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GC-MS and FTIR Profiling of Fruits of *Artocarpus lakoocha* for Identification of Bioactive Compounds

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Abstract: *Artocarpus lakoocha*, a wild edible fruit, commonly known as Monkey Jack fruit has numerous benefits and reportedly used by various tribals' namely Gadaba, Juang, Bhumia, Sanatala, Paika etc. of Odisha. However, there is limited information available regarding the fruit's chemical composition. Present study aims to identify the volatile compounds present in this underexplored fruit. GC-MS analysis has been to know the chemical content of *Artocarpus lakoocha* fruit. The methanol extract, which is light yellow in colour, contains fifty volatile compounds. The most prevalent compound is 5-Hydroxymethyl furfural (5-HMF), comprising 38.26% of the extract. Other significant components include 3,5-Dimethylpyrazole-1-meth(DMP), Furazan-3-ol, 4-amino-, 1,2,3,5-Cyclohexanetetrol, 4H-Pyran-4-one, 2,3-dihydro-, Sucrose; Glycerine; D-Allose; Ribitol; Hexanoic Acid; 9,12,15 Octadecadienoic acid and Oxirane, tertradecyl . FTIR analysis of the dried fruit powder revealed absorption bands indicating the presence of hydroxyl groups, C-H stretches, ketenes, isothiocyanates, alkynes, aldehydes, alkenes, alcohols, phenols, amines, tertiary and primary alcohols, fluoro compounds, and aromatic compounds. These findings suggest that the fruit possesses significant antioxidant potential and contains various compounds with anticancer, antimicrobial, and food flavouring properties. Consequently, it may have applications in pharmaceutical and health-related fields.

Keywords: *Artocarpus lakoocha* **;** wild edible fruit; GC-MS; volatile compound, Antioxidant.

Introduction

A wide variety of medicinal plants and derivatives are marketed and used to treat health conditions mostly by Tribals' (Gadaba, Juang, Bhumia, Sanatala, Paika etc.) of Odisha. These plants often contain beneficial compounds with antibacterial, anti-inflammatory, antioxidant, and cytotoxic properties (Hayetet al., 2009, Jasprica et al., 2007, Kader et al., 2016). The scientific consensus on free radicals and their link to various diseases indicates that excessive production and inadequate antioxidant defences accelerate aging and disease onset (Lebaka et.al, 2021). Monkey jack (*Artocarpus lakoocha*), a tropical fruit from the Moraceae family, is native to India and found in different forest regions of Odisha. Rich in nutrients, this fruit is an important part of the human diet. It contains essential vitamins like vitamin C and beta-carotene, which are potent antioxidants. Additionally, minerals such as zinc, copper, manganese, and iron contribute to its antioxidant properties, helping in maintaining health, preventing coronary heart disease, and combating cancer (Hossain et al., 2016).Antioxidants, which include enzymatic and non-enzymatic substances, inhibit chain reactions in lipids, proteins, or DNA. Notable antioxidant molecules are phenolic compounds, which can neutralize singlet and triplet oxygen or break down peroxides(Goze et al., 2009, Lebaka et.al, 2021).Natural products possess 40% more chemical scaffolds compared to synthetic chemistry(Harvey 2000), giving them a significant edge in providing new commercial drug candidates. The challenge lies in efficiently and effectively tapping into this diverse resource (Anand et al., 2019). Plants stand out as a promising source among the various origins of natural active compounds. (Harvey 2000, Joray et al., 2015).Traditional plant-based medicines are frequently derived from raw plant extracts, which comprise complex mixtures of different phytochemicals (Pandey et al., 2008). These phytochemicals possess distinctive and intricate structures, and are utilized in the treatment of both chronic and infectious diseases (Sahoo et al., 2013).Fruit plays a significant role in global economic systems and food security from multiple perspectives (Schreinemachers et al., 2018). Although numerous unexplored wild fruit species contain a vast array of bioactive secondary metabolites, only a small fraction have been investigated and demonstrated to be valuable sources of bioactive agents. In the pursuit of novel compounds and for quality assurance purposes, it is imperative to develop appropriate screening methods (Keskes et al., 2017). The extraction and characterization of many such bioactive compounds from various medicinal plants have culminated in the production of certain highly efficacious medicines (Yadav et al.,2017).However, there is lack of reports on the bioactive compounds present in fruits of *Artocarpus lakoocha*. This present study aimed at highlighting potential bioactive compounds in fruits of *Artocarpus lakoocha.*

Materials and Methods

- **Collection Of Materials**

The fruits of *Artocarpus lakoocha* was collected from “Chandaka - Dampara Wildlife Sanctuary” forest areas (21.31211°N 85.80488°E ±14m ; 20.34944°N 85.76509°E ±10m) and was identified using the authentic herbarium and “The Flora of Odisha” book (Saxena HO. And Brahmam 1995).

- **Preparation of Powder and Extract**

The meticulous fruit sample preparation and extraction process yields high-quality, diverse extracts for analysis. This comprehensive method, using multiple solvents of varying polarities, isolates a wide range of bioactive compounds, potentially advancing phytochemistry and natural product research. The collected fruits were washed properly with running tap water and pulp was separated and dried in hot air oven at 60°C for 48hours. The dried material was powdered and stored in air tight container. The corase powder were extracted by using continuous maceration for 12-18hr using methanol (GC-MS grade). Filtered extract was evaporated to remove the solvent via rotary evaporator.

- **GC-MS analysis of Bioactive compounds**

GCMS analysis was conducted on the dried powder of *Artocarpus lakoocha* to identify volatile bioactive compounds. The examination utilized a Thermo Scientific Trace 1300 gas chromatograph coupled with an ISQ-QD mass spectrometer, featuring an EB-5MS column (30 mm × 0.25 mm ID × 25μm). Helium gas (99.999%) served as the carrier gas, maintained at a steady flow rate of 1ml/minute, with a 1μl injection volume. The injection port was set at 250 °C, while the oven temperature was programmed to start at 60 °C for 3 minutes, then increase by 5 °C/min to 110 °C, holding for 3 minutes. The entire GC process lasted 47 minutes. Bioactive compounds identification from the extract was based on mass spectra from NIST library data.

