



Original article

Phytochemical analysis of *Moringa Oleifera* leaves extracts by GC-MS and free radical scavenging potency for industrial applicationsNitesh Bhalla^{a,b,*}, Nitin Ingle^b, Srilakshmi V. Patri^a, D. Haranath^c^a Department of Chemistry, National Institute of Technology, Warangal 506004, Telangana, India^b IFFCO Group, Seville Products LLC, Plot 24, Street 3B, Umm Ramool, PO Box 10596, Dubai, United Arab Emirates^c Department of Physics, National Institute of Technology, Warangal 506004, Telangana, India

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ABSTRACT

Natural extracts have been of very high interest since ancient time due to their enormous medicinal use and researcher's attention have further gone up recently to explore their phytochemical compositions, properties, potential applications in the areas such as, cosmetics, foods etc. In this present study phytochemical analysis have been done on the aqueous and methanolic *Moringa* leaves extracts using Gas Chromatography-Mass spectrometry (GCMS) and their free radical scavenging potency (FRSP) studied using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical for further applications. GCMS analysis revealed an extraction of range of phytochemicals in aqueous and methanolic extracts. In aqueous, extract constituents found with high percent peak area are Carbonic acid, butyl 2-pentyl ester (20.64%), 2-Isopropoxyethyl propionate (16.87%), Butanedioic acid, 2-hydroxy-2-methyl-, (3.14%) (also known as Citramalic acid that has been rarely detected in plant extracts) and many other phytochemicals were detected. Similarly, fifty-four bio components detected in methanolic extract of *Moringa* leaves, which were relatively higher than the aqueous extract. Few major compounds found with high percent peak area are 1,3-Propanediol, 2-ethyl-2- (hydroxymethyl)- (21.19%), Propionic acid, 2-methyl-, octyl ester (15.02%), Ethanamine, N-ethyl-N-nitroso- (5.21%), and 9,12,15-Octadecatrienoic acid etc. FRSP for methanolic extract was also recorded much higher than aqueous extract. The half-maximal inhibitory concentration (IC₅₀) of *Moringa* aqueous extract observed is 4.65 μl/ml and for methanolic extract 1.83 μl/ml. These extracts can act as very powerful antioxidants, anti-inflammatory ingredient for various applications in diverse field of food, cosmetics, medicine etc.

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1. Introduction

Plants and extracts of their various sections have been used for their medical characteristics and to cure specific ailments as well as general tonics, meals, and other methods to increase the body's immunity and vigor since ancient times (Ullah et al., 2020; Ageel et al., 1986; Gamal et al., 2010; Purena et al., 2018). However, since last few decades the interest of researchers has gone up dramatically to understand their detailed compositions and also to explore

and establish their potential applications in diverse areas. In fact, it is the need of the hour to leverage the vital power of the nature to combat proliferating diseases like cancer, heart attacks, diabetes, rapid skin aging etc. and upcoming varieties of new alarming health concerns like recent concerns of Coronavirus disease in 2019 (COVID-19), which affects the respiratory system acutely (Varahachalam et al., 2021; Paliwal et al., 2020).

Different parts like seeds, roots, stem, bark, leaves, flower and fruits of the plant have their own phytochemical compositions and potential medicinal properties. *Moringa* have various species across the globe which are known for their variety of usages few examples of *Moringa* species are *Moringa longituba*, *Moringa drouhardii*, *Moringa ovalifolia* etc. (Leone et al., 2015). *Moringa Oleifera* is one of the magical plants considered in India due to its high medicinal properties. However, there is still a lot to unleash the potential of *Moringa Oleifera* by understanding their phytochemicals and variation in extraction due to solvents, understanding their potential properties and to establish their applications in var-

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ious fields. The present study is focused to investigate the phytochemical composition of the Moringa Oleifera leave's aqueous and alcoholic extracts by GC-MS and their free radical scavenging potencies, which make them useful for their further applications in cosmetic products to prevent skin damage due to free radicals generated because of pollution, UV from sunlight, smoke, also for animal feed etc.

2. Materials and methods

2.1. Materials

Methanol of 99.8% purity, HPLC grade from SD fine chemical limited, distilled water having pH 5.3–7 and conductivity 2µS/cm. Raw leaves powder of Moringa Oleifera of India origin grown in the climate of tropics and sub tropics were used for the investigation. Extra pure 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) from SRL Pvt. Ltd was used for the free radical scavenging studies.

2.2. Preparation of aqueous and Methanolic extracts

The suspension of 5% Moringa Oleifera leaves were prepared in distilled water and in Methanol separately and were subjected to

continuous stirring at 45–50 °C for 8 h respectively. The resultant samples were filtered through Whatman filter paper 1 to extract the phytochemicals. The filtrates were concentrated by evaporating the solvents and were used to prepare the samples for further analysis.

2.3. GC-MS analysis

The analysis of extracted phytochemicals of M. Oleifera leaves were done using GC-MS Agilent Technologies-7820A GC system. Gas Chromatogram coupled with Mass Spectrometer of Agilent Technologies-5977MSD equipped with an Agilent Technologies GCMS capillary column HP-5MS (30 m × 0.25 mm ID × 0.25µ) composed of 5% diphenyl 95% Dimethyl polysiloxane. An electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1 mL/min and an injection volume of 1 µl was employed at split ratio of 50:1, injector temperature was at 60 °C and ion source temperature was at 250°C. Mass spectra were recorded using voltage of 70 eV. The relative percentage amount of each component were calculated by comparing its average peak area to the total areas, software of GC-MS Mass Hunter used for spectra and chromatograms analysis.

