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Research Article

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GC-MS-based metabolomics analysis unravels the therapeutic potential of *Neolamarckia cadamba* fruit peel

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ABSTRACT

Kadam (*Neolamarckia cadamba* (Roxb.) is an evergreen tropical tree widely grown in Asia, particularly in India. *Neolamarckia cadamba* commonly known as kadam, cadamba or burflower tree. The roots, leaves, barks, and fruits of *N. cadamba* possess medicinal properties and are commonly used in the pharmaceutical industry. Fruit peels are the main waste and may contain various biologically active compounds. However, no prior knowledge about the therapeutic compounds of the peel. The objective of the present study was to unveil therapeutic compounds from the peel by Gas Chromatography–Mass Spectrometry (GC-MS) based metabolomics analysis. Metabolites from the kadam fruit peel were isolated and derivatized using MSTFA, characterized by the GC-MS analysis. Raw spectral data were pre-processed, and peak identification was performed using SHIMADZU Postrun analyse software. The metabolites in *N. cadamba* fruit peel were identified by comparing the peaks with the mass spectral reference database NIST v20. The results showed that the peel of kadam fruit contains 149 metabolites, which were further categorized into 46 different metabolite classes, with 52 different metabolic pathways and 63 biological functions. The principal roles of the metabolites were identified by functional annotation and enrichment analysis. It revealed that metabolites were responsible for anti-inflammation, anti-oxidant, anti-microbial, and anti-cancer properties. In summary, the peel of kadam fruit also contains various therapeutic compounds like other cadamba parts (i.e., roots, leaves, barks, and fruits). Further, comparing the peel with other parts discloses the peel-specific metabolites. The results obtained in this study could be useful for the pharmaceutical industry.

KEYWORDS: Fruit peel, GC-MS, Metabolites, *Neolamarckia cadamba*, Therapeutic compounds

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INTRODUCTION

Neolamarckia cadamba (Roxb.) Bosser, also known as a “miracle tree”, is a fast-growing tall evergreen tree found in South and Southeast Asia (Pandey & Negi, 2016). Various tissues (i.e., roots, leaves, barks, and fruits) of *N. cadamba* possess medicinal properties and used to treat various diseases, including fever, dysentery, leprosy, skin, and blood. It also has wound healing, anti-oxidant, and hepatoprotective properties (Kapil *et al.*, 1995; Umachigi *et al.*, 2007). The kadam is frequently mentioned in Indian literature for ayurvedic treatments and mainly has pharmacological effects like anti-diarrheal and detoxifying, analgesic, and seminal fluids (Bandyopadhyay & Mukherjee, 2009). The kadam leaf’s aqueous extract has been used in traditional medicine to treat menorrhagia, pain, swelling, and wounds. The bark’s decoction can treat colitis, diarrhoea, and dysentery and can help treat skin infections (Ambujakshi *et al.*, 2009).

The tree produces spherical fruits with an edible pulp at the center surrounded by a thin covering of seeds (Narzary *et al.*, 2013). Fruits are juicy, globose, orange, and turn yellow when ripe. The fruit diameters range from 5-7 cm. (Government of India & Family Welfare, 1999). The kadam fruit has higher quantities of magnesium, zinc, calcium, and iron when compared to numerous commonly consumed fruits. As a result, the kadam fruit is a fantastic source of essential minerals, and in terms of its mineral content, it may be compared to other well-known and pricey fruits like apple, pear, and so on (Pal *et al.*, 2014).

Recent studies have confirmed that fruit and vegetable peel waste can be a valuable source of bioactive compounds due to the presence of steroids, phenolics, tannins, flavonoids, triterpenoids, glycosides, carotenoids, ellagitannins, anthocyanins, vitamin C, and essential oil (Pathak, 2020). If extracted properly, these compounds can add value to the by-products of fruits. Peels of fruits can be converted into various economically valuable products with superior medicinal, nutritional, and antioxidant properties through multiple processes. The non-edible kadam fruit peel (as a by-product) is not yet explored for its medicinal value and metabolites information.

Despite several published findings on the metabolites and biological functions of *N. kadam* 's bark, root, and leaves, few studies have been reported on the kadam fruit. After the kadam fruit has been consumed as juice or pulp, the peel is typically ignored. Also, considering the limited availability of kadam trees and their seasonal fruit, avoiding plant waste is crucial. However, the significant bioactive compounds present in the ripened fruit peel and its biological function are unknown. In light of the above facts, the present study aimed to unveil the therapeutic compounds from *N. cadamba* fruit peel by Gas chromatography–Mass Spectrometry (GC-MS) based metabolomics analysis.

