Original Article

Therapeutic potential of water treated by *Moringa oleifera* leaves

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Abstract

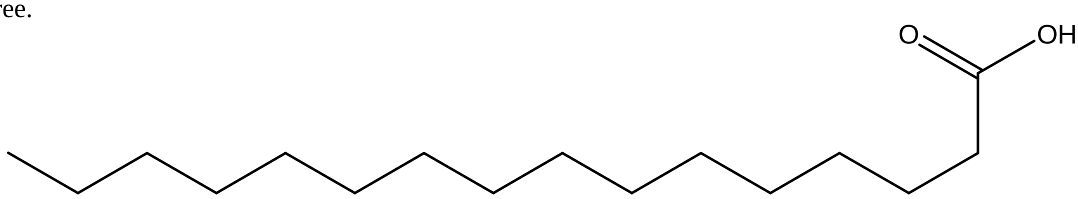
Water is the universal solvent, of course it cannot dissolve everything, but it does dissolve more substances than any other liquid. Water molecules have a polar arrangement of oxygen and hydrogen atoms—one side (hydrogen) has a positive electrical charge and the other side (oxygen) had a negative charge. This allows the water molecule to become attracted to many other different types of molecules**,** due to this attribute it can dissolve many phytochemical components from medicinal plants even at normal room temperature. Moringa, a well known plant have rich source of vitamin and protein , it also have pharmacological properties to fight against numerous diseases such as anticancer, hypotensive, antimicrobial, anti-inflammatory, antidiabetic, antihelmentic and many more but due to its acrid taste people avoid it·s consumption it diet The aim of present study was to screen and to identify useful phytoconstituents present in MLTW. GC-MS chromatogram showed the presence of more than 60 different phytochemical compounds. The active fraction of the MLTW was identified as 1,2,3-Benzenetriol or pyrogallol (antimalarial), N-hexadenoic acid (Cytotoxic) and many more compounds was found to be present in Moringa leaves treated water (MLTW),which may serve as bioactive agent for the humans as well as animals.

Keywords: Moringa Oleifera, Moringa leaves treated water (MLTW) ; Pyrogallol; GC-MS chromatogram.

1.0 INTRODUCTION

Plants always play a vital role for mankind irrespective of the era and area. *Moringa oleifera* is the most nutrient-rich plant on earth. Apart from nutritional uses, Moringa is used to boost immunity and possesses anti-tumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antihypertensive, cholesterol-lowering, and anti-diabetic activity. Due to lack of awareness about the nutritional benefits of Moringa plant and its unpalatable taste it restricts the general public especially the rural areas of Uttar Pradesh, to use against Malnutrition and disease management.

The current research focuses incorporation of bioactive component of Moringa leaves in drinking water without imparting bitter and acrid taste so general public can use this water without hesitation and get maximum benefits of this Miracle tree.

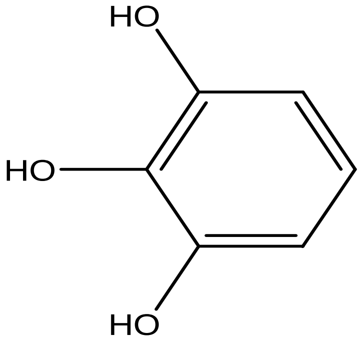


N-hexadecanoic acid

Figure 1

The N-hexadecanoic acid is a common fatty acid, found in plants [1]. Harada *et al.* [2] has reported that N-hexadecanoic acidselectively inhibit DNA topoisomerase-I and thereby prevents the proliferation of human fibroblast cells.
Hence, the proposed mechanism of cytotoxic activity of N-hexadecanoic acid is due to its interaction with DNA topoisomerase-I and thereby prevents proliferation of cells.

Pyrogallol also known as pyrogallic acid or 1,2,3-trihydroxybenzene (Figure 2), an organic phenol compound that exists naturally in many plants such as *oak*, *eucalyptus*, *Terminalia chebula* and *Myriophyllum spicatum* [3-5]. People are naturally exposed to pyrogallol through several ways including consumption of tea, use of hair colouring creams, inhaling smoke produced during cooking mutton, fish and so on [6].