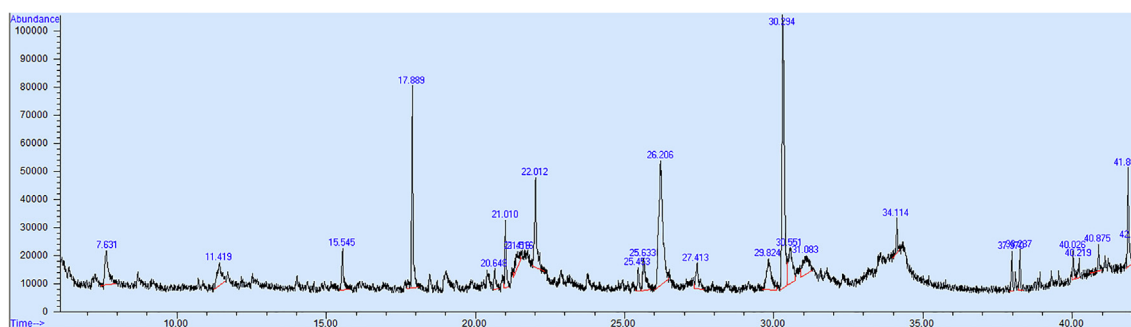


Fig. 1. GC MS of Moringa O. leaves aqueous extract.

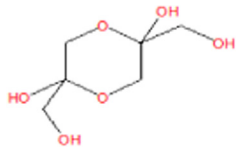
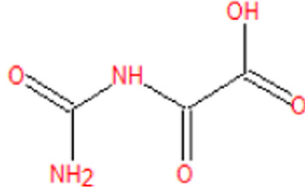
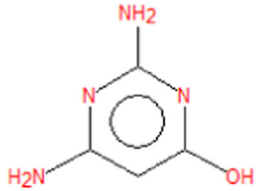
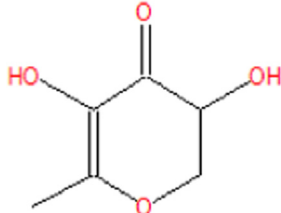

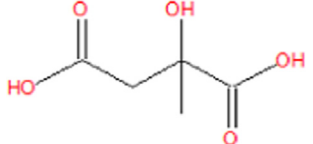
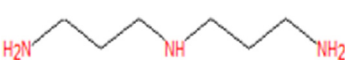

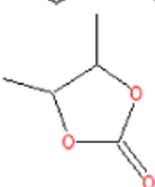
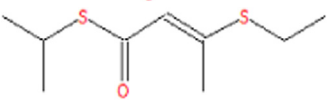
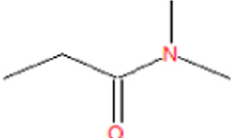
Table 1

List of phytochemicals identified in aqueous extract of Moringa O. leaves, their retention time and peak area% with molecular weight (grams/mole).

Sr. No.	RT	Peak Area %	Library/ID	Molecular Weight (g/mol)
1	7.63	3.8551	1,3-Dihydroxyacetone dimer	180
2	11.42	3.2396	Acetic acid, [(aminocarbonyl)amino]oxo-	132
3	15.54	2.2433	4(1H)-Pyrimidinone, 2,6-diamino-	126
4	17.89	8.9858	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144
5	20.65	1.1214	2-Hexynoic acid	112
6	21.01	3.1422	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	148
7	21.42	1.9275	3,3'-Iminobispropylamine	131
8	21.52	0.0774	1-Hexanamine	101
9	22.01	6.1627	1,3-Dioxolan-2-one, 4,5-dimethyl-	116
10	25.45	1.3083	2-Butenethioic acid, 3-(ethylthio)-, S-(1-methylethyl) ester	204
11	25.63	3.0349	Propanamide, N,N-dimethyl-	101
12	26.21	16.8738	2-Isopropoxyethyl propionate	160
13	27.41	2.5622	D-Mannoheptulose	210
14	29.82	4.6738	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	142
15	30.29	20.6431	Carbonic acid, butyl 2-pentyl ester	188
16	30.55	5.0379	Tetra acetyl-d-xylonic nitrile	343
17	31.08	3.445	.alpha.-D-Glucose	180
18	34.11	1.2587	1H-Cyclopenta[c]furan-3(3aH)-one, 6,6a-dihydro-1-(1,3-dioxolan-2-yl)-, (3aR,1-trans,6a-cis)-	196
19	37.97	1.5253	3-[1-(4-Cyano-1,2,3,4-tetrahydronaphthyl)]propanenitrile	210
20	38.24	1.4067	Quinolinium, 1-ethyl-, iodide	285
21	40.03	0.9462	N-Isopropyl-3-phenylpropanamide	191
22	40.22	0.7335	Propanamide	73
23	40.88	0.8805	1,2-Ethanediamine, N-(2-aminoethyl)-	103
24	41.87	4.3169	1,4-Benzenediol, 2-methyl-	124
25	42.05	0.5981	Ethene, ethoxy-	72

Table 2

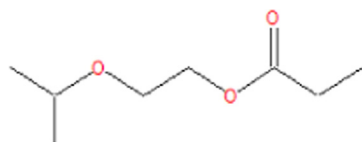
Phytochemicals in aqueous extract of Moringa O. leaves, their structure from NIST library and known potential applications wherever applicable.