MATERIALS AND METHODS

Sample Collection and Metabolite Extraction

Fruit samples collected from the three-year-old *N. cadamba* tree maintained at Forest College and Research Institute in Mettupalayam, Tamil Nadu, India (altitude of 300 m with a longitude of 11.19'N, latitude of 77.56'E) were used for the GC-MS analysis. In this study, the kadam fruit peel was examined. To preserve the samples for further analysis, they were immediately frozen using liquid nitrogen and kept at -80 °C. For the GC-MS analysis, three replications of samples were used. Metabolites were extracted from the peel and derivatized according to Lisec *et al*. (2006).

GC-MS Analysis

Using Shimadzu single quadrupole GCMS-QP2020 NX Gas Chromatograph-Mass Spectrometer (GC-MS), the samples that had been derivatized were examined. At an injection temperature of 250 °C, one μl sample was injected. The column (Rxi-5Sil MS column) temperature was set to start at 40 °C for 2 min, then was raised to 320 °C at a rate of 8 °C per min for 10 min. At a split ratio of 1:30, samples were injected. Following is the programming for the mass spectrometer: Ion source at 220 °C, interface at 300 °C, and solvent cut at 6.0 min, and the mass range was 40 m/z to 600 m/z.

Data Analysis

We used the Shimadzu Postrun Analyzer v2020 to profile the metabolites. The National Institute of Standards and Technology (NIST) v2020 is used for compound identification and mass spectrum interpretation. Identified metabolites were classified using ClassyFire online tool (Djoumbou Feunang *et al.*, 2016). Pathway analysis was done using Metaboanalyst v5 (Pang *et al.*, 2015). Metabolite's biological functions were predicted using MBROLE 2.0. online tool (López-Ibañez Infante, 2021).

RESULTS

GC-MS Profiling of Kadam Fruit Peel

With the untargeted approach using the GC-MS detection platform, we identified 149 plant-based metabolites in the peel of kadam fruit (Table 1). Ten metabolites showed highest peak in the total ion chromatogram, including sucrose (17 %), citric acid (14.48 %), quininic acid (6.84%), pinitol (6.67%), catechine (4.46 %), pcicose (3.66%), glucose (3.56 %), chlorogenic acid (3.44 %), myoinositol (2.22 %) and Altrose (1.27 %) (Figure 1). The highest accumulated metabolites belonged to the following classes, sugars, organic acids, and flavans.

Functional Annotation of Metabolites and their Enrichment Analysis

We categorized 149 identified metabolites in peel into 46 chemical classes (Table 1). We have analysed all the metabolites in three ways: chemical classification, metabolic pathways, and biological function annotation. In the chemical classification, carbohydrates are identified as a predominant chemical class (56 compounds), followed by alcohols (13 compounds) and then amino acids (6 compounds) (Figure 2). In metabolic pathways mapping, a total of 52 different metabolic pathways were identified. We identified significant pathways with a high impact and a low FDR value. This includes the indole alkaloid pathway, alanine, aspartate, glutamate metabolism, monobactam biosynthesis, and glyoxylate and dicarboxylate metabolism (Figure 3). In functional annotation, the metabolites were classified into 61 functions: sweetening agents, food acidity, osmolyte, and food stabiliser (Table 2). From these annotations, we have identified many pharmaceutically significant metabolites, including glucaric acid (antineoplastic agent), mandelic acid (antibacterial), chloramphenicol (antibiotic), succinic acid (anti-ulcer), chlorogenic acid (hepatoprotective agent) and mannitol (antiglaucoma drug).