1,2,3-trihydroxybenzene or Pyrogallol

Figure 2

Pyrogallol was found for the first time in the natural extract of the seeds of *Abrus precatorius*[7].
Due to the problem of drug resistance, numerous researches have been conducted on the field of herbal medicine to discover new effective antimalarial drugs [8]

Pyrogallol possesses antibacterial, antipsoriatic, antifungal and oxidative properties. It generates free radicals such as molecular oxygen (O_2), peroxide nitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), and hydroxyradical (OH^-) by the Haber-Weiss reaction Inui et al.

Pyrogallol has a capability of generating free radicals [9,10], which may cause the inhibition of parasite growth. However, the pyrogallol-related toxicity remains alarge gap that needs further studies.
This study aims to explore and analyze the chemical constituents of Moringa leaves treated water (MLTW). In the given study Moringa leaves treated water (MLTW) was analyzed for identification of phytoconstituents by Gas Chromatography-Mass Spectrometry (GC-MS). The major biologically active compounds obtained from Moringa leaves treated water (MLTW) can be used for their Cytotoxic potential and anti-Malarial activity.

2.0 MATERIALS AND METHODS

2.1 Procurement and verification of plant materials

In December 2022, *Moringa Oleifera* Lam [Moringaceae] leaves were obtained from Shri Ramswaroop Memorial University Campus, Tindola, Lucknow-Deva Road, Barabanki, Uttar Pradesh, India. The sample of leaves was authenticated from CIMAP, Lucknow, India-226026. (Reference no. CISIR/CIMAP/Moringa0254).

2.3 Equipment

Borosilicate beaker of suitable size is used as basic equipment.

2.4 Methodology

In this process, the freshly collected leaves (about 5gm.) of *Moringa oleifera* were cleaned and washed with 20% w/v sodium chloride solution and was soaked in 150 ml. of distilled water. The sampling was done using disposable sterile hand gloves. The sample was kept for overnight from 8pm to 6am. After that 20 ml of MLTW was collected in a standard sterilizes container before sending it to analysis. Pre and post experimental analysis of water have been done according to international standards from an ISO certified lab. The quality of water was assessed on the following parameters;

1. Physical Parameters :

Colour
Odour
Taste
Turbidity
Total Dissolved solids
pH

Table 1: Pre and Post Experimental analysis of water

Parameters	At 8pm	At 6am	WHO guidelines
pH	6.53	7.2	6.5-8.5
Appearance	Colourless	Colourless	Colourless
Odour	Odorless	Odorless	Odorless
Taste	Tasteless	Tasteless	Tasteless
Turbidity (NTU)	2.5	4.7	5
Total Dissolved solids	235	355	1000

2.5 GC-MS analysis of Phytoconstituents

Moringa leaves treated water (MLTW) of *Moringa oleifera* leaves was examined using Gas Chromatography-Mass Spectroscopy (GC-MS). The (GC-MS) instrument was equipped with Shimadzu QP-2010 Ultra with capillary standard, a non-polar column 60 M TRX 5-MS. Helium gas was employed as vehicle gas with a mobile phase flow rate kept at 1.25 ml min^{-1} [10].

3.0 RESULTS AND DISCUSSION

3.1 Identification of Phytocomponent by GC-MS of aqueous extract of *M. Oleifera* leaves

The GC-MS profile of the Moringa leaves treated water (MLTW) of *M. Oleifera* leaves is shown in Figure 3, which reflects more than 60 peaks of biomolecules. They present the phytocomponents, their retention time, peak area percentage and Molecular weight. The chemical name of active components are tabulated in Table 2.