Ingredients	Structure	Key Applications (if any readily available)
1,3-Dihydroxyacetone dimer		Synthesis of polymeric biomaterials (Zelikin and Putnam, 2005)
Acetic acid, [(aminocarbonyl) amino]oxo-		
4(1H)-Pyrimidinone, 2,6-diamino-		Hydroxy and amino Pyrimidines are of great interest in natural products and in the development of new drugs for various diverse areas like anti-cancer (Skoweranda et al., 1990)
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-		Strong Anti-oxidant (Yu et al., 2013; Čechovská et al., 2011)
2-Hexynoic acid		As precursor for synthesizing various biological metabolites like leucotrienes, oxylipins (Starostin et al., 2000)
Butanedioic acid, 2-hydroxy-2-methyl-, (S)-		One of the rarely identified component in plant extracts. Mobilize the Phosphates in soil for agricultural applications to enhance the P availability (Khorassani et al., 2011)
3,3'-Iminobispropylamine		This is also among the rare component identified in plant have various role in plant as well as medicinal antitumor active (Rodriguez-Garay et al., 1989; Sunkara et al., 1988; Nishio et al., 2019)
1-Hexanamine		
1,3-Dioxolan-2-one, 4,5-dimethyl-		Precursor for various drugs and cyclic Carbonates have applications in Electrochemical energies (Takebe et al., 1984; Hagiyaama et al., 2008)
2-Butenethioic acid, 3-(ethylthio)-, S-(1-methylethyl) ester		
Propanamide, N,N-dimethyl-		

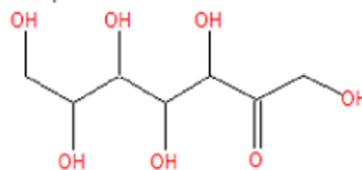
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Table 2 (continued)

2-Isopropoxyethyl propionate

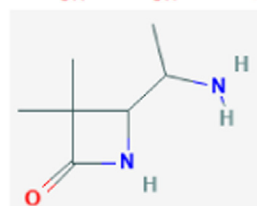


D-Mannoheptulose



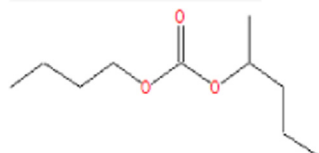
D-Mannoheptulose widely studied for its activity against breast cancer and to suppress the D-glucose induced insulin release. (Al-Ziaydi et al., 2020; Courtois et al., 2001)

Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-

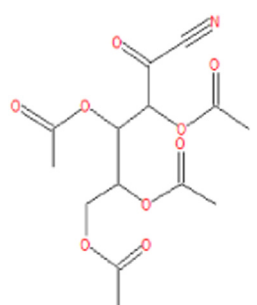


Anti-inflammatory, ulcerogenic, and analgesic activities (Siddiqui et al., 2010)

Carbonic acid, butyl 2-pentyl ester

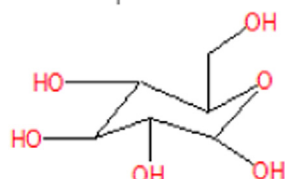


Tetra acetyl-d-xylic nitrile



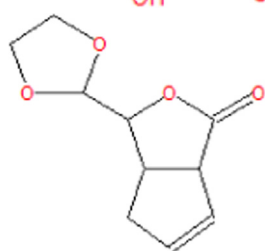
Anti-tumor and Anti-oxidant (Imad et al., 2015; Kanhar and Sahoo, 2018)

Alpha-D-Glucose



Source of energy, highly effective and used in food, medicine and its derivatives also have many medicinal use. (Shendurse and Khedkar, 2016; Cancelas et al., 2000)

1H-Cyclopenta[c]furan-3(3aH)-one, 6,6a-dihydro-1-(1,3-dioxolan-2-yl)-, (3aR,1-trans,6a-cis)-



3-[1-(4-Cyano-1,2,3,4-tetrahydronaphthyl)] propanenitrile

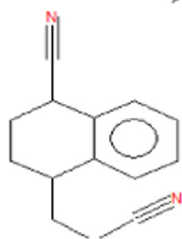
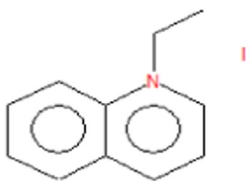
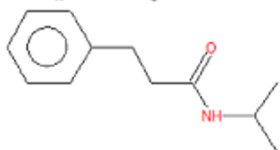
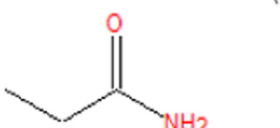
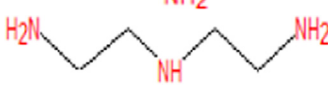
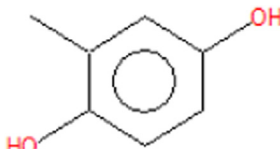
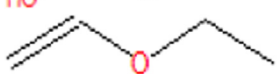


Table 2 (continued)

Quinolinium, 1-ethyl-, iodide		
N-Isopropyl-3-phenylpropanamide		
Propanamide		Propanamide derivatives studied for antimicrobial and antiviral efficacy (Ölgen et al., 2008)
1,2-Ethanediamine, N-(2-aminoethyl)-		
1,4-Benzenediol, 2-methyl-		
Ethene, ethoxy-		

2.4. Phytochemicals identification

The phytochemicals were identified based on their retention time, percentage of peak area and pattern of mass spectra and its comparison with the data of library of NIST11.LIB of National Institute of Standards and Technology (NIST).