Table 1: List of the compounds identified in the peel of cadamba fruit

S. No.	RT	CID	Compounds	MW	Chemical classification
1	6.275	6329	Methylamine	31.06	Amines
2	6.51	65098	Norvaline	117.2	Amino acids, peptides, and analogues
3	7.36	4113	O-Methylhydroxylamine	47.06	Organooxygen compounds
4	7.68	6341	Ethylamine	45.08	Amines
5	7.815	174	1,2-Ethanediol	62.07	Alcohols and polyols
6	8.885	1060	Pyruvic acid	88.06	Alpha-keto acids and derivatives
7	9.915	5959	Chloramphenicol	323.1	Nitrobenzenes
8	9.935	10442	1,3-Propanediol	76.09	Alcohols and polyols
9	10.345	1176	Urea	60.06	Ureas
10	11.32	64960	1,5-Anhydro-D-glucitol	164.2	Carbohydrates and carbohydrate conjugates
11	11.935	827	Pentitol	152.2	Carbohydrates and carbohydrate conjugates
12	12.05	8064	1,4-Butanediol	90.12	Alcohols and polyols
13	12.08	5951	Serine	105.1	Amino acids, peptides, and analogues
14	12.2265	1150	Tryptamine	160.2	Tryptamines and derivatives
15	12.305	753	Glycerol	92.09	Carbohydrates and carbohydrate conjugates
16	12.79	69507	Fructose-6-phosphate	260.1	Carbohydrates and carbohydrate conjugates
17	12.88	1110	Succinic acid	118.1	Dicarboxylic acids and derivatives
18	13.085	752	Glyceric acid	106.1	Carbohydrates and carbohydrate conjugates
19	14	1004	Phosphoric acid	98	Non-metal phosphates
20	14.54	444266	Maleic acid	116.1	Dicarboxylic acids and derivatives
21	14.845	262	2,3-Butanediol	90.12	Alcohols and polyols
22	14.845	8117	DI (Hydroxyethyl) ether	106.1	Ethers
23	14.845	8146	Diethylene glycol monoethyl ether	134.2	Ethers
24	15.177	525	Malic acid	134.1	Beta hydroxy acids and derivatives
25	15.23	643798	Citraconic acid	130.1	Fatty acids and conjugates
26	15.895	181976	3-Hydroxyglutaric acid	148.1	Beta hydroxy acids and derivatives
27	16.195	785	Hydroquinone	110.1	Benzenediols
28	16.335	6503	Tromethamine	121.1	Amines
29	16.51	5460677	D-ribonic acid	166.1	Carbohydrates and carbohydrate conjugates
30	16.57	5960	Aspartic acid	133.1	Amino acids, peptides, and analogues
31	16.754	33032	Glutamic acid	147.1	Amino acids, peptides, and analogues
32	16.935	875	Tartaric acid	150.1	Carbohydrates and carbohydrate conjugates
33	17.15	6602431	D-xylonic acid	166.1	Carbohydrates and carbohydrate conjugates
34	17.54	444972	Fumaric acid	116.1	Dicarboxylic acids and derivatives
35	17.76	169019	D-Threitol	122.1	Carbohydrates and carbohydrate conjugates
36	17.855	25310	L-Rhamnose	164.2	Carbohydrates and carbohydrate conjugates
37	17.915	222285	Erythritol	122.1	Carbohydrates and carbohydrate conjugates
38	17.925	17106	L-Fucose	164.2	Carbohydrates and carbohydrate conjugates
39	18.085	499	DL-Pyroglutamic acid	129.1	Amino acids, peptides, and analogues
40	18.085	7405	L-Pyroglutamic acid	129.1	Amino acids, peptides, and analogues
41	18.43	440921	l-Arabinofuranose	150.1	Carbohydrates and carbohydrate conjugates
42	18.53	1032	Propionic acid	74.08	Carboxylic acids
43	18.695	439535	2,3,4-Trihydroxybutanoic acid	136.1	Carbohydrates and carbohydrate conjugates
44	18.705	128869	D-Galactonic acid	196.2	Medium-chain hydroxy acids and derivatives
45	18.715	128889	methyl beta-D-fructofuranoside	194.2	Carbohydrates and carbohydrate conjugates
46	18.795	3469	2,5-Dihydroxybenzoic acid	154.1	Benzoic acids and derivatives
47	18.795	7420	3-Hydroxybenzoic acid	138.1	Benzoic acids and derivatives
48	19.154	311	Citric acid	192.1	Tricarboxylic acids and derivatives
49	19.495	51	2-Oxoglutaric acid	146.1	Gamma-keto acids and derivatives
50	19.575	102192447	2,2′-Dithiobisethanol 1-(2-methyl-2-bromopropionate) 1′-acrylate	357.3	Dicarboxylic acids and derivatives
51	19.675	345824	Quininic acid	203.2	Quinoline carboxylic acids
52	19.68	6508	Quinic acid	192.2	Alcohols and polyols
53	19.7	6912	Xylitol	152.2	Carbohydrates and carbohydrate conjugates
54	19.7	64689	beta-D-Glucose	180.2	Carbohydrates and carbohydrate conjugates
55	19.91	91738890	1,3,5-Benzetriol	270.5	Phenoxy compounds
56	19.99	441036	D-Psicose	180.2	Carbohydrates and carbohydrate conjugates
57	20.07	10975657	D-Ribose	150.1	Carbohydrates and carbohydrate conjugates
58	20.244	5793	D-Glucose	180.2	Carbohydrates and carbohydrate conjugates
59	20.405	33037	Glucaric acid	210.1	Carbohydrates and carbohydrate conjugates
60	20.565	441032	D-Altrose	180.2	Carbohydrates and carbohydrate conjugates
61	20.565	10219674	L-Altrose	180.2	Carbohydrates and carbohydrate conjugates
62	20.62	2723872	D-Fructose	180.2	Carbohydrates and carbohydrate conjugates
63	20.63	560035	3-Deoxy-d-mannitol	166.2	Fatty alcohols
64	20.715	2724705	Levoglucosan	162.1	Oxepanes