- **FTIR(Fourier-transform infrared spectroscopy)Analysis**

Fourier-transform infrared spectroscopy (FTIR) analysis was conducted to detect the presence of the functional groups in fruits of *Artocarpus lakoocha*. The analysis employed a Spectrum Two FTIR spectrometer (Perkin Elmer, USA) with a circular K-Br cell Window and 0.05mm round Teflon Spacer, utilizing Spectrum software version 10.4.3. The FTIR analysis parameters included a wavenumber range of 4000-450 cm-1, sensitivity exceeding 0.5%, 4 cm-1 resolution, and a sample mass of approximately 5-6 mg. For each sample analysis, background spectra were obtained in air, and the ATR was thoroughly cleaned with Methanol to ensure no residual bands were visible in the background spectra.

Results

- **Chemical components identified in *Artocarpus lakoocha* through GC-MS**

The fruit extract of *Artocarpus lakoocha*, obtained using methanol, exhibits a light yellow hue. An examination of the volatile constituents in the *Artocarpus lakoocha* extract revealed the presence of eighty distinct compounds (Fig-1). The most abundant of these components was 5-Hydroxymethyl furfural (5-HMF), which accounted for 38.26% of the total volatile content. Additional major components

(Table 1) identified were3,5-Dimethylpyrazole-1-meth(DMP);Furazan-3-ol, 4-amino-; 1,2,3,5-Cyclohexanetetrol;4H-Pyran-4-one, 2,3-dihydro-; D- Allose ; 2,4-Dimethyl-3-pentanol acet ; Thymine;1,6-Anhydro-.beta.-D-glucofu ; Sucrose ; (R)-(-)-14-Methyl-8-hexadecy ; Oxirane, tetradecyl-; Furylhydroxymethyl ketone; Carbonic acid, but-2-yn-1-yl;Glycerine; 2-Furancarboxaldehyde, 5-me;Cyclohexane,1-ethyl-4-methyl,Digitoxin.

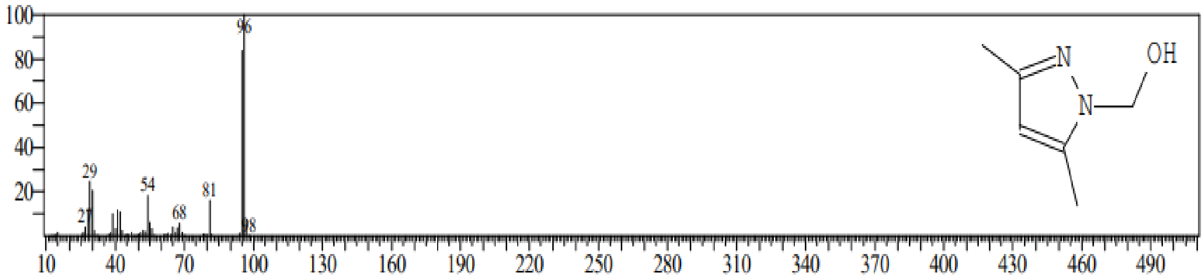
- **FTIR (Fourier Transform Infrared Spectrophotometer) Analysis**

Dried fruit powder of *Artocarpus lakoocha* showed characteristic absorption band at 3284.63cm^{-1} for hydroxyl group (-OH group),2922.45 cm^{-1} for C-H stretch,2167.35 cm^{-1} for Ketenes (C=C=O),2099.7 cm^{-1} for isothiocyanate (N=C=S stretching), 1986.83cm^{-1} for Alkynes (C=C asymmetric stretch),1731.96 cm^{-1}for Aldehyde (C=O stretching), 1627.8 cm^{-1} for Alkene (C=C stretching), 1417.34cm^{-1} for alcohol(O-H bending), 1365.92cm^{-1}for phenol (O-H bending), 1334.33cm^{-1}for alcohol (O-H bending),1243.73cm^{-1}for amines(C-N stretching),1148.42 cm^{-1} for tertiary alcohol(C-O stretching),1073.87cm^{-1} for primary alcohol(C-O stretching),1010.83cm^{-1} for fluoro compound (C-F stretching), 861.72cm^{-1} for 1,2,4 trisubstituted (C-H bending), 816.95cm^{-1} for p-substituted aromatic compound(C-H bending),763.44cm^{-1} for monosubstituted aromatic compound(C-H bending), 572.08 cm^{-1}for alkyl halide(C-Br stretching) were found(Table 2).

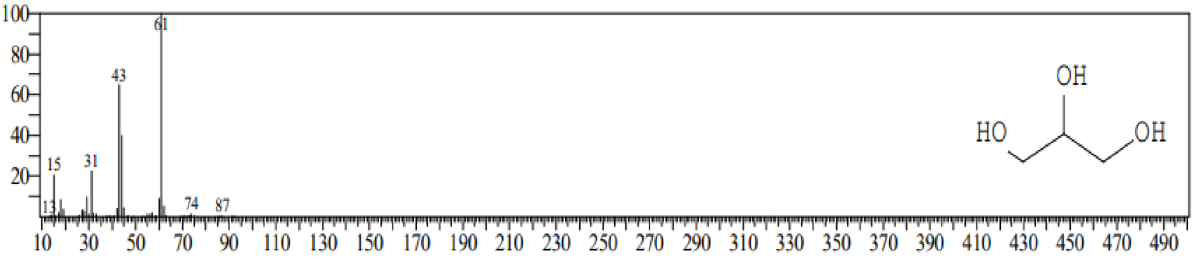
Table 1: Volatile compounds identified in *Artocarpus lakoocha* fruit by GC-MS.

SL. No.	Retention Time(RT)	Compound Name	Molecular Formula	Molecular weight	%Area
1.	2.167	3,5-Dimethylpyrazole-1-meth	$C_6H_{10}N_2O$	126.16 g/mol	7.22
2.	2.813	Glycerine	$C_3H_8O_3$	92.09 g/mol	1.14
3.	2.87	2-Furancarboxaldehyde, 5-me	C6H6O2	110.1106 g/mol	1.4
4.	3.028	2,4-Dihydroxy-2,5-dimethyl-3	$C_6H_8O_4$	144.12 g/mol	0.84
5.	4.054	Thymine	$C_5H_6N_2O_2$	126.11 g/mol	1.46
6.	4.262	Furylhydroxymethyl ketone	$C_6H_6O_3$	126.11 g/mol	0.99
7.	4.955	Ribitol	$C_5H_{12}O_5$	152.14578 g/mol	0.19
8.	5.197	4H-Pyran-4-one, 2,3-dihydro-	$C_6H_8O_4$	144.12 g/mol	3.18

