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PROFILING BIOACTIVE COMPOUNDS IN *CARICA PAPAYA* L. USING GC-MS FOR SICKLE CELL DISEASE MANAGEMENT

Nilima U. Rangari[1] and Aparna S. Margonwar[2]
Department of Botany, Nevjabai Hitkarini College Bramhapuri, (MS) 441206
Department of Botany, Shri. Sadgru Saibaba Science and commerce College, Ashti (MS)
Contact details of corresponding author: email ID: nur358@gmail.com

Abstract
Carica papaya L. plant have potential phytochemical compound that help into the management of sickle cell disease. The present study investigated the bioactive component responsible for sickle cell disease management in the methanol extract of the unripe fruit of *Carica papaya* L. This extract of unripe fruit phytochemical was identified by GC-MS using a library search. A total 54 phyto-component were identified and 07 are directly related to sickle cell disease management. The main bioactive component present in methanolic extract are piracetam, 4H-Pyran-4-one, 2,3-dihydro-3,5dihydroxy-6-methyl-,(S)-5-Hydroxymethyl-2[5H]-furanone, Pterin-6-carboxylic acid, L-(+)-Ascorbic acid 5,6-dihexadecanoaten, 10(E),12(Z)-Conjugated linoleic acid, 9(E), 11(E)- Conjugated linoleic acid in term of their role and action with management sickle cell anemia, Other antioxidant compounds identified in the GC-MS analysis could play a significant role in future clinical trials for management of sickle cell disease.

Key Words: *Carica papaya L., Sickle Cell disease, GC-MS, bioactive component*

Introduction

Sickle-cell disease (SCD) is a autosomal hereditary disorder caused by the presence of abnormal hemoglobin (HbS) in red blood cells, which occurs when glutamic acid is replaced by valine. This genetic mutation leads to the deformation of red blood cells from their usual biconcave shape into a sickle like form under certain conditions, such as low oxygen levels, dehydration, or stress (Patrick, TM et al., 2013). It is a systemic condition marked by the accelerated destruction of red blood cells, which results in chronic anemia and vaso-occlusion, leading to intense pain and other complications. Sickled red blood cells often obstruct small blood vessels, leading to stagnation of blood flow, depriving tissues and organs of oxygen and essential nutrients, and ultimately causing organ dysfunction or irreversible tissue damage (Pauling, et al., 1949). The management of sickle-cell disease (SCD) includes medication such as hydroxycarbamide (also called hydroxyurea), which helps reduce the frequency of painful crises and may lower the need for blood transfusions by promoting the production of fetal hemoglobin (Acquaye et al., 1982) Supportive treatments often involve analgesics, antimalarials, antibiotics, and antioxidants (Heeney et al., 2008) There has been growing interest in the use of plants in treating SCD.

Carica papaya L., which belongs to family Caricaceae is widely cultivated across tropical and subtropical regions, with some cultivation occurring in temperate zones. In India, papaya fruit is used to treating stomach disorders, obesity, and urinary tract infections (Krishna et al., 2008).The therapeutic benefits and functional properties of papaya are attributed to the presence of various phytochemicals, including phenolic compounds, carotenoids, glucosinolates, and alkaloids (Ikram et al., 2015; Pathak et al., 2018). The GC-MS; technique is widely regarded for its speed, accuracy, and effectiveness in detecting a wide range of compounds, such as alcohols, alkaloids, nitro accuracy, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids, while requiring only a small volume of plant extracts. Therefore, in this study, the GC-MS method was employed to identify the bioactive component

used in treatment of sickle cell anemia from unripe fruit of *Carica papaya* L.

Materials and Methods

Unripe fruit of *Carica papaya* L. was collected from Kurkheda tehsil located in the Gadchiroli district during April 2023. The unripe fruit was identified and authenticate in the Department of Botany Nevjabai Hitkarini College, Bramahpuri. Unripe fruit was subsequently washed under tap water in laboratory for 5 min. The fruit was finely chopped into small pieces and air dried at room temperature until loss moisture. These dried pieces were crushed into powder by using electrical blender machine.

Preparation of extract

The protocol for preparation of the unripe fruit sample of *Carica papaya* L. for extraction was according to the standard maceration method (Chibuye et al., 2023) but with minor modifications. The powdered sample 2 gm was soaked in 20 ml methanol for 48 hours. The extract was filtered by Filtros^{TM}2001 qualitative circles, 1.18 mm in thickness, and the clear filtrate was sending for GC-MS in SAIF IITM Chennai.