2.5. Anti-oxidant/free radical scavenging potency (FRSP) using diphenyl picrylhydrazyl (DPPH)

The Anti-oxidant efficacy and in other words free radical scavenging potency (FRSP) of the aqueous and methanolic extract of the Moringa leaves powder were determined by using DPPH. Methanolic solution of 1.52×10^{-4} M of DPPH was used in 1:1 ratio with Moringa aqueous and methanolic extracts at varying concentrations, studied the scavenging activity as a function of time. FRSP percentage rate was studied after incubating the mixture of DPPH and extracts for 30 min and recording the UV absorption at 517 nm. The same was used to calculate the concentration of antioxidant required to reduce the concentration of DPPH to 50% (IC_{50}). Higher value of IC_{50} indicates the lower antioxidant activity

and vice versa. The control samples were prepared using DPPH solution and mixing only with respective solvents (Aqua/Methanol) at 1:1 ratio and measured at 517 nm.

$$FRSP\% = (\text{Control}_{\text{abs}} - \text{Sample}_{\text{abs}}) \times 100 / \text{Control}_{\text{abs}}$$

3. Results

3.1. Phytochemical identification by GC MS of aqueous extract of *M. Oleifera* leaves powder

The GC-MS profile of the aqueous extract of *M. Oleifera* leaves is shown in Fig. 1, which reflects 25 peaks of biomolecules. Table 1 presents the phytochemicals, their retention time, peak area percentage and Molecular weight. Chemical structure of active components and their known key applications like medicinal, cosmetics etc. are tabulated in Table 2. Few major compounds found with high percent peak area are Carbonic acid, butyl 2-pentyl ester (20.64%), 2-Isopropoxyethyl propionate (16.87%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (8.98%), and 1,3-Dioxolan-2-one, 4,5-dimethyl- (6.16%) additional compounds with reasonable

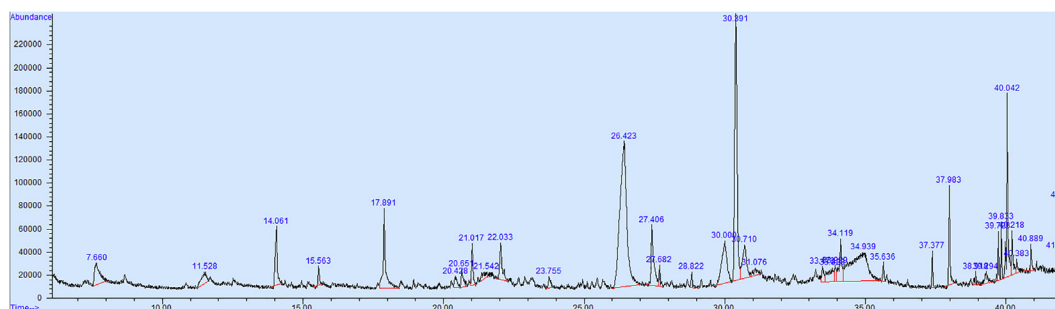


Fig. 2. GC MS of Moringa O. leaves Methanolic extract.

Table 3

List of phytochemicals identified in methanolic extract of Moringa O. leaves, their retention time and peak area% with molecular weight (grams/mole).

Sr.No	RT	Peak Area %	Library/ID	Molecular Weight (g/mol)
1	7.66	2.4651	Dihydroxyacetone	90
2	11.5285	1.8656	Glycerin	92
3	11.7064	0.5327	Erythritol	122
4	14.0612	2.5684	Monomethyl malonate	118
5	15.5626	0.6434	4,5-Diamino-6-hydroxypyrimidine	126
6	17.8911	4.1801	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144
7	18.9343	0.2105	Furan, 2,3-dihydro-4-methyl-	84
8	20.4278	0.6806	Catecholborane	120
9	20.6515	0.8121	2-Fluoropyridine	97
10	21.017	1.4375	1,2,3-Propanetriol, 1-acetate	134
11	21.2273	0.1746	3,4-Furandiol, tetrahydro-, trans-	104
12	21.4101	0.5114	1-Nitro-.beta.-d-arabinofuranose, tetraacetate	363
13	21.5424	0.1172	1,8-Diamino-3,6-dioxoactane	148
14	22.033	1.7997	1,7-Diaminoheptane	130
15	22.6606	0.454	N,N-Dimethylacetamide	131
16	22.8934	0.5466	2-Oxoglutaric acid	146
17	23.1495	0.9008	Oxazolidine, 2-ethyl-2-methyl-	115
18	23.7541	0.7112	Heptanal	114
19	25.4554	0.4293	6-Methoxy-3-pyridazinethiol	142
20	25.6675	0.5971	3-Piperidinol	101
21	26.4192	21.1909	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	134
22	27.4032	3.4763	Benzeneacetonitrile, 4-hydroxy-	133
23	27.6817	0.395	Benzenebutanal, .gamma.,4-dimethyl-	176
24	28.8207	0.4491	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	180
25	30.0026	5.2161	Ethanamine, N-ethyl-N-nitroso-	102
26	30.3938	15.0279	Propanoic acid, 2-methyl-, octyl ester	200
27	30.7101	3.2947	3-Deoxy-d-mannonic lactone	162
28	31.0768	0.3814	d-Glycero-d-ido-heptose	210
29	31.1206	0.3314	D-erythro-Pentose, 2-deoxy-	134
30	32.4351	0.5345	N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide	273
31	33.2282	0.5847	Formamide, N,N-dimethyl-	73
32	33.4717	0.5651	d-Talonic acid lactone	178
33	33.7365	0.482	Sorbitol	182
34	33.9282	0.5189	Allo-Inositol	180
35	34.1181	1.595	D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl)amino)-2,3,4,6-tetra-deoxy-.alpha.-D-arabino-hexopyranosyl)-	379
36	34.3555	1.1254	Allo-Inositol	180
37	34.5368	1.1121	Scyllo-Inositol	180
38	34.7792	2.0264	Muco-Inositol	180
39	34.8409	0.8749	Allo-Inositol	180
40	34.8998	2.0545	Inositol	180
41	35.6364	0.4822	Cyclohexane, 1-methyl-4-(2-hydroxyethyl)-	142
42	37.3767	0.8519	Hexadecanoic acid, methyl ester	270
43	37.9829	2.5703	n-Hexadecanoic acid	256
44	38.9185	0.3737	Phenol, 2-methyl-	108
45	39.2935	0.967	(1S)-Propanol, (2S)-[(tert.butylloxycarbonyl)amino]-1-phenyl-	251
46	39.7227	1.0307	9-Octadecenoic acid (Z)-, methyl ester	296
47	39.8325	0.9664	Phytol	296
48	40.0418	5.0063	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278
49	40.2184	1.2051	Octadecanoic acid	284
50	40.3832	0.4257	4-Allyl-3-(dimethylhydrazono)-2-methylhexane-2,5-diol	228
51	40.8886	0.6056	Benzyl .beta.-d-glucoside	270
52	41.0816	0.1698	4,6-dimethyl-2-propyl-1,3,5-dithiazinane	191
53	41.8877	0.545	1,3-Benzenediol, 2-methyl-	124
54	42.0626	1.4694	9-Octadecenamide, (Z)-	281