(*Contd...*)

Table 1: (*Continued*)

S. No.	RT	CID	Compounds	MW	Chemical classification
65	20.855	439665	D-Threose	120.1	Carbohydrates and carbohydrate conjugates
66	20.855	5460672	L-Threose	120.1	Carbohydrates and carbohydrate conjugates
67	21.205	11850	Galactitol	182.2	Carbohydrates and carbohydrate conjugates
68	21.365	643757	cis-Aconitic acid	174.1	Tricarboxylic acids and derivatives
69	21.52	439312	D-Tagatose	180.2	Carbohydrates and carbohydrate conjugates
70	21.82	6036	D-Galactose	180.2	Carbohydrates and carbohydrate conjugates
71	21.945	12306016	D-tagatofuranose	180.2	Carbohydrates and carbohydrate conjugates
72	22.238	164619	D-Pinitol	194.2	Alcohols and polyols
73	22.2985	8742	Shikimic acid	174.2	Alcohols and polyols
74	22.77	11005	Myristic acid	228.4	Fatty acids and conjugates
75	23.52	439507	D-Allose	180.2	Carbohydrates and carbohydrate conjugates
76	23.67	515	2-Methylcitric acid	206.2	Tricarboxylic acids and derivatives
77	23.73	89640	Loganic acid	376.4	Terpene glycosides
78	24.125	439215	D-Galacturonic Acid	194.1	Carbohydrates and carbohydrate conjugates
79	24.357	892	Inositol	180.2	Alcohols and polyols
80	24.69	152109	4-O-beta-D-Mannopyranosy l-D-mannopyranose	342.3	Carbohydrates and carbohydrate conjugates
81	24.69	161276	Secologanin	388.4	Terpene glycosides
82	24.92	60961	Adenosine	267.2	Purine nucleosides
83	25.005	1826	5-Hydroxyindole-3-acetic acid	191.2	Indolyl carboxylic acids and derivatives
84	25.165	985	Palmitic acid	256.4	Fatty acids and conjugates
85	26.13	689043	Caffeic acid	180.2	Hydroxycinnamic acids and derivatives
86	26.145	736715	Urocanic acid	138.1	Imidazoles
87	27.35	5281	Stearic acid	284.5	Fatty acids and conjugates
88	27.695	5780	Sorbitol	182.2	Carbohydrates and carbohydrate conjugates
89	27.845	127686	Bungeiside C	430.4	Carbohydrates and carbohydrate conjugates
90	27.845	151261	D-ribulose	150.1	Carbohydrates and carbohydrate conjugates
91	27.845	91696780	3-alpha-Mannobiose	948.8	Fatty acyl glycosides
92	27.98	67901	Trifluoroacetaldehyde hydrate	116	Fluorohydrins
93	28.095	6251	Mannitol	182.2	Carbohydrates and carbohydrate 9conjugates
94	28.115	5958	Glucose 6-phosphate	260.1	Carbohydrates and carbohydrate conjugates
95	28.25	1061	Phosphate	94.97	Non-metal phosphates
96	28.705	94176	D-Erythrose	120.1	Carbohydrates and carbohydrate conjugates
97	29.07	441478	beta-D-Glucopyranuronic acid	194.1	Carbohydrates and carbohydrate conjugates
98	29.53	971	Oxalic acid	90.03	Dicarboxylic acids and derivatives
99	29.8	24879693	Polygalatenoside A	430.4	Carbohydrates and carbohydrate conjugates
100	29.835	5202	Serotonin	176.2	Tryptamines and derivatives
101	30.065	439260	Norepinephrine	169.2	Benzenediols
102	30.285	6902	D-arabinopyranose	150.1	Carbohydrates and carbohydrate conjugates
103	30.285	439195	L-Arabinose	150.1	Carbohydrates and carbohydrate conjugates
104	30.485	206	Hexose	180.2	Carbohydrates and carbohydrate conjugates
105	30.675	439503	Salicin	286.3	Carbohydrates and carbohydrate conjugates
106	30.81	14900	Glyceryl palmitate	330.5	Monoradylglycerols
107	31.105	6989	Thymol	150.2	Monoterpenoids
108	31.105	7427	Trehalose	342.3	Carbohydrates and carbohydrate conjugates
109	31.58	107802	3-Hydroxypentanoic acid	118.1	Fatty acids and conjugates
110	32.12	10712	D-(+)-Cellobiose	342.3	Carbohydrates and carbohydrate conjugates
111	32.161	5988	Sucrose	342.3	Carbohydrates and carbohydrate conjugates
112	32.28	10314695	Rosiridin	332.4	Terpene glycosides
113	32.33	135191	D-Xylose	150.1	Carbohydrates and carbohydrate conjugates
114	32.92	439193	Isomaltose	342.3	Carbohydrates and carbohydrate conjugates
115	32.97	441422	Gentiobiose	342.3	Carbohydrates and carbohydrate conjugates
116	33.07	9378	2-Hydroxy-3-(4-hydroxyphenyl) propanoic acid	182.2	Phenylpropanoic acids
117	33.14	91696999	Catechine	651.2	Flavans
118	33.5	24699	Glyceryl monostearate	358.6	Monoradylglycerols
119	33.69	1135	Thymine	126.1	Pyrimidines and pyrimidine derivatives
120	33.725	6441280	trans-5-O-(4-coumaroyl)-D-quinic acid	338.3	Alcohols and polyols
121	33.79	70966	Vanillylamine	153.2	Methoxyphenols
122	34.16	87691	Loganin	390.4	Terpene glycosides
123	34.63	20695	4-Hydroxypyrimidine	96.09	Pyrimidines and pyrimidine derivatives
124	34.635	1054	Pyridoxine	169.2	Pyridoxines
125	34.925	442534	Paeoniflorin	480.5	Terpene glycosides
126	35.025	440658	6-O-(alpha-D-Galactopyranosyl) -D-glucopyranose	342.3	Carbohydrates and carbohydrate conjugates