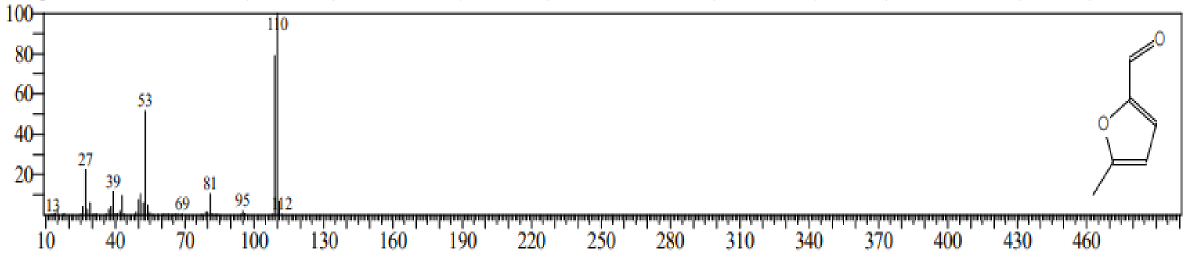
9.	6.535	5-hydroxymethylfurfural	$C_6H_6O_3$	126.11 g/mol	38.26
10.	6.819	Cyclohexane,1-ethyl-4-methyl	C_9H_{18}	126.2392 g/mol	1.88
11.	7.235	Cyclobutanecarboxylic acid	$C_{11}H_{20}O_2$	184.12 g/mol	0.85
12.	7.722	4-Pyrimidinol, 5-methoxy-	$C_5H_6N_2O_2$	126.11 g/mol	1.19
13.	7.883	2,4-Dimethyl-3-pentanol acet	$C_9H_{18}O_2$	158.24 g/mol	3.14
14.	8.583	2-Hexenoic acid, ethyl ester	$C_8H_{14}O_2$	142.20 g/mol	0.77
15.	8.875	Furazan-3-ol, 4-amino-	$C_2H_3N_3O_2$	245.24 g/mol	3.96
16.	9.885	Sucrose	$C_{12}H_{22}O_{11}$	342.30 g/mol	2.16
17.	10.134	Carbonic acid, but-2-yn-1-yl	$C_{14}H_{24}O_3$	166.1739 g/mol	0.71
18.	10.643	D-Allose	$C_6H_{12}O_6$	180.16 g/mol	3.14
19.	11.748	1,5-Anhydro-d-altritol	$C_6H_{12}O_5$	164.157 g/mol	0.52
20.	12.153	1,6-Anhydro-.beta.-D-glucofu	$C_6H_{10}O_5$	162.1406 g/mol	2.18
21.	12.387	1,2,3,5-Cyclohexanetetrol	$C_6H_{12}O_4$	148.16 g/mol	3.52
22.	16.866	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241g/mol	1.03
23.	19.579	9,12,15-Octadecadienoic acid (Z	$C_{18}H_{32}O_2$	280.4455 g/mol	1.34
24.	29.873	(R)-(-)-14-Methyl-8-hexadecy	$C_{17}H_{34}O$	254.5 g/mol	1.52
25.	29.9	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240.4247 g/mol	1.59