percentage of peak area are Tetra acetyl-d-xylonic nitrile (5.03%), Azetid-2-one 3,3-dimethyl-4-(1-aminoethyl)- (4.67%), 1,3-Dihydroxyacetone dimer (3.85%), Alpha-D-Glucose (3.44%), and Butanedioic acid, 2-hydroxy-2-methyl-, (3.14%) which is rarely been detected in plant extracts and have various applications.

3.2. Phytocomponent identification by GC-MS of Methanolic extract of *M. Oleifera* leaves

The GC-MS profile of the methanolic extract of *M. Oleifera* leaves is shown in Fig. 2, which reflects 54 peaks of biomolecules. Table 3 presents the phytocomponents, their retention time, peak area percentage and Molecular weight. Chemical structure of active components and their known key applications like medicinal, cosmetics etc. are tabulated in Table 4. Few major compounds

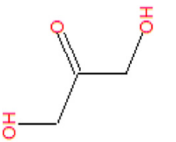
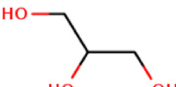
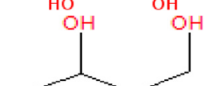
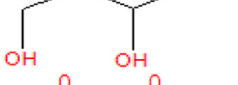
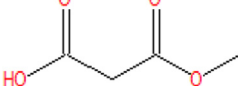
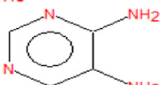
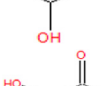
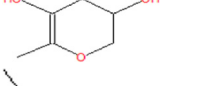

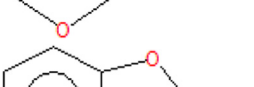
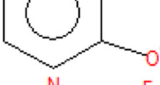
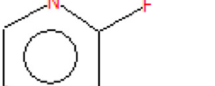
found with high percent peak area are 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- (21.19%), Propionic acid, 2-methyl-, octyl ester (15.02%), Ethanamine, N-ethyl-N-nitroso- (5.21%), and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (5.00%) additional compounds with reasonable percentage of peak area are 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (4.18%), Benzeneacetonitrile, 4-hydroxy- (3.47%), 3-Deoxy-d-mannonic lactone (3.29%), n-Hexadecanoic acid (2.57%) and Monomethyl malonate (2.56%).

3.3. Free radical scavenging efficacy of aqueous and Methanolic extracts of *M. Oleifera* leaves at varying concentrations:

The aqueous extract of Moringa leaves have the IC₅₀ at concentration of 4.65 µl/ml after incubating for 30 min Fig. 3 whereas the IC₅₀ of Methanolic extract was found 1.83 µl/ml Fig. 4 which is sig-

Table 4

Phytochemicals in methanolic extract of Moringa O. leaves, their structure from NIST library and known potential applications wherever applicable.