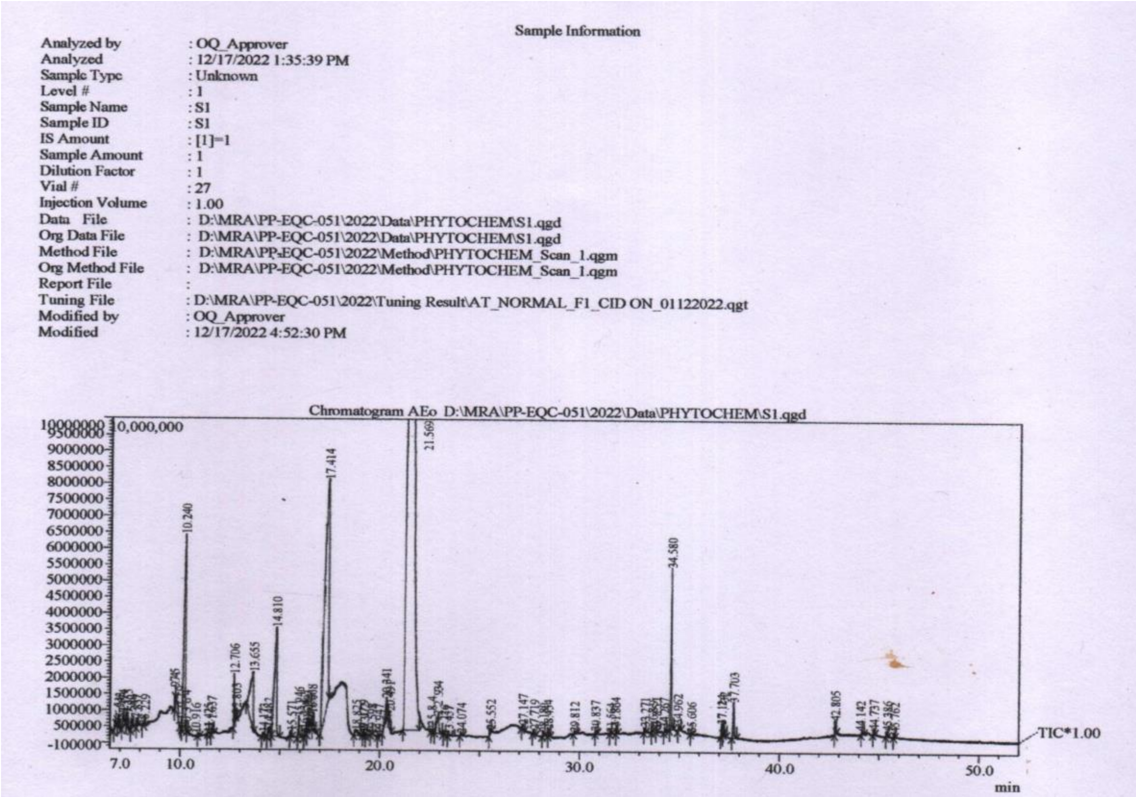


Figure 3. GC MS of *M. oleifera* leaves aqueous extract

Table 2: Phytoconstituents identified in MLTW through GC-MS analysis

Peak#	R. Time	F. Time	Area	Area%	Height	Height%	A/H	Name
1	6.751	6.805	775190	0.13	215742	0.42	3.59	Furan, tetrahydro-2,5-dimethoxy-
2	6.943	7.020	1629824	0.27	388547	0.76	4.19	Furan, tetrahydro-2,5-dimethoxy-
3	7.174	7.295	1928377	0.32	439177	0.86	4.39	Butanoic acid, 2-ethyl-, methyl ester
4	7.355	7.430	1562234	0.26	424176	0.83	3.68	Furan, tetrahydro-2,5-dimethoxy-
5	7.580	7.670	1246681	0.21	327999	0.64	3.80	L-Lactic acid
6	7.837	7.915	800274	0.13	271335	0.53	2.95	2(5H)-Furanone
7	8.229	8.275	443765	0.07	210699	0.41	2.11	1,2-Cyclopentanedione
8	9.745	9.770	458817	0.08	212881	0.42	2.16	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on
9	10.029	10.080	166370	0.03	67038	0.13	2.48	
10	10.240	10.320	35137190	5.79	6065999	11.83	5.79	2H-Pyran-2,6(3H)-dione
11	10.364	10.415	132409	0.02	59944	0.12	2.21	Oxazolidine, 2,2-diethyl-3-methyl-
12	10.916	10.985	197986	0.03	79282	0.15	2.50	2,5-Furandione, dihydro-3-methylene-
13	11.425	11.465	107322	0.02	45577	0.09	2.35	Cyclotrisiloxane, hexamethyl-
14	11.637	11.705	853494	0.14	278199	0.54	3.07	Butanedioic acid, monopropargyl ester
15	12.706	12.780	4242585	0.70	1537737	3.00	2.76	Furyl hydroxymethyl ketone
16	12.803	12.850	255459	0.04	129662	0.25	1.97	2,4,5-Trihydroxypyrimidine
17	13.655	13.690	17694199	2.92	1757469	3.43	10.07	2-Furancarboxylic acid
18	14.173	14.265	597790	0.10	144319	0.28	4.14	Aluminum, triethyl-
19	14.482	14.570	1679063	0.28	289378	0.56	5.80	Ethanamine, N-ethyl-N-nitroso-
20	14.810	15.000	27674543	4.56	3377958	6.59	8.19	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6
21	15.571	15.630	687580	0.11	193154	0.38	3.56	Benzoic acid
22	15.946	16.095	2819918	0.46	674884	1.32	4.18	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-
23	16.234	16.315	793827	0.13	237142	0.46	3.35	2-Pentanol, propanoate
24	16.393	16.470	1604660	0.26	368396	0.72	4.36	Catechol
25	16.499	16.560	172872	0.03	70246	0.14	2.46	1-Methyl-2-piperidinemethanol