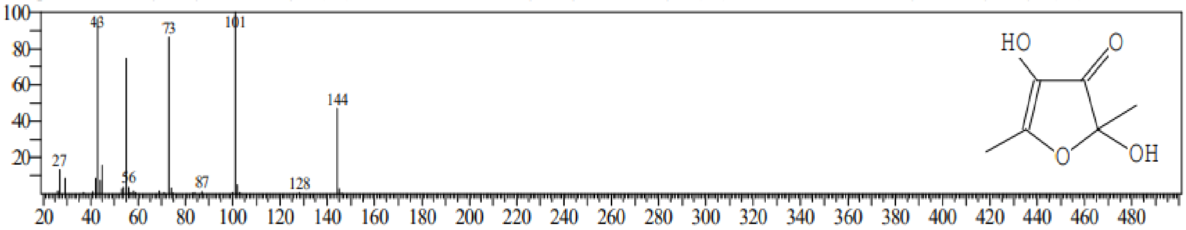
a.) 3,5-Dimethylpyrazole-1-meth



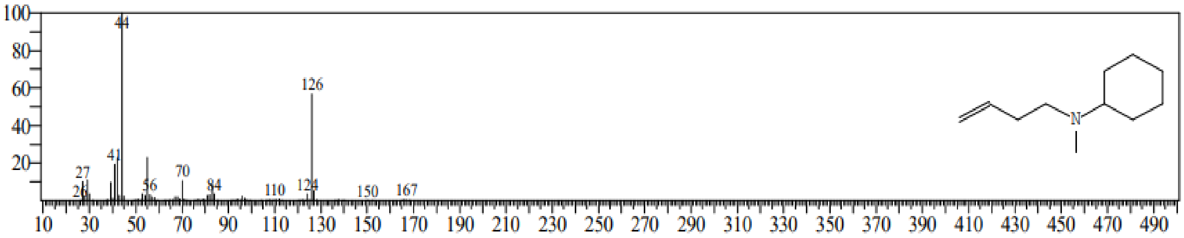
b.) Glycerine



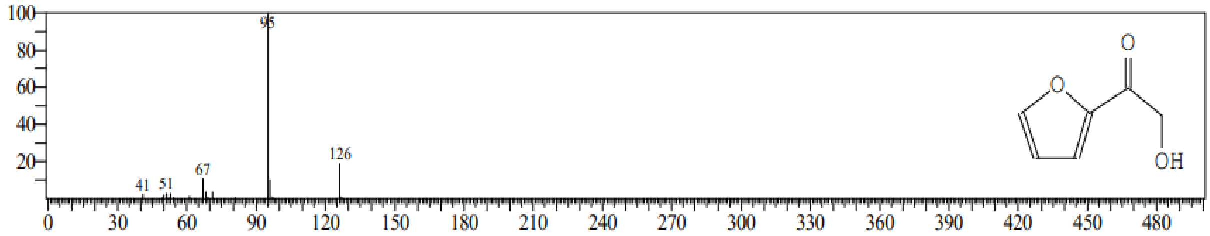
c.) 2-Furancarboxaldehyde, 5-me



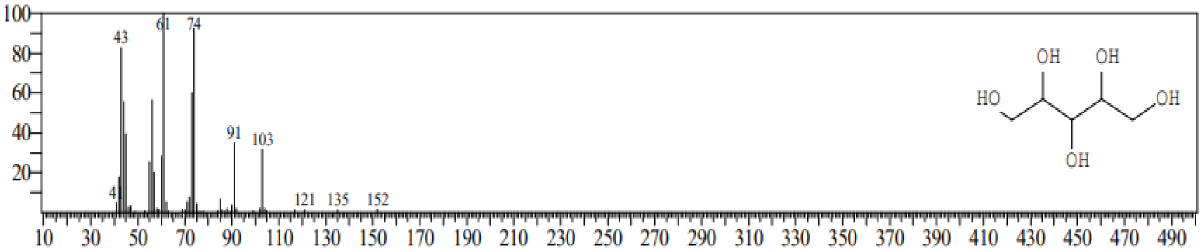
d.)2,4-Dihydroxy-2,5-dimethyl-3



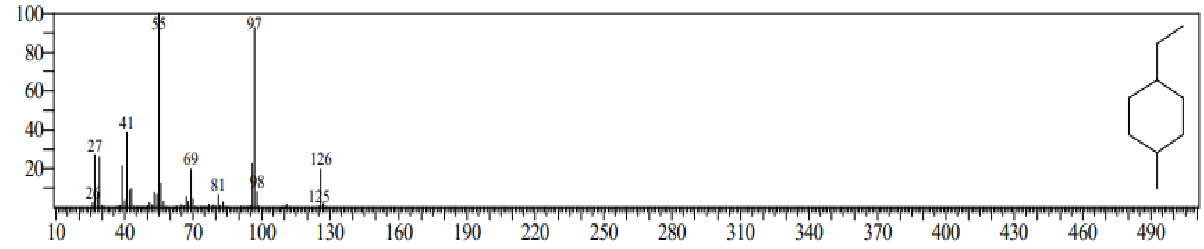
e.) Thymine



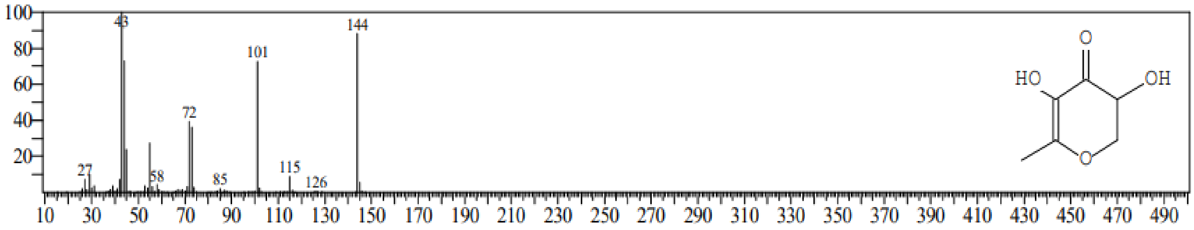
f.) Furylhydroxymethyl ketone



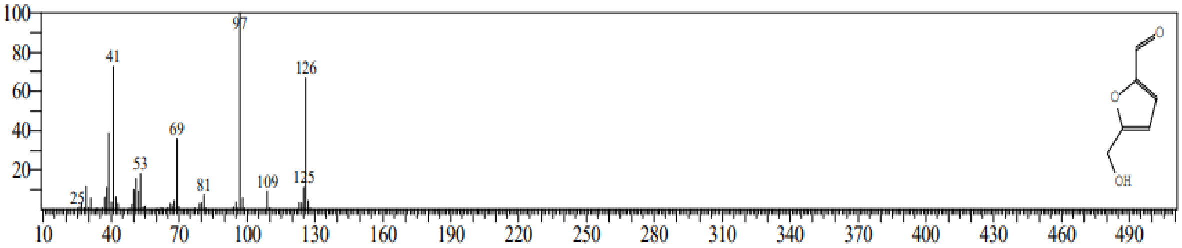
g.) Ribitol



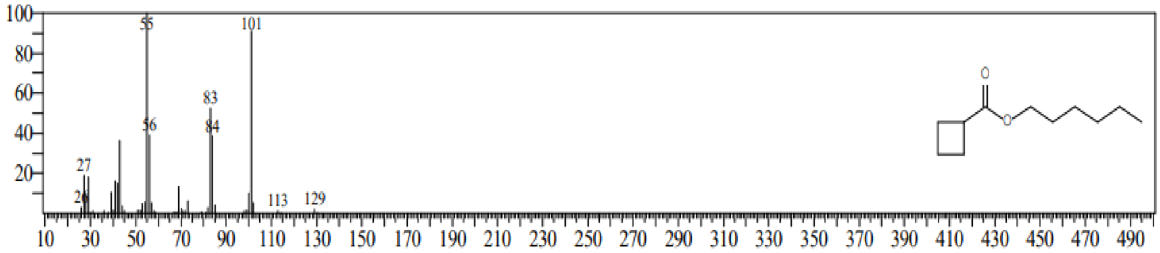
h.) 4H-Pyran-4-one, 2,3-dihydro-



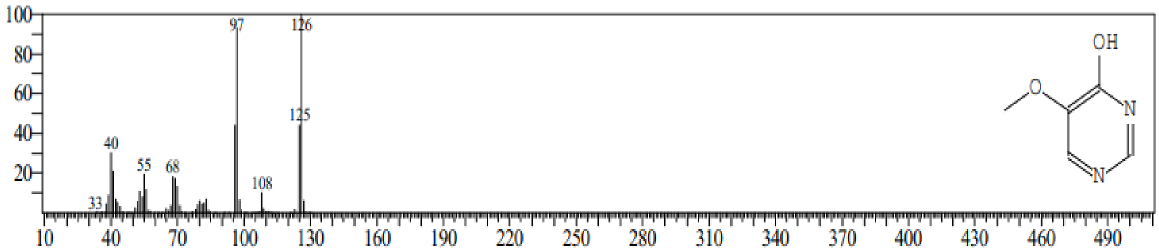
i.) 5-hydroxymethylfurfural