(*Contd...*)

Table 1: (*Continued*)

S. No.	RT	CID	Compounds	MW	Chemical classification
127	35.255	9799386	3-O-Feruloylquinic acid	368.3	Alcohols and polyols
128	35.74	90478782	5-p-Coumaroylquinic acid, (Z)-	338.3	Alcohols and polyols
129	35.96	18950	D-Mannose	180.2	Carbohydrates and carbohydrate conjugates
130	36.09	101995872	Foliachinenoside I	412.4	Fatty acyl glycosides
131	36.42	1794427	Chlorogenic acid	354.3	Alcohols and polyols
132	36.45	493591	Maltitol	344.3	Fatty acyl glycosides
133	36.57	9798666	Cryptochlorogenic acid	354.3	Alcohols and polyols
134	36.715	135398635	Guanosine	283.2	Purine nucleosides
135	36.88	94715	D-Glucuronic Acid	194.1	Carbohydrates and carbohydrate conjugates
136	37.495	39197	(3-Propoxyphenyl) carbamic acid 1-methyl-2-(1-pyrrolidinyl) ethyl ester hydrochloride	342.9	Phenylcarbamic acid esters
137	37.575	6029	Uridine	244.2	Pyrimidine nucleosides
138	37.85	72277	Epigallocatechin	306.3	Flavans
139	38.215	441033	D-Gulose	180.2	Carbohydrates and carbohydrate conjugates
140	38.69	6255	Maltose	342.3	Carbohydrates and carbohydrate conjugates
141	38.965	1188	Xanthine	152.1	Purines and purine derivatives
142	39.35	69948	N-Methyl-2,2,2-trifluoroacetamide	127.1	Carboxylic acid derivatives
143	40.33	3336	Fendiline	315.5	Diphenylmethanes
144	40.41	85782	3,4-Dihydroxymandelic acid	184.2	Benzenediols
145	41.98	73323	Xanthosine-5′-monophosphate	364.2	Purine ribonucleotides
146	44.25	439533	Taxifolin	304.3	Flavans
147	44.25	443758	(+)-Epitaxifolin	304.3	Flavans
148	46.745	1052	Pyridoxamine	168.2	Pyridoxamines
149	47.075	1292	Mandelic acid	152.2	Benzene and substituted derivatives

Table contains the retention time of the metabolites (RT), pubchem compounds identifier (CID), name of the compounds, molecular weight (MW) and its chemical classification.