26	16.668	16.815	2195863	0.36	535786	1.04	4.10	Dianhydromannitol
27	17.414	17.470	106915253	17.63	6877949	13.41	15.54	5-Hydroxymethylfurfural
28	18.825	19.040	1668519	0.28	177572	0.35	9.40	Methyl 3-hydroxytetradecanoate
29	19.179	19.250	245478	0.04	92722	0.18	2.65	5-Acetoxymethyl-2-furaldehyde
30	19.312	19.365	119192	0.02	43817	0.09	2.72	1,2-Benzenedicarboxylic acid
31	19.598	19.645	118433	0.02	29470	0.06	4.02	3-(5,5,6-Trimethylbicyclo[2.2.1]heptan-2-yl)c
32	19.947	20.005	232415	0.04	68897	0.13	3.37	2-Methyl-1-di(tert-butyl)silyloxypropane
33	20.341	20.410	3890393	0.64	699164	1.36	5.56	Pivaloin
34	20.491	20.570	1798851	0.30	401901	0.78	4.48	Glutaric acid, 1-naphthyl tridecyl ester
35	21.569	21.985	348432734	57.44	13686466	26.69	25.46	1,2,3-Benzenetriol
36	22.584	22.655	172161	0.03	52435	0.10	3.28	1-Acetylpiperazine
37	22.934	23.120	5105418	0.84	620746	1.21	8.22	2-Propenoic acid, 3-phenyl-
38	23.216	23.315	147665	0.02	35628	0.07	4.14	Bicyclo[2.2.2]oct-2-ene, 5-(ethoxycarbonylme
39	23.457	23.560	217349	0.04	42022	0.08	5.17	2-Furancarboxaldehyde, 5-(2-furanylmethyl)-
40	24.074	24.185	420867	0.07	85986	0.17	4.89	Benzaldehyde, 4-(acetyloxy)-3-ethoxy-
41	25.552	25.600	592483	0.10	125790	0.25	4.71	benzoic acid, 2-chloro-4-[[4-(pentyloxy)benzo
42	27.147	27.270	625029	0.10	157756	0.31	3.96	Acetic acid, 2-(5-methyl-1,3,4-oxadiazol-2-yl)
43	27.719	27.835	514740	0.08	160410	0.31	3.21	Dodecanoic acid
44	28.180	28.265	571195	0.09	145699	0.28	3.92	5-amino-3,4-dihydroisoquinolin-1(2H)-one
45	28.454	28.545	785961	0.13	243283	0.47	3.23	2,4-imidazolidinedione, 3-phenyl-
46	29.812	29.890	400182	0.07	59925	0.12	6.68	
47	30.837	30.905	301109	0.05	96284	0.19	3.13	5-[(Pentachlorophenoxy)methyl]furan-2-carba
48	31.564	31.630	175916	0.03	56391	0.11	3.12	(2S,4aS,5R,8aS)-2-Allyl-5-((Z)-pent-2-en-4-y
49	31.864	31.920	524928	0.09	243271	0.47	2.16	Tetradecanoic acid
50	33.271	33.335	336330	0.06	138600	0.27	2.43	Pentadecanoic acid
51	33.689	33.770	315709	0.05	51638	0.10	6.11	5-Keto-2,2-dimethylheptanimine
52	33.851	33.890	144543	0.02	79270	0.15	1.82	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
53	34.267	34.375	514087	0.08	159512	0.31	3.22	Oleic Acid
54	34.580	34.710	15209996	2.51	4983406	9.72	3.05	n-Hexadecanoic acid
55	34.962	35.075	833432	0.14	217855	0.42	3.83	Cirsiumaldehyde
56	35.606	35.670	181581	0.03	62145	0.12	2.92	E-2-Hexenyl benzoate
57	37.124	37.180	1076379	0.18	365375	0.71	2.95	9,12-Octadecadienoic acid (Z,Z)-
58	37.232	37.370	1484309	0.24	459662	0.90	3.23	Oleic Acid
59	37.703	37.875	3939414	0.65	1118827	2.18	3.52	Octadecanoic acid
60	42.805	42.865	923453	0.15	424011	0.83	2.18	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
61	44.142	44.250	476182	0.08	121161	0.24	3.93	2-Heptanone, 6-(2-furanyl)-6-methyl-
62	44.737	44.820	469478	0.08	160125	0.31	2.93	Octadecanoic acid, 2,3-dihydroxypropyl ester
63	45.386	45.455	490964	0.08	182651	0.36	2.69	13-Docosenamide, (Z)-
64	45.762	45.810	377492	0.06	155782	0.30	2.42	Squalene
			606607912	100.00	51286330	100.00		