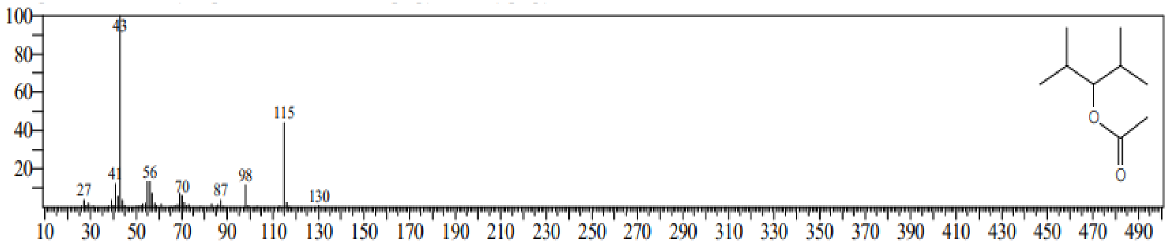
j.) Cyclohexane,1-ethyl-4-methyl



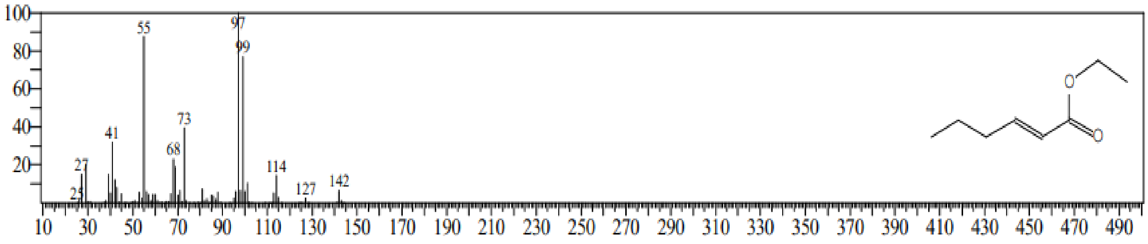
k.) Cyclobutanecarboxylic acid



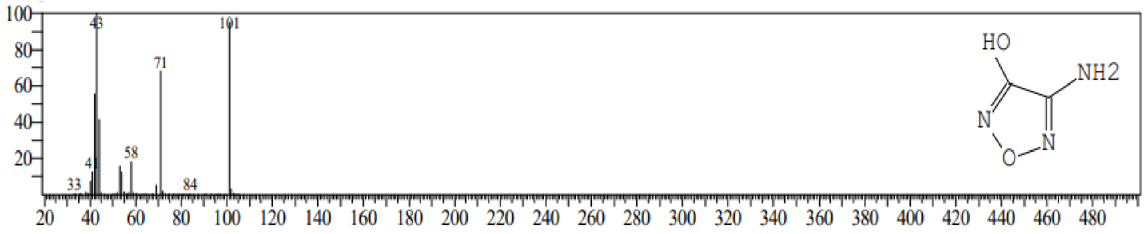
l.) 4-Pyrimidinol, 5-methoxy-



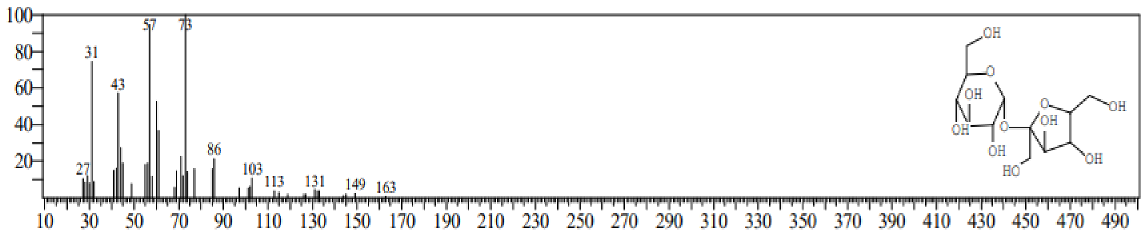
m.) 2,4-Dimethyl-3-pentanol acet



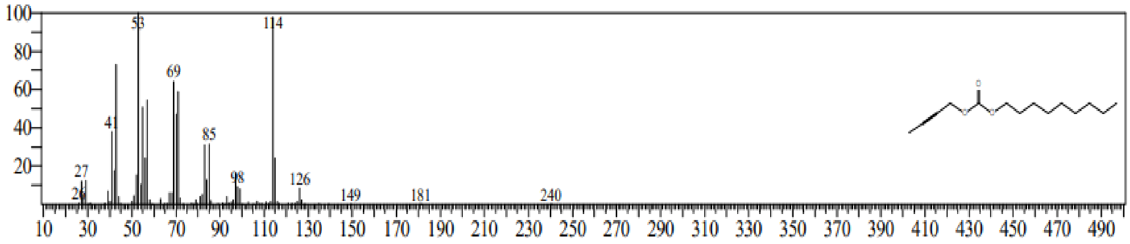
n.) 2-Hexenoic acid, ethyl ester



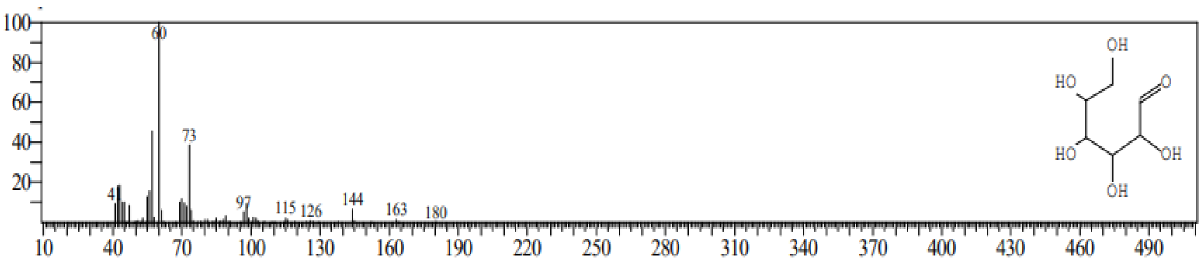
o.) Furazan-3-ol, 4-amino-



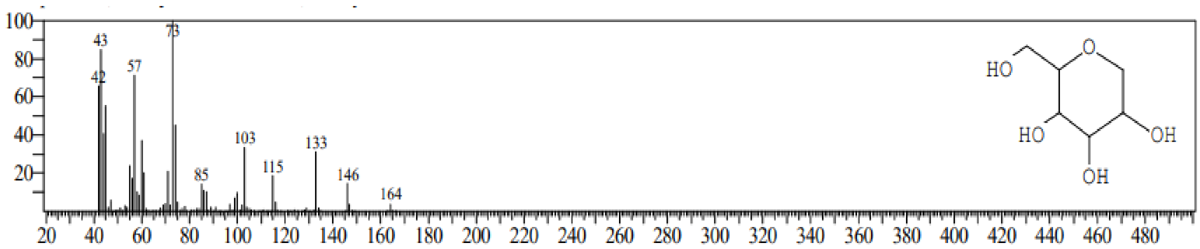
p.) Sucrose



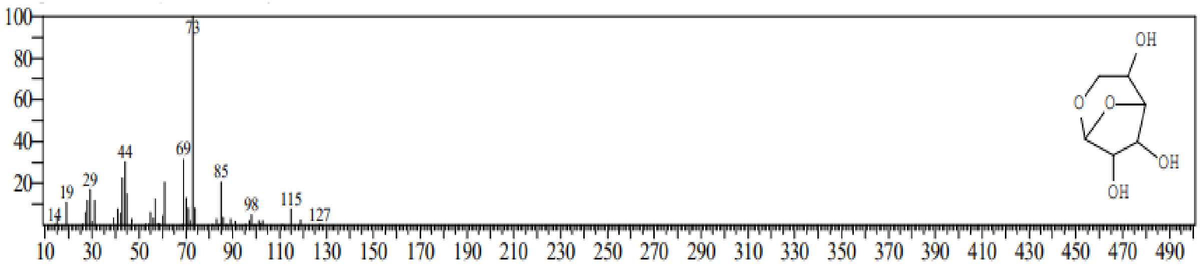
Q.) Carbonic acid, but-2-yn-1-yl nonyl ester