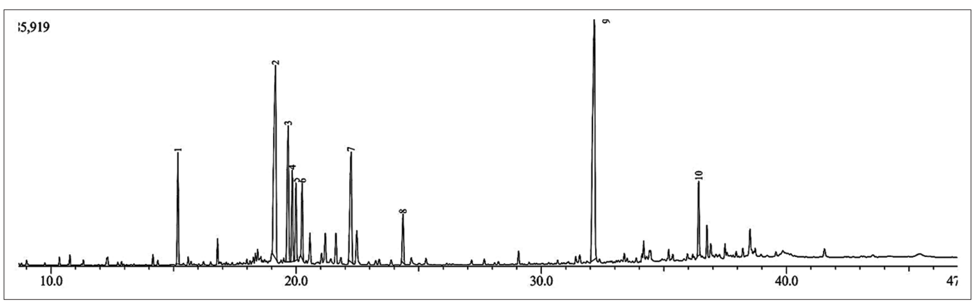

Figure 1: GC-MS total ion chromatogram of kadam fruit peel. Note: Numbers above the peaks indicate the abundant compounds. 1. Malic acid, 2. Citric acid, 3. Quinic acid, 4. Pcicose, 5. Catechine 6. D-Glucose, 7. D-Pinitol, 8. Inositol, 9. Sucrose 10. Chlorogenic acid

DISCUSSION

In our study, 149 metabolites were identified from the kadam fruit peel, which is the first metabolites analysis on the kadam fruit peel using GC-MS. The peak's area represents the concentration of particular metabolites in the sample; based on that, and we identified the highest area compounds such as sucrose, citric acid, quininic acid, pinitol, catechine, pcicose, glucose, chlorogenic acid, myo-inositol, and Altrose (Table 1). The medicinal value of these compounds was already reported by many researchers i.e., in many food and pharmaceutical industries, citric acid is used as an emulsifier, flavorant, sequestrant, buffering, acidulant, and preservative (Verhoff, 2000; Nangare *et al.*, 2021). Quininic acid is one of the organic acids, and it was also extracted from *Eucalyptus globulus* and employed as an astringent and a precursor for the synthesis of novel medications (Shi *et al.*, 2018). Pinitol is one of the more well-researched insulin mimickers, and also it has antidiabetic, anti-inflammatory, antioxidant, and immunomodulatory properties (Bates *et al.*, 2000; Sripathi *et al.*, 2011; Poongothai & Sripathi, 2013). The polyphenol compound catechins are well-studied metabolites with proven activities such as anti-oxidant (Parisi *et al.*, 2013), UV protection (Zhang *et al.*, 2017), anti-microbial (Goyal *et al.*, 2017), anti-allegenic (Ohmori *et al.*, 1995), anti-inflammation, antiviral (Ide *et al.*, 2014), anti-cancer (Kumar *et al.*, 2015). Additionally, they enhance cell activity and activate skin barrier passage (Puri *et al.*, 2016; Bae *et al.*, 2020). D-Pcicose is a rare sugar; it prevents and controls obesity and hyperglycemia (Hossain *et al.*, 2015). Chlorogenic acid is also a polyphenol compound, which has significant and bioactive nutrient polyphenol that has numerous beneficial and therapeutic properties, including anti-oxidant, hepatoprotective, cardioprotective, anti-inflammatory, anti-pyretic, anti-obesity, anti-microbial, anti-hypertension, and central neural system stimulator activities (Naveed *et al.*, 2018). Myo inositol is the sugar alcohol which is mainly present in fruits. It also has prevention and control properties such as bipolar disorder