Ingredients	Structure	Key applications (if any readily known)
Dihydroxyacetone		Dihydroxyacetone (DHA) are being used in Sunless tanning type of products. (Huang et al., 2017)
Glycerin		Used as humectant, Moisturizer having application in cosmetics and Medicines (Sagiv et al., 2001)
Erythritol		Antioxidant Improve blood vessel function in people with type 2 diabetes (den Hartog et al., 2010)
Monomethyl malonate		
4,5-Diamino-6-hydroxypyrimidine		Analogues are used for various Medicinal properties like antimicrobial (Abbas et al., 2017)
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-		Strong Anti-oxidant (Yu et al., 2013; Čechovská et al., 2011)
Furan, 2,3-dihydro-4-methyl-		
Catecholborane		Versatile compound for various organic synthesis (Brown and West, 2001; Paquette et al., 2009)
2-Fluoropyridine		
1,2,3-Propanetriol, 1-acetate		
3,4-Furandiol, tetrahydro-, trans-		
1-Nitro-.beta.-d-arabinofuranose, tetraacetate		

(continued on next page)

Table 4 (continued)

Ingredients	Structure	Key applications (if any readily known)
1,8-Diamino-3,6-dioxaoctane		
1,7-Diaminoheptane		Malaria treatment (Kaiser et al., 2001)
N,N-Dimethylacetamide		Novel antiviral agent (He et al., 2005)
2-Oxoglutaric acid		Biosynthesis of Carotenoids in Chloroplasts (Liu et al., 2018)
Oxazolidine, 2-ethyl-2-methyl-		
Heptanal		
6-Methoxy-3-pyridazinethiol		
3-Piperidinol		Anti-tuberculosis agent (Markad et al., 2015)
1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-		
Benzeneacetonitrile, 4-hydroxy-		
Benzenebutanal, gamma,4-dimethyl-		
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-		Anti arthritic activity (Rhew et al., 2020)
Ethanamine, N-ethyl-N-nitroso-		Boost immune system. (Zaitseva et al., 2018)
Propanoic acid, 2-methyl-, octyl ester		Anti-Microbial properties (Hamed et al., 2019)

Table 4 (continued)

Ingredients	Structure	Key applications (if any readily known)
3-Deoxy-d-mannonic lactone		
d-Glycero-d-ido-heptose		Inhibition of insulin secretion & Hexokinase (Scruel et al., 1998)
D-erythro-Pentose, 2-deoxy-		
N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide		
Formamide, N,N-dimethyl-		
d-Talonic acid lactone		
Sorbitol		Help to prevent hyperglycemia (Wick et al., 1951) Protect and rejuvenation of skin from oxidative stress (Manca et al., 2018)
Allo-Inositol		Control the il6 level to reduce the inflammation (Bizzarri et al., 2020)
D-chiro-Inositol, 3-O-(2-amino-4-((carboxymethylamino)-2,3,4,6-tetra-deoxy-alpha.-D-arabino-hexopyranosyl)-		Type 2 Diabetes Treatment (Pintaudi et al., 2016)
Allo-Inositol		Control the il6 level to reduce the inflammation (Bizzarri et al., 2020)
Scyllo-Inositol		Treat mild to moderate Alzheimer's disease. (Choi et al., 2010)
Muco-Inositol		Oligomer of muco-inositol act as glycosidase inhibitors (Freeman and Hudlicky, 2004)

(continued on next page)

Table 4 (continued)

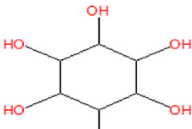
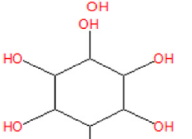
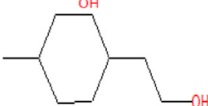

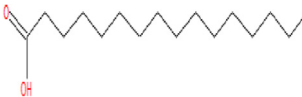
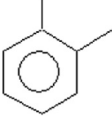
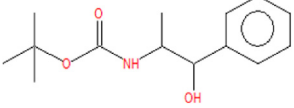
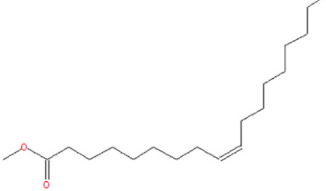

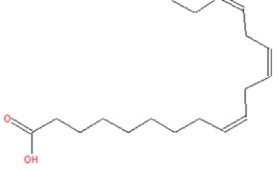
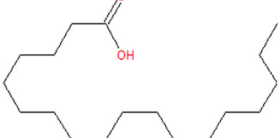
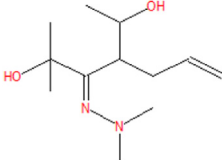
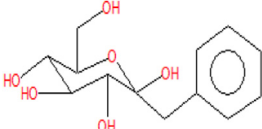
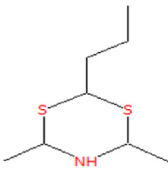
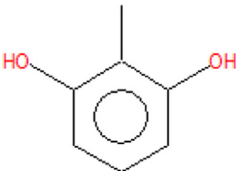
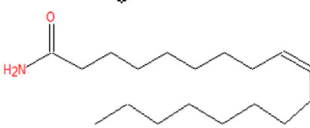
Ingredients	Structure	Key applications (if any readily known)
Allo-Inositol		Control the il6 level to reduce the inflammation (Bizzarri et al., 2020)
Inositol		Inositol and its derivative are known for treatment of Polycystic ovary syndrome (PCOS) (Kamenov and Gateva; Mariano and Gianfranco, 2014)
Cyclohexane, 1-methyl-4-(2-hydroxyethyl)-		
Hexadecanoic acid, methyl ester		Anti-inflammatory (Saeed et al., 2012)
n-Hexadecanoic acid (palmitic acid)		Anti-inflammatory (Aparna et al., 2012)
Phenol, 2-methyl-		
(1S)-Propanol, (2S)-[(tert.butylloxycarbonyl)amino]-1-phenyl-		Human Immunodeficiency virus -1 (HIV-1) Protease Inhibitors (Ghosh et al., 2015)
9-Octadecenoic acid (Z)-, methyl ester		Lubricant (Faujdar and Singh, 2021)
Phytol		Control Ganoderma boninense (Plant disease) (ABDUL AZIZ,) Antioxidant, Anti-inflammatory (Islam et al., 2018)
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-		Reduce complications in Covid-19 patients. (Weill et al., 2020) Neuroprotective Properties. (Blondeau et al., 2015)
Octadecanoic acid		Play role in food reward (Li et al., 2020) Lowers High density lipoprotein (HDL) cholesterol (van Rooijen et al., 2021)
4-Allyl-3-(dimethylhydrazono)-2-methylhexane-2,5-diol		
Benzyl .beta.-d-glucoside		