GC-MS

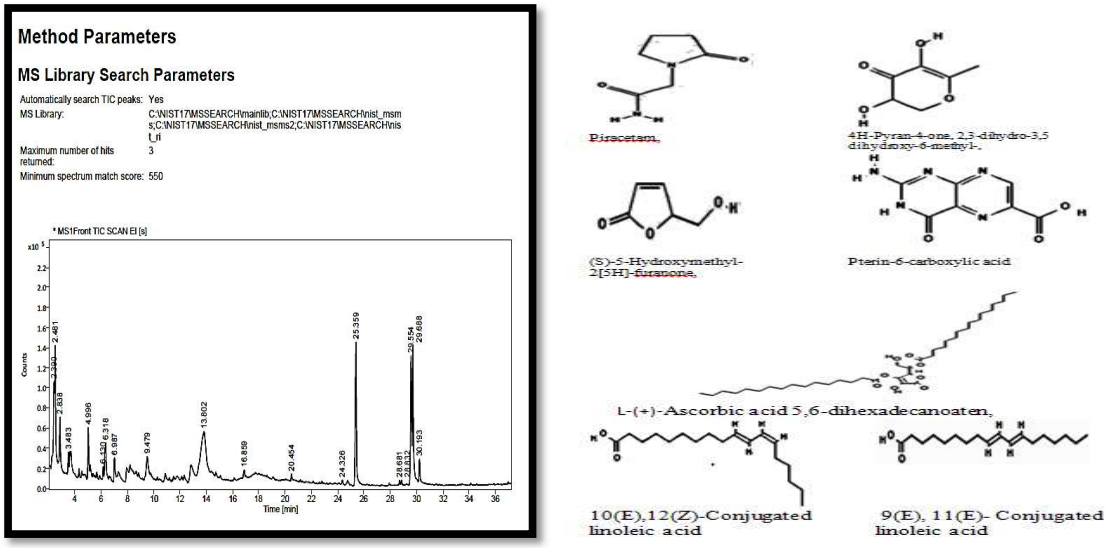
Agilent Model 8890 GC System with Single Quadrupole Mass Spectrometer (5977B MSD) analyser system available in the sophisticated analytical instrument facility of Indian institute of Technology Madras (SAIF IITM), Chennai used for the separation and identification of thermally stable volatile compounds. The SAIF IITM laboratory was provided spectral library and database. The data were processed with Agilent 8890 GC-MS System software Open Lab CDS 2.5 version by comparing the compound with database licensed National Institute of standard and technology (NIST 2017). Table no. 1 shows name compound, Retention time, CAS number, molecular weight, molecular formula, Peak Area percentage, Probability. The bioactive components for management of sickle cell disease were searched from Pubchem Data base, National Library of medicine data base, Drug Bank Data base, Searching research article.

Result and Discussion:

Gas chromatography-mass spectrometry (GC-MS) is powerful technique combining gas chromatography and mass spectrometry to identify and analyses compounds in a sample, particularly volatile ones. In the analysis of *Carica papaya* L., 54 compounds were identified as listed in Table No. 1. and The GC- MS chromatogram is presented mention in Fig.1. The GC-MS analysis of *Carica papaya* L. identifies 7 bioactive compound shows Fig. 2 like Piracetam, an organonitrogen nootropic compound chemically known as 2- (2-oxopyrrolidin-1 yl) acetamide, shares a structure similar to pyroglutamic acid, a derivative of the neurotransmitter gamma aminobutyric acid (GABA). It has been shown to enhance the viscosity of oxygenated sickle cells by modifying hemoglobin interactions, potentially reducing red blood cell sickling and improving polymerization dynamics (Toshio et al., 1981). The compound 4H-Pyran-4-one, 2-3-dihydroxy-6-methyl- has demonstrated antioxidant properties that could help reduce oxidative stress in sickle cell anemia. This stress, caused by the cyclical sickling and unsickling of red blood cells, contributes to endothelial dysfunction and inflammation (Xiangying et al., 2013). Hydroxymethyl-2[5H]-furanone, a derivative of 5 Hydroxymethyl-2-furfural (5HMF), shows potential as a therapeutic agent for sickle cell anemia. It may act as an "anti-sickling" agent by inhibiting the polymerization of deoxygenated sickle hemoglobin (HbS) helping to maintain the normal shape of red blood cells, improve blood flow, and reduce painful vasoocclusive crises (Xu et al., 2017). Pterin-6-carboxylic acid, a derivative product of folic acid, plays a role in restoring folate levels essential for proper erythropoiesis. In sickle cell disease (SCD), where red blood cells have a significantly shorter lifespan, folate stores are often depleted due to accelerated cell turnover. While folate supplementation is known to support hemoglobin levels and reticulocyte response in anemia, a 1983 study found no significant effects on hematological profiles or growth in children with SCD (Ndefo et al., 2008). L-(+)-Ascorbic acid 2,6-dihexadecanoate is a lipid-soluble derivative