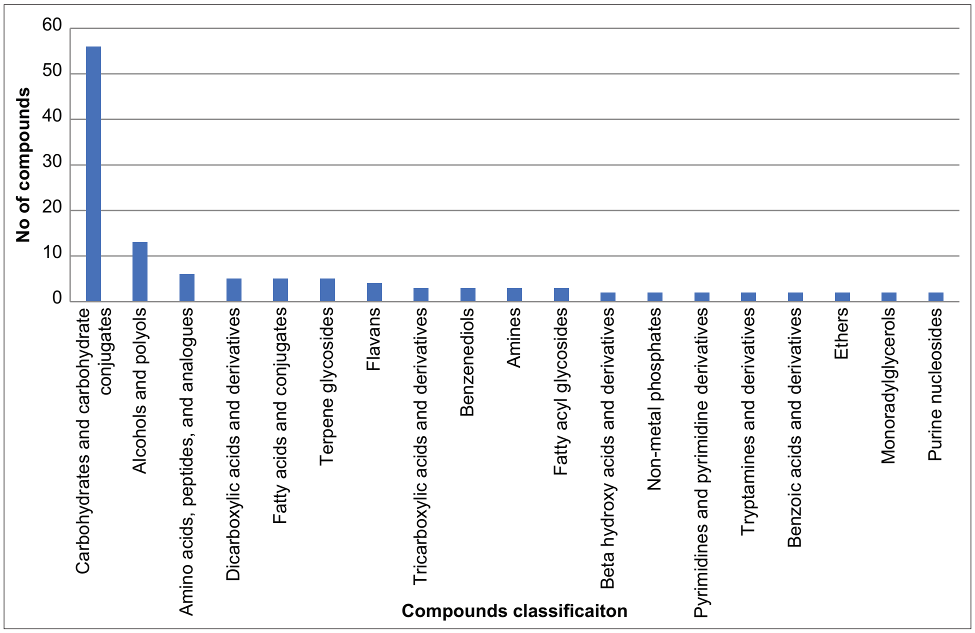

Figure 2: Metabolites classification in the peel of *N. cadamba*. (46 classes. In this figure, we selected classes with the total number of compounds equal to or more than two for graphical representation. The X-axis indicates metabolite classes, and the Y-axis shows the number of metabolites identified in the peel of cadamba

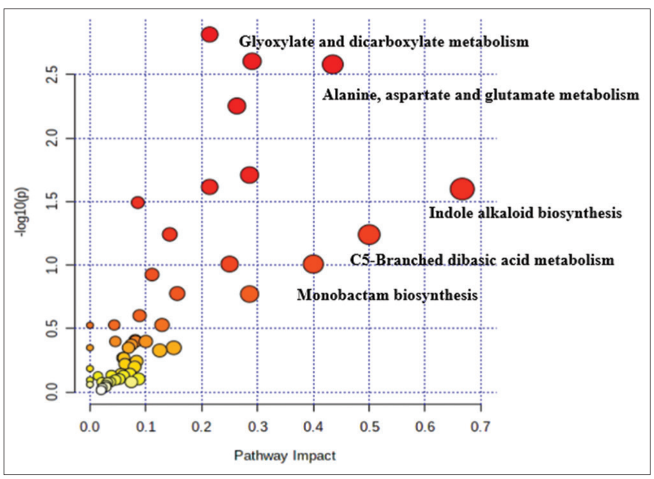

Figure 3: Pathway analysis of identified metabolites from *N. cadamba* peel. A total of 150 metabolites were mapped into 52 different metabolic pathways. Each circle represents a metabolic pathway. Red colour indicates a higher impact, and yellow colour represents the lower impact pathways. The size of the circle indicates the number of metabolites in the pathway. Here, we labeled high-impact metabolic pathways

(Bartoli *et al.*, 2021), depression (Taylor *et al.*, 2004), obsessive-compulsive disorder (Benjamin *et al.*, 1995), polycystic ovary syndrome (Unfer *et al.*, 2017), Alzheimer's disease and diabetic neuropathy. The above-discussed compounds have numerous therapeutic applications, which indicate that the peel of kadam fruit contains significant bioactive compounds.

In our study, different metabolic classes were also identified. In the kadam fruit peel, carbohydrates are predominant in chemical classification (Table 2). Similar results were supported by quantitative studies on the kadam fruit (Surani *et al.*, 2022) but not on the peel. Carbohydrates play a significant role in plant protection, immunity, and plant-microbe interactions. Total metabolites or crude extraction of different parts of kadam revealed its biological significance, including anti-helminthic (Acharyya *et al.*, 2011), antifungal (Divyakant *et al.*, 2011), antifilarial (Kumar *et al.*, 2013), antimalarial (Santiarworn, 2005), antibacterial (Mishra & Siddique, 2011), antidiabetic activity (Bussa & Jyothi, 2010), antidiarrheal activity (Alam *et al.*, 2008), hypolipidemic activity (Kumar *et al.*, 2010) and diuretic and laxative activities (Mondal *et al.*, 2009). However, it is yet unclear which specific metabolite groups are in response to these biological functions. GC-MS or other metabolites profiling and quantification techniques will help identify the significant metabolites responsible for these biological activities. Generally, flavonoids *viz.*, quercetin, apigenin is known to have analgesic and anti-inflammatory activities, we also identified quercetin in the peel of kadam fruit, and the existing report revealed similar results (Ambujakshi *et al.*, 2009; Bachhav *et al.*, 2009). According to studies, the kadam is used for its hepatoprotective properties. Chlorogenic acid isolated from the cadamba plant is responsible for hepatoprotective activity (Kapil *et al.*, 1995). Additionally, we discovered 3.4% chlorogenic acid in the peel of kadam (Figure 1). Further, we identified many biological roles of identified metabolites using the ChEBI database. These medicinal properties are due to alkaloids, flavonoids, saponins, phenolics, and carbohydrates (Ahmed *et al.*, 2011; Malothu *et al.*, 2012). There has not yet been enough thorough research on the active metabolites that give cadamba its wide range of pharmacological effects. A study also showed that monoterpene indole alkaloids (MIA) are responsible for these activities (Kumar *et al.*, 2010). We mapped metabolites against the pathways to confirm this statement, and the results showed that most of the compounds are part of the indole alkaloid pathway. Our studies identified many