Table 4 (continued)

Ingredients	Structure	Key applications (if any readily known)
4,6-dimethyl-2-propyl-1,3,5-dithiazinane		
1,3-Benzenediol, 2-methyl-		
9-Octadecenamide, (Z)-		Anti-depressive effects (Ge et al., 2015)

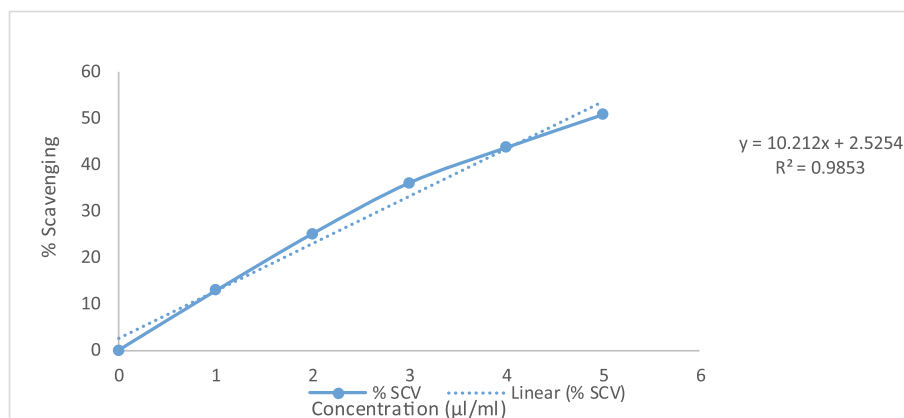


Fig. 3. IC₅₀ of Moringa O. leaves aqueous extract.

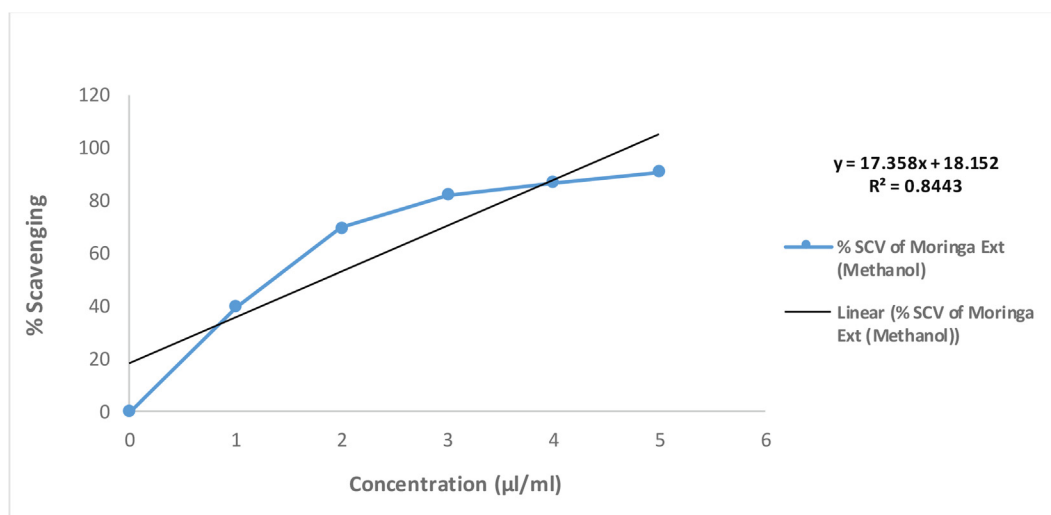


Fig. 4. IC₅₀ of Moringa O. leaves Methanolic extract.

nificantly lower than the IC₅₀ of Moringa aqueous extract. Various concentrations (1 µl/ml to 5 µl/ml) of Moringa aqueous and Methanolic extracts were also evaluated and compared at the

defined interval of incubation time up to 160 min to identify the maximum FRSP for each concentration and the results are shown in Fig. 5.

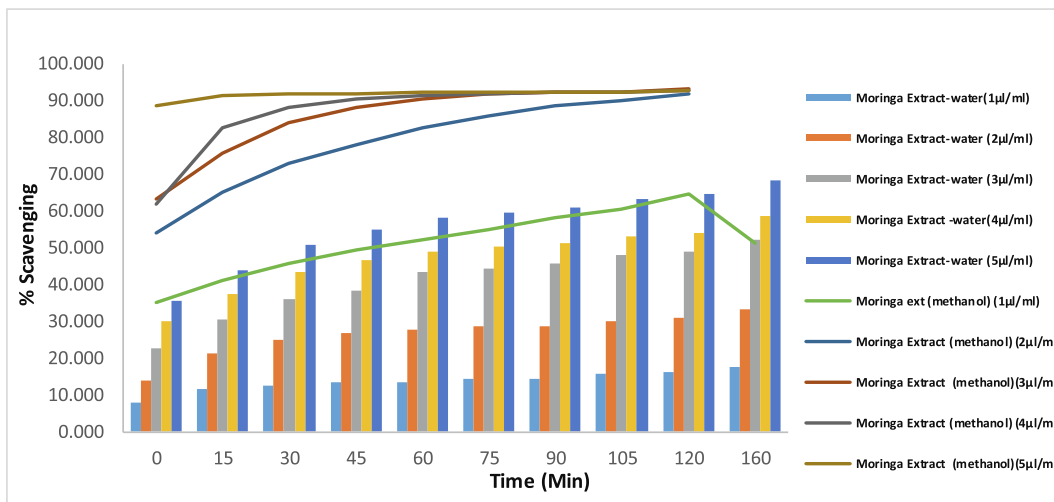


Fig. 5. Free radical scavenging potency (FRSP) comparison of Aqueous and Methanolic extract of varying concentration at various incubation time points.