of vitamin C, where two hexadecanoate fatty acid chains are esterified to the ascorbic acid molecule. This modification allows it to cross red blood cell membranes more easily than water-soluble ascorbic acid and results in slower metabolism, potentially offering longer-lasting effects. It has been shown to increase hemoglobin concentration and hematocrit in sickle cell anemia, while also improving iron absorption, utilization, and metabolism for hemoglobin synthesis in sickle cell patients. (Frederick et al., 2019). Both 10(E), 12(Z)-Conjugated linoleic acid and 9(E), 11(E)-Conjugated linoleic acid have shown potential in reducing inflammation, which plays a key role in pain crises in sickle cell anemia. These anti-inflammatory compounds may help in managing the disease (Yunkyoung et al., 2009; Putera, et al., 2023). Oxidative stress plays a key role in sickle cell disease (SCD), contributing to vascular damage, hemolysis and inflammation. While recent drug advancements have improved survival and reduced morbidity, further understanding of oxidative stress mechanisms could enable more targeted therapies. This study highlights several antioxidant bioactive compounds with potential benefits for sickle cell disease, including Thymine (Saragatsis and Pontiki, 2024), Linoelaidic acid (Natalia et al., 2008), Z-1, 9-Hexadecadiene (Doriane et al., 2008), and Octadecanoic acid (Sadhasivam et al., 2011).

Conclusion: The GC-MS result analysis *Carica papaya* L. shows effective bioactive compounds with potential therapeutic effects for sickle cell disease management. Some notable compounds found Piracetam, which may reduce red blood cell sickling, and 4H-Pyran-4-one, 2-3-dihydroxy-6-methyl-, known for its antioxidant properties. Some other compounds, such as, Hydroxymethyl-2[5H]-furanone and Pterin-6-carboxylic acid, may plays inhibit hemoglobin polymerization and restore folate levels. Additionally, the lipid-soluble Vitamin C derivative L-(+)-Ascorbic acid 2, 6-dihexadecanoate could improve hemoglobin and iron metabolism. Conjugated linoleic acids may help manage pain crises. This study also highlights several antioxidant compounds for further research, aiming at targeted therapies in Sickle cell



Disease management in future.

Fig. 2: Bioactive compound present in Carica papaya L. for use of management of sickle cell anemia

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Table 1: Phytochemical compound, retention time (Min.), CAS No. Molecular weight, Molecular formula, Peak Area, Peak Area (%).

Sr. No	Name of the compound	RT (Min.)	CAS #	Molecular weight	Molecular formula	Peak area	Peak Area (%)	Prob. %
1	Thiophene, tetrahydro-3-methyl-	2.39	4740-00-5	102.198	C5H10S	121196.399	3.47	16.57
2	2-Methyl-3-(methylthio)-1-propene		52326-10-0	102.198	C5H10S			14
3	Thiophene, tetrahydro-2- methyl-		1795-09-1	102.198	C5H10S			10.44
4	Nitric acid	2.481	7697-37-2	63.013	HNO3	224659.892	6.44	61.32
	Nitric acid		7697-37-2					
	Nitric acid		7697-37-2					61.32
5	6-Oxabicyclo[3.1.0]hexan-3-one	2.838	74017-10-0	98.1	C5H6O2	167989.997	4.82	28.3
6	Piracetam		7491-74-9					18.84
	Piracetam		7491-74-9	142.16	C6H10N2O			18.84
7	dl-Homoserine	3.483	1927-25-9	119.12	C4H9NO3	61296.323	1.76	13.17
8	3-(N'-Acetylhydrazinecarbonyl) propanoic acid			106.12	C3H10N2O			10.62
9	Succinamic acid		638-32-4	117.1	C4H7NO3			6.86
10	3-Acetylthymine	4.996		168.15	C7H8N2O3	185138.437	5.31	18.3
11	Thymine		65-71-4	126.11	C5H6N2O2			18.3
12	Cyclohexanamine, N-3-butenyl-N-methyl-		108144-20-3	167.29	C11H21N			8.33
13	3-Methyl-3-butene-1-thiol	6.13	58156-49-3	102.2	C5H10S	35611.611	1.02	50.25
14	2-Propanamine, Nmethyl-N-nitroso-		30533-08-5	102.14	C4H10N2O			15.32
15	3,3-Dimethylthietane		13188-85-7	102.2	C5H10S			13.54

