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GCMS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS AND SCREENING FOR ANTI-ANAEMIC ACTIVITY OF THE METHANOLIC EXTRACT OF *MORINGA OLEIFERA* LEAVES

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ABSTRACT

The present study was aimed to determine phytochemical constituents with the aid of Gas Chromatography Mass Spectroscopy technique in methanolic extract of locally grown *M. oleifera* leaves for their anti-anaemic activity in Ranchi district of Jharkhand. The bioactive compounds present in methanolic leaves extract, identified by GCMS chromatogram showed 42 peaks indicating the presence of 42 bioactive compounds. It revealed 1,2,3-Propanetriol be the major phytocompound present in *Moringa* followed by 2-Ethylbutyric acid, nonyl ester (9.44%), Phytol (6.89%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethy) ethyl ester (6.32%), 2-Ethylbutyric acid, eicosyl ester (5.16%) and other remaining compounds ranging from 4.76% to 0.21%. Herbal powder was formulated and given to some patients. Pre and post haemoglobin level was measured using a haemocytometer. There was an increase in the level of haemoglobin after consumption of herbal powder. The result proved ability of this plant in curing nutrition deficiency anaemia. Medicinal plants are backbone of traditional healthcare system and anti-anaemic activity of the plant is due to presence of different bioactive compounds. They can also act as anti-oxidants and beneficial in management of hypertension, heart diseases, stroke and heart failure etc.

KEYWORDS: GCMS, Phytoconstituents, *Moringa oleifera*, herbal powder, haemoglobin, anaemia.

INTRODUCTION

The dependency of man on plants evolved gradually along with the development of civilization. The herbal plants biodiversity and cultural biodiversity of India is highly rich that contributes to traditional system of medicine India is the largest producer of medicinal herbs and is called as botanical garden of the world. The state of Jharkhand occupies a unique position in the tribal map of India having 62 scheduled tribes including 15 primitive tribes.[1]

Traditional medicines are important source of herbal medication guidance but modern medicine must prove these guidelines through scientific approaches before using them in practice.

Moringa, also known as drumstick tree or horseradish tree, is a plant species that is native to India, Pakistan and other parts of South Asia. It has been used in traditional medicines for centuries due to its various medicinal properties.
Medicinal plants are backbone of traditional herbal medicine, having bioactive substances which make them potentioal for