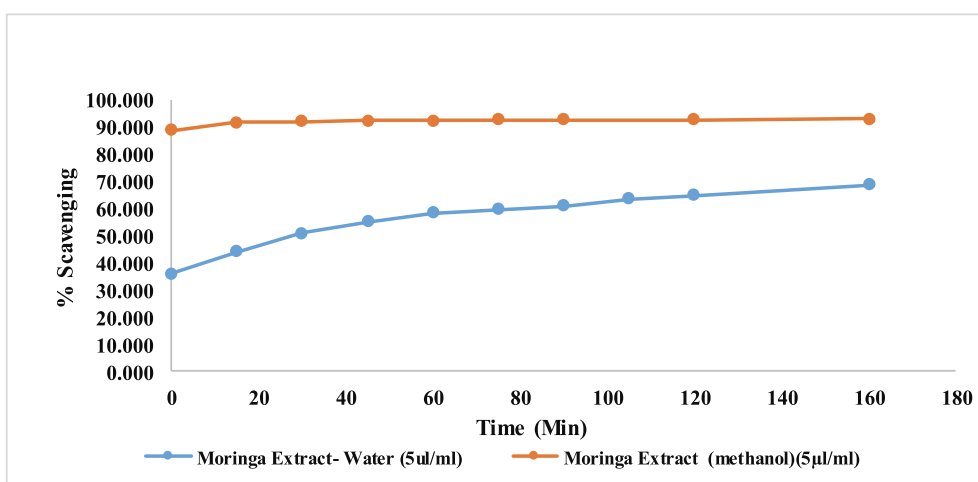


Fig. 6. Free radical scavenging potency (FRSP) comparison of Aqueous and Methanolic extract of 5 µl/ml concentration at various incubation time points.

4. Discussion

This study is among the very few reports where the phytochemical profile of Moringa extracts with their free radical scavenging potency studies have been reported. In this study, higher number of phytochemicals in methanolic extract (Table 3) of Moringa extract in comparison to aqueous (Table 1) could be due to difference in their polarity, which could have led to difference in the extraction of phytochemicals. These, phytochemicals have various industrial applications and medicinal properties like anti-tumor, anti-cancer, Insulin regulation, anti-oxidant etc. which makes it really a magical plant for food, medicine and suitable natural ingredient to explore its further applications in diverse areas like in cosmetics, personal care products etc.

Natural antioxidants are always of very high importance for health as a part of food and as a part of cosmetics for topical applications to combat the detrimental affects of free radicals on internal and external organs like skin aging etc. Both aqueous and methanolic Moringa extracts have excellent anti-oxidant/FRSP. However, in relative terms Methanolic extract found to have lower IC₅₀ (1.83 µl/ml) Fig. 4 reflecting higher free radical scavenging ability in comparison to of aqueous extract having higher IC₅₀

(4.65 µl/ml) Fig. 3. This could be mainly due to higher number of polyphenolic component extracted in Methanolic extracts and relatively at higher percentages, which is contributing towards higher scavenging potency at lower concentration. It has been found that Methanolic extract at 5 µl/ml gives the 88.5% FRSP and does not change significantly over the period of time and reaches maximum level of 92.8% FRSP. Whereas aqueous extract at 5 µl/ml show 35.8% FRSP at the initial time point and which further scavenge the DPPH free radical significantly up to 68.4% in 160 min Fig. 6, however still less FRSP of Moringa Aqueous extract than the Methanolic extract could be due to the same reason as explained above of having more polyphenolic components in Methanolic extract of Moringa as reflected in GC-MS results.

5. Conclusions

This study has investigated the Moringa aqueous extract and identified twenty-five phytochemicals and similarly in Methanolic extract fifty-four phytochemical components were identified. The higher number of these constituents in methanol may be due to polarity difference, which contributed to solubilize the various molecules. Few of phytochemicals reported here are rarely

detected in plant extracts like 3,3'-Iminobispropylamine, Butanedioic acid, 2-hydroxy-2-methyl- etc. and have potential applications. In further studies these extracts have shown substantial free radical scavenging potency, IC₅₀ of Moringa aqueous extract observed is 4.65 µl/ml and for methanolic extract 1.83 µl/ml. Also, found that Methanolic extract at 5 µl gives the 88.5% FRSP at initial time point and does not change significantly over the period of time and reaches maximum level of 92.8% FRSP. Whereas aqueous extract at 5 µl show 35.8% FRSP at the initial time point and which further scavenge the DPPH free radical significantly up to 68.4% in 160 min. These high FRSP make them very suitable ingredients for various applications like food, animal feed medicines, cosmetic etc. We are in the process of establishing the same in products of topical application to have skin benefits like prevention of skin damage, aging etc.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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