				0				
16	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.318	28564-83-2	144.1253	C6H8O4	45810.299	1.31	90.8
17	2-Propyl-tetrahydropyran-3-ol			144.21	C8H16O2			1.74
18	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one		10230-62-3	144.12	C6H8O4			1.4
19	(S)-5-Hydroxymethyl-2[5H]-furanone	6.987	78508-96-0	100.073	C4H4O3	111127.633	3.19	40.64
20	6-Hydroxy-2,6-dihydropyran-3-one			114.10	C5H6O3			18.51
21	2H-Pyran, 3,4-dihydro-		110-87-2	84.12	C5H8O			5.04
22	5-(2-Hydroxyethyl)-4-methylthiazole	9.479	137-00-8	143.21	C6H9NOS	71701.947	2.06	37.12
23	1,3-Diazacyclooctane-2-thione		5269-85-2	144.24	C6H12N2S			4.67
24	1-Hexanamine, 6,Ndihydroxy-		104556-12-9	119.16	C5H13NO2			4.49
25	Sucrose	13.802	57-50-1	342.30	C12H22O11	638814.47	18.31	55.84
26	2-Deoxy-D-galactose		1949-89-9	164.16	C6H12O5			11.78
27	1,4,2,5 Cyclohexanetetrol		35652-37-0	148.16	C6H12O4			3.59
28	Benzenamine, 3-azido- N,N-dimethyl-4-nitro-	16.859	1437-65-6	207.19	C8H9N5O2	23572.963	0.68	52.15
29	Pterin-6-carboxylic acid		948-60-7	207.15	C7H5N5O3			15.06
30	1-(4-Methoxyphenyl)piperazine		38212-30-5	192.26	C11H16N2O			4.1
31	Methanone, [1-(5-fluoropentyl)-1Hbenzimidazol-2-yl]-1-naph	20.454		329.5	C21H28FNO	18789.604	0.54	15.06
32	Tetradecanoic acid		544-63-8	228.37	C14H28O2			10.63
33	Undecanoic acid		112-37-8	186.2	C11H22O			9.81

				9	2			
34	Pentanoic acid, 4-methyl-	24.326	646-07-1	116.16	C6H12O2	19647.076	0.56	12.28
35	2-(3-Methylguanidino)ethanol			117.15	C4H11N3O			5.59
36	Decanoic acid, 3-methyl-		60308-82-9	186.2912	C11H22O2			5.15
37	Hexadecanoic acid	25.359	57-10-3	255.42	C16H31O2-	780341.796	22.37	70.05
38	l-(+)-Ascorbic acid 2,6-dihexadecanoaten-		28474-90-0	652.9	C38H68O8			11.06
39	Pentadecanoic acid		1002-84-2	242.40	C15H30O2			6.03
40	Z-1,9-Hexadecadiene	28.681		222.41	C16H30	18744.584	0.54	8.18
41	Z-1,9-Dodecadiene			166.30	C12H22			5.28
42	Z-1,6-Undecadiene			152.28	C11H20			4.87
43	3-Nonyn-2-ol 664	28.832	26547-25-1	140.22	C9H16O	19048.043	0.55	6.48
44	4-Chloro-3-n-butyltetrahydropyran		35952-06-8	204.73	C11H21ClO			4.97
45	3-Tetradecyn-1-ol		55182-74-6	210.36	C14H26O			4.58
46	Linoelaidic acid	29.554	506-21-8	280.4	C18H32O2	403828.729	11.58	24.08
47	10(E),12(Z)-Conjugatedlinoleic acid		2420-56-6	280.4	C18H32O2			22.21
48	9(E),11(E)-Conjugatedlinoleic acid		544-71-8	280.45	C18H32O2			16.12
49	cis-9-Hexadecenal	29.688	56219-04-6	238.41	C16H30O	449570.206	12.89	18.82
50	7,11-Hexadecadienal			236.39	C16H28O			11.4

51	13-Octadecenal, (Z)-		58594-45-9	236.39	C16H28O			8.73
52	Octadecanoic acid	30.193	57-11-4	284.5	C18H36O2	91438.598	2.62	52.5
53	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester		106-11-6	372.58	C22H44O			33.91
54	i-Propyl 16-methylheptadecanoate			326.6	C21H42O2			3.32

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