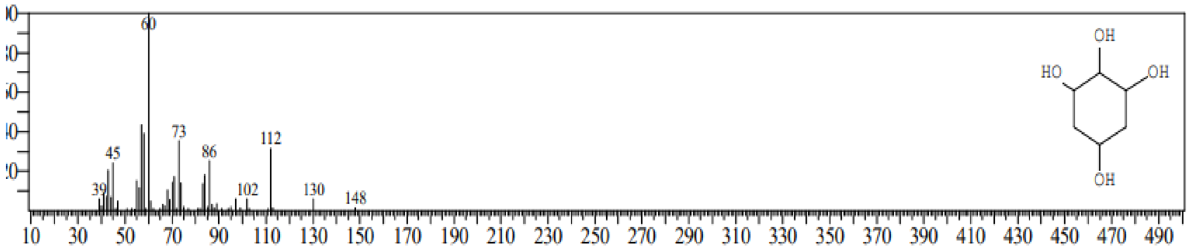
r.) D-Allose



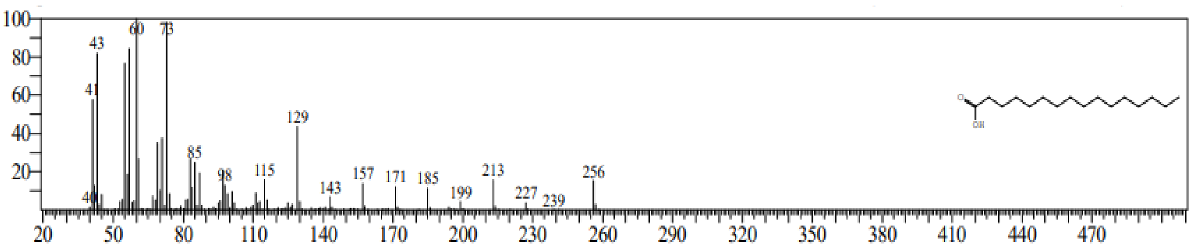
s.) 1,5-Anhydro-d-altritol



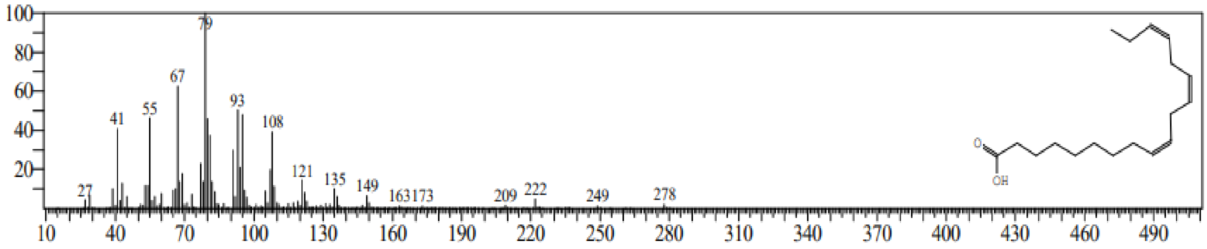
t.)1,6-Anhydro-.beta.-D-glucofuranose



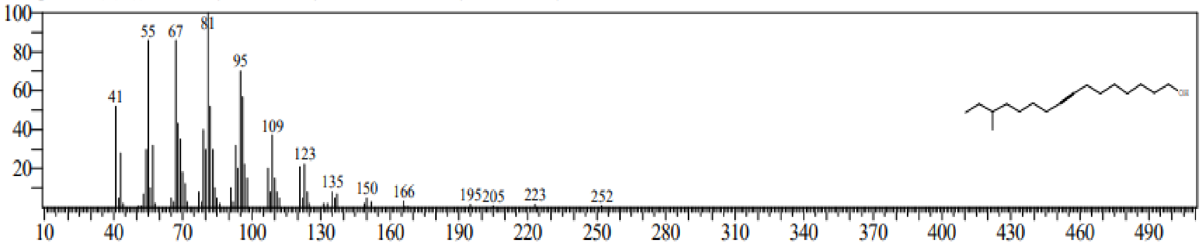
u.) 1,2,3,5-Cyclohexanetetrol



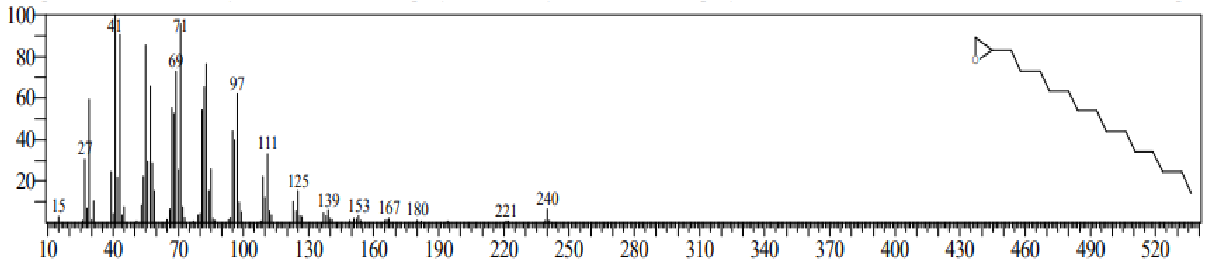
v.) n-Hexadecanoic acid



w.)9,12,15-Octadecatrienoic acid



x.) (R)-(-)-14-Methyl-8-hexadecyn-1-ol



y.) Oxirane, tetradecyl-

Table 2: Peak and functional groups in fruits of *Artocarpus lakoocha*

Peak No.	X (cm-1)	Class of Compound	Intensity	Assignment
1	3284.63	Alkynes	s,sharp	C-H stretching
2	2922.45	Carboxylic acid	s,broad	O-H stretching
3	2167.35	Ketenes	m	C=C=O stretching
4	2099.7	Isothiocyanate	s	N=C=S stretching
5	1986.83	Alkynes	s	C=C asymmetric stretch
6	1731.96	Aldehyde	s	C=O stretching
7	1627.8	Alkene	m-s	C=C stretching
8	1417.34	Alcohol	m	O-H bending
9	1365.92	phenol	m	O-H bending
10	1334.33	Alcohol	m	O-H bending
11	1243.73	amine	m	C-N stretching
12	1148.42	tertiary alcohol	s	C-O stretching
13	1073.87	primary alcohol	s	C-O stretching
14	1010.83	fluoro compound	s	C-F stretching