Table 2: Function of metabolites identified using Chemical Entities of Biological Interest (ChEBI) database

S. No.	Annotation	Pubchem CID
1	Sweetening agent	6251, 6912, 5780, 5988, 2723872, 493591
2	Food acidity regulator	525, 444972, 311
3	Food humectant	6251, 5780
4	Bacterial metabolite	9378, 65098, 7420
5	Solvent	753, 1004, 174
6	Osmolyte	753, 5988
7	EC 1.13.11.33 (arachidonate 15-lipoxygenase) inhibitor	3469, 689043
8	Laxative	5780, 493591
9	Protic solvent	10442, 8064
10	EC 1.1.1.189 (prostaglandin-E2 9-reductase) inhibitor	985
11	Food bulking agent	6251
12	Osmotic diuretic	6251
13	Cofactor	1054, 785
14	Antioxidant	439507, 222285, 85782, 689043, 72277
15	Food stabiliser	6251
16	Food thickening agent	6251
17	EC 3.1.4.11 (phosphoinositide phospholipase C) inhibitor	892
18	Food anticaking agent	6251
19	Drug metabolite	85782, 1826
20	Cathartic	5780
21	EC 2.5.1.18 (glutathione transferase) inhibitor	689043
22	Nutrient	892
23	Nutraceutical	1110, 33032
24	Allergen	6251, 6912
25	Flour treatment agent	1176
26	Food component	72277
27	EC 3.1.1.1 (carboxylesterase) inhibitor	11005
28	Human xenobiotic metabolite	785, 1292
29	Detergent	753
30	MALDI matrix material	3469
31	Buffer	6503
32	Toxin	174
33	EC 1.13.11.34 (arachidonate 5-lipoxygenase) inhibitor	689043
34	EC 3.5.1.98 (histone deacetylase) inhibitor	689043
35	Alpha-adrenergic agonist	439260
36	Hapten	6251, 6912
37	Protein synthesis inhibitor	5959
38	Prodrug	439503, 8064
39	Antiglaucoma drug	6251
40	Anti-ulcer drug	1110
41	Chelator	311
42	Hepatoprotective agent	9798666
43	Human metabolite	6341
44	Vasoconstrictor agent	439260
45	Sympathomimetic agent	439260
46	Analgesic	60961
47	EC 1.14.99.1 (prostaglandin-endoperoxide synthase) inhibitor	439503
48	Antimicrobial agent	311
49	Plant metabolite	72277, 69507, 1052, 151261, 785, 94715, 69507, 1052, 9378, 65098, 7420
50	Antipyretic	439503
51	Anti-arrhythmia drug	60961
52	Vasodilator agent	60961
53	Carcinogenic agent	785
54	Non-narcotic analgesic	439503
55	Non-steroidal anti-inflammatory drug	439503
56	Epitope	64689, 10712, 441422
57	Antibacterial drug	5959
58	Antibiotic	5959
59	Antibacterial agent	1292
60	Metabolite	73323
61	Antineoplastic agent	33037

therapeutic metabolites from fruit peel and their biological roles using GC-MS based metabolomics and bioinformatics analyses. Further, therapeutic potential of these compounds needs to be tested by *in vitro* and *in vivo* studies.

CONCLUSION

The current study was the first to look into the identification of metabolites in the kadam fruit peel using GC-MS analysis. We have identified 149 compounds and explored the biological functions of these compounds. It revealed that the peel of kadam fruit also contains various therapeutic compounds like other kadam parts (i.e., roots, leaves, barks, and fruits). Further, comparing the peel with other parts discloses the peel-specific compounds. Collectively, the results obtained in this study could be useful for the pharmaceutical industry.

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