

Artocarpus heterophyllus seeds and flakes contain the chemical 2,3-dihydro-3,5-dihydroxy-6-methyl 4H-pyran-4-one, which is present in both wild and domesticated varieties. Flakes of *Artocarpus heterophyllus*, both wild and domesticated, contained 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, according to GC-HRMS analysis. n-Hexadecanoic acid, a substance found in cultivar flakes and wild seeds. Both cultivated and wild flakes and seeds contain the chemical 2, 3-dihydroxypropyl ester-octadecanoic acid.

The potential of one flavonoid fraction, 4H-pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl, to inhibit the fungus that destroys rubberwood wood, has attracted a lot of interest (Teoh and Mashitah, 2015) [8]. A bioactive compound called 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran4-one, which is composed of flavonoid fractions, was found to be quite popular due to its antibacterial properties. Particularly, its antifungal activity towards the rubberwood's wood-degrading fungi has drawn a lot of attention (Teoh *et al*., 2011) [9]. Through the use of GC-MS analysis and HPLC analysis, a chemical known as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) was shown to be the main element in fraction H7. This analysis reinforced the antioxidant activity of DDMP by comparing the HPLC chromatograms of fraction H7 (or DDMP) before and after reaction with DPPH radical, ABTS radical, or ferric ion (Yu *et al*., 2013).

The following 27 compounds were found in the ethanolic extract of jackfruit powder; they were the most common and significant in terms of relative percentage: Lup-20(29)-en-3-one, 9,19-Cyclo-9 lanostane-3,25-diol, 5-Hydroxymethylfurfural, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 9,19-Cyclolanost-24-en-3-ol, acetate, (3)-, and n-Hexadecanoic acid are some of the chemicals (Srinivasan and Kumaravel, 2016) [11].

The Jackfruit peels (*Artocarpus integer*) included hexadecanoic acid, squalene, calophyllolide, thialisopyine, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H pyran-4-one as well as other phytochemicals and secondary metabolites (Antony Allwyn Sundarraj and Thottiam Vasudevan Ranganathan, 2017) [12].

The fatty acid alcohol (Z) 6, (Z) 9-pentadecadien-1-ol's antimicrobial properties (Sabithira and Udayakumar, 2017) [13]. A phytosterol molecule known as cycloartenol has a number of pharmacological properties, including benefits against Alzheimer's disease that are anti-inflammatory, anti-tumor, antioxidant, and anti-biosis. It is also one of the key components used to create new sterol compounds (Zhong-Lian Zhang *et al*., 2017) [14].

The two varieties of jackfruit had 66 different chemicals altogether. 2-methylbutyl pentanoate (31.87%), hexyl 2-methylbutanoate (23.594%), ethyl 3-methylbutanoate (10.923%), butyl aceoate (3.337%), butyl butanoate (3.164%), and butyl hexanoate (3.1%) were the principal components identified in the fragrance extraction of hard jackfruit (Hongbo *et al*., 2013) [15].

Squalene is frequently used in the cosmetics business. The -sitosterol has been found to directly lower fasting blood glucose levels through cortisol suppression, which has a positive effect on a diabetic state (McAnuff *et al*., 2005) [16]. The chemical γ-butyrolactone exhibits anticancer, anti-inflammatory, antibacterial, antifungal, antioxidant, immunosuppressive, neuroprotective, and hypoglycemic effects (Joonseong Hur *et al*., 2021) [17]. It has anti-bacterial, anti-inflammatory, and anti-proliferative effects of 4H pyran-4-one, 4,5-dihydroxy-6-methyl (Nandikar *et al*., 2018) [18]. FDA-approved medications containing γ-butyrolactone are used in clinics as diuretics, cancer treatments, contraceptives, heart disease treatments, and glaucoma treatments, among other ailments. Gamma-butyrolactone-containing substances can regulate how quickly plant roots and buds grow. Antioxidant chemicals that can be used to create medicinal products for the treatment and management of metabolic disorders are abundant in the hard shell of the Aegle marmelos fruit (Ankita Chaubey and Ashok K Dubey, 2020) [19].

Conclusion

In the current study, sixty-two bioactive compounds from methanol extracts of seed and flakes from both wild and cultivated *Artocarpus heterophyllus* were discovered by GC-MS. Both wild and cultivated seed and flakes showed a substantial difference. The existence of numerous bioactive substances in *A. heterophyllus* demonstrated its significance for pharmaceuticals. Therefore, determining its bioactivity and toxicity profile is necessary for further research. This is the first study on the flakes and seeds of genotypes of cultivated and wild jackfruit.

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Conflict Of Interest

There are no conflicts of interest for the author (s).

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