15	861.72	1,2,4-trisubstituted	s	C-H bending
16	816.95	P-substituted aromatic compond	s	C-H bending
17	763.44	mono substituted aromatic compound	S	C-H bending
18	572.08	Alkyl Halide	s	C-Br stretching

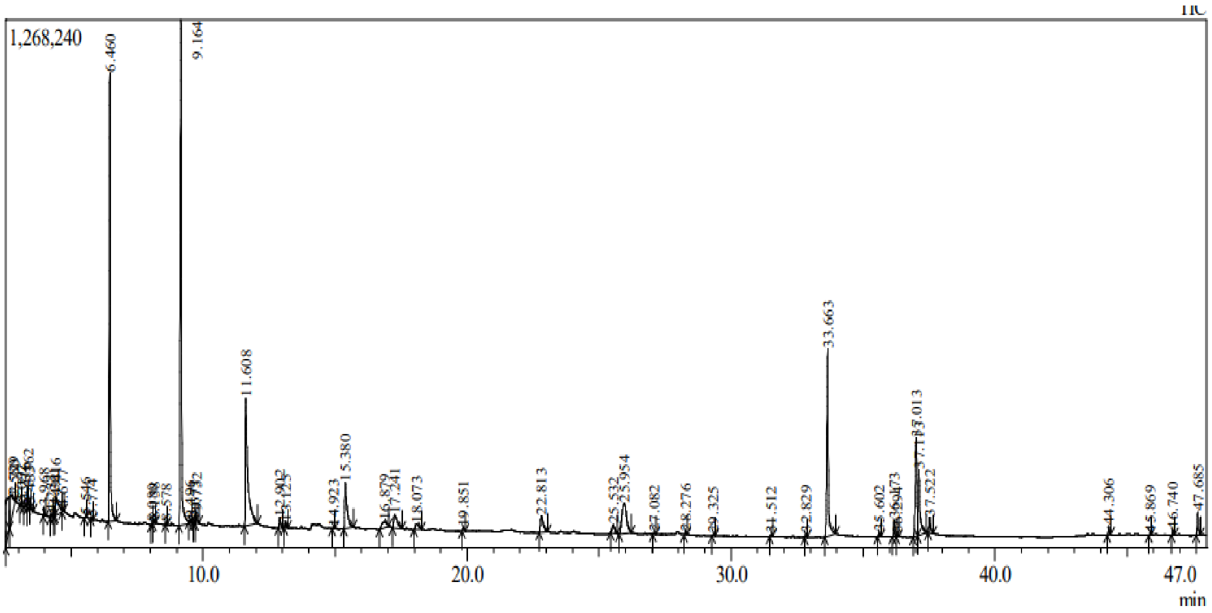


Figure 1: GC-MS of fruits of *Artocarpus lakoocha*

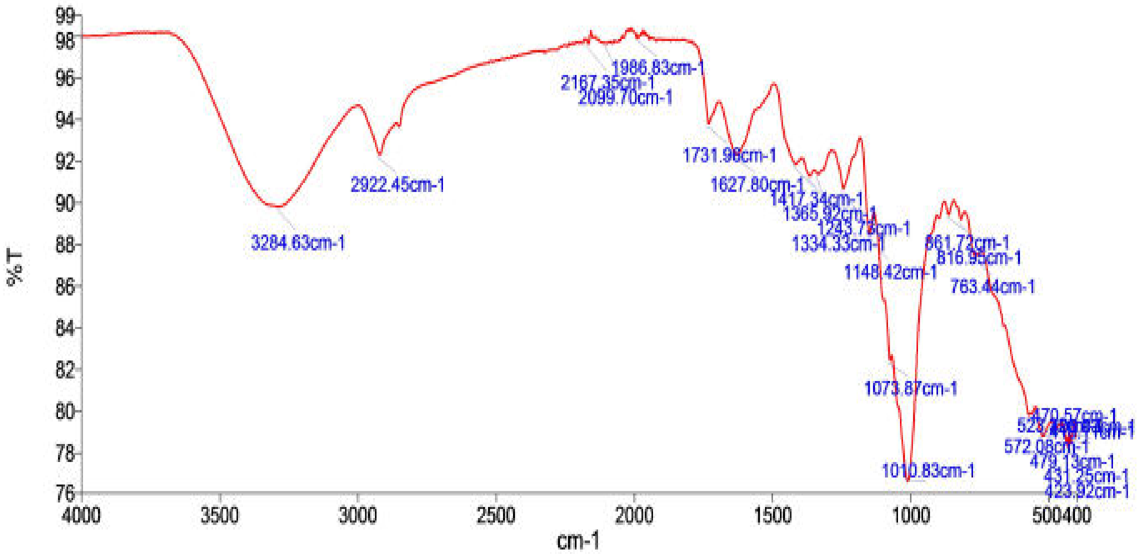


Figure 2: ATR-FTIR spectrum of fruits of *Artocarpus lakoocha*

Discussion

In the present study, the most prevalent compound identified in fruit of *Artocarpus lakoocha* was 5-HMF, constituting 38.26% of the relative amount. This substance is naturally present in honey and also found in variety of processed foods such as fruit juice, ketchup, and UHT milk. (Saeed et al., 2018). HMF serves as a versatile compound that functions as a precursor for various applications. It can be utilized in the production of polymers, pharmaceutical products, and liquid fuels. Additionally, HMF is valuable in synthesizing multiple groups of substances, including dialdehydes, ethers, and other organic compounds (Gomes et al., 2015).

Another predominant compound found was Dimethylpyrazole-1-meth (DMP). By slowing down the formation of nitrate-N and reducing nitrate reductase activity. DMP acts positively to mitigate environmental stress. DMP effectively suppresses soil nitrification, thereby reducing soil nitrate levels and nitrate reductase activity. DMP plays a significant role in soil nitrogen transformation processes and has the potential to improve nitrogen fertilizer efficiency, thereby promoting environmentally sustainable, low-carbon agriculture (Lu Y et.al. 2022). 3,5-dimethyl pyrazole which can have potential bacterial growth inhibition on different types of strains microorganisms: *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Anca and Constantin2018).