3.0 CONCLUSION

Moringa leaves treated water (MLTW) of the plant *Moringa Oleifera* was analyzed in order to find out its therapeutic potential as it ensures uniformity and quality control. For the same objective, the current paper was designed to identify the biologically active compounds present in Moringa leaves treated water (MLTW).

The analysis revealed diverse medicinal compounds and heterocyclic compounds such as furan, oxazolidine, catechol, pivaloin and different types of fatty acids such as tetradecanoic acid, pentadecanoic acid, oleic acid, n-hexadecanoic acid , octadecanoic acid and squalene, benzoic acid. Some compound such as 1,2,3-benzenetriol, n-Hexadecanoic, 5-Hydroxymethylfurfural and 2H-pyran-2,6(3H)-dione, amoung these compounds 1,2,3-benzenetriol also known as Pyrogallol shows it potential for antimalarial agent and another compound n-hexadecanoic acid shows it potential as cytotoxic agent. The N-hexadecanoic acid is a common fatty acid, found in plants. It is already reported that N-hexadecanoic acid selectively inhibit DNA topoisomerase-I and thereby prevents the proliferation of human fibroblast cells. The results of our study were supported by previous reports that the cytotoxic activity of the leaf extract is due to the presence of several phytochemicals in the leaf. Docking study also predict the DNA topoisomerase-I inhibitory activityof N-hexadecanoic acid. Hence, the proposed mechanism of cytotoxic activity of N-hexadecanoic acid is due to its interaction with DNA topoisomerase-I and thereby prevents proliferation of cells. These findings further confirm the medicinal value of MLTW may serve as cytotoxic as well as antimalarial.

GC-MS reports will be promising in the pharmaceutical sector in the identification of a variety of Phytoconstituents in distinct plant extracts, polyherbal extracts and the standardization of particular plant materials.

The benefits of identifying Phytoconstituents are not just limited to the Pharma arena but also to health sectors involved in manufacturing dietary supplements to support overall health and other products as well as the cosmetic industry. Academically, this analysis unlocks more opportunities to study plants for their diverse contents and improved awareness and knowledge of the traditional application. Furthermore, it helps in a scientific backup of indigenous use and creates a chance to use modern data with traditional formulation and produce unique, safe and effective medicines. Yet, more research is necessary to prove the efficacy and safety of the bioactive compound, this will be the objective of our future studies.

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All authors reviewed and approved the manuscript.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE
The authors have no ethical conflicts to disclose.

AVAILABILITY OF DATA AND MATERIAL
All data generated during this study were included in this article.

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