Furazan-3-ol, 4-amino- have the antiplasmodial activity, significantly influenced by the replacement of the phenyl ring at the fourth position of the furazan ring (Hermann et.al. 2021).

Compounds in the furazan class have shown antimicrobial properties. Research by Wang et al. (2019) indicates that certain furazan derivatives exhibit activity against various bacteria and fungi. Furazan derivatives can serve as intermediates in the synthesis of pesticides. Their ability to inhibit specific enzymes can be harnessed for crop protection.

D-allose, an uncommon sugar (Huijuan et al, 2020), demonstrates anti-inflammatory properties through its antioxidant effects and is utilized to decrease cerebral edema. This rare sugar's impact on the brain involves reducing inflammatory cytokines and preventing the development of cerebral swelling. Its effectiveness extends to both vasogenic and cytotoxic forms of brain edema, including cases resulting from water intoxication (Irie et al 2024).Ingesting foods or drinks fortified with sucrose has been linked to enhanced cognitive functions, including increased mental alertness, improved memory, faster reaction times, better attention, and superior mathematical problem-solving abilities. Additionally, it has been shown to decrease feelings of tiredness in both healthy individuals and those with Alzheimer's disease (Zamora et al., 2013). Sucrose can improve the absorption of electrolytes and water when ingested with fluids, particularly during physical activity (Sun et.al., 2015).

Thiamine (Vitamin B1) is essential due to its extensive effects on the human body, as a cofactor in enzymatic processes, impacts cell metabolism directly and indirectly. Dietary deficiency of thiamine disrupts crucial biochemical and metabolic processes, including glucose metabolism, bioenergetic processes, mitochondrial function, and DNA synthesis due to low transketolase activity and ribose-5-phosphate production in the pentose phosphate pathway. It also impairs neurotransmitter synthesis. Clinically, deficiency leads to conditions such as beriberi, characterized by cardiomyopathy with edema and lactic acidosis, and Wernicke–Korsakoff syndrome, including Wernicke's encephalopathy. Thiamine deficiency is further linked to neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's (Małgorzata et al 2023).

Hexadecenoic acid, identified at (R/T 29.9), functions as an antioxidant, hypocholesterolemic agent, nematicide, pesticide, antiandrogen, and haemolytic substance as reported by Rajeswari (2012).

Oxirane tetradecyl- found in this species acts as a disinfectant with bactericidal, fungicidal, and sporicidal properties. It demonstrates efficacy against a wide range of microorganisms, including viruses. This compound is utilized for fumigating food products and textiles, as well as for the gaseous sterilization of heat-sensitive pharmaceutical and surgical items (Ajayi 2021).

Glycerine, a type of carbohydrate also known as a polyol or sugar alcohol. Plants naturally create glycerine during the process of carbohydrate digestion. This clear liquid has a thick texture and a sugary taste with a laxative property that is commonly used to treat constipation. Products with glycerol, like lotions and syrups, are soft, flexible, and creamy because of glycerol's hygroscopic nature. Glycerine provides advantages for skin care such as hydrating, regulating water balance, and preserving the structure of intercellular lipids. Glycerol aids in skin exfoliation by hydrating enzymes (Mast 2018).

Artocarpus lakoocha contains hexanoic acid, an organic compound also present in fruits like *Morinda citrifolia*, *Ananas comosus* and *Rubus idaeus* (Aprea et al., 2015; Ferlinahayati et al., 2016). Research has shown that hexanoic acid exhibits anticancer properties and may boost energy metabolism (Miyamoto et al., 2016; Narayanan et al., 2015). Additionally, studies have demonstrated the anticancer effects of *Artocarpus lakoocha* extract on murine macrophage cell line (Hankittichai et al., 2020) which plays a major role in tumour progression and metastasis. It is possible that hexanoic acid contributes to the anticancer activity observed in *Artocarpus lakoocha*.

The *Artocarpus lakoocha* fruit contains another important compound known as 1,6-Anhydro-beta-D-glucofuranose. This anhydro sugar is generally produced through glucose pyrolysis (Hu et al., 2017). Nevertheless, it can also be found naturally occurring in the bark of Oak trees, honey derived from *Acacia*, and *Punica granatum* (Yelliantty et al, 2021).

Ribitol, a compound found in *Artocarpus lakoocha* used as supplementation for increased glutathione and decreased oxidative phosphorylation and gluconeogenesis. In contrast, ribose elevated oxidized glutathione (GSSG) levels, indicating higher oxidative stress. Ribitol also promoted nucleotide biosynthesis. The TCA cycle disruption, shown by ribitol-induced rises in succinate and fumarate and a decline in citrate, demonstrates cancer cells' adaptability to

nutritional changes, suggesting potential for targeted cancer therapies, especially in combination with other treatments. In breast cancer cells, ribitol-enhanced matriglycan correlates with metabolic changes, causing TCA cycle dysregulation with reduced citrate and increased succinate and fumarate levels (Tucker et al., 2022). This particular compound may be beneficial for breast cancer.
The FTIR analysis inference the presence of Alkynes, carboxylic, Ketens, Aldehyde, Alcohol and phenol groups, based on that the fruit of *Artocarpus lakoocha* found to be rich source of antioxidants.

Conclusion

The study's findings indicate that up to eighty different chemicals were detected by GC-MS as volatile components in the *Artocarpus lakoocha* fruit extract. It was found that 5-hydroxymethyl furfural (5-HMF) was the component with the highest concentration among these. Apart from 5-HMF other main compounds i.e. include 3,5-Dimethylpyrazole-1-meth (DMP), Furazan-3-ol, 4-amino-, 1,2,3,5-Cyclohexanetetrol, 4H-Pyran-4-one, 2,3-dihydro-, Sucrose; Glycerine; D-Allose; Ribitol; Hexanoic Acid; 9,12,15 Octadecadienoic acid and Oxirane, tetradecyl and a number of compounds with flavouring potential were present. Long-chain fatty acids, numerous organic acids, and photochromic chemicals were also present in *Artocarpus lakoocha*. The substances found in this study might indicate potential biological function, demonstrating the fruits have considerable for use in food processing, medicine, and functional foods, among other areas.

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Declaration of conflicting interests

No Conflict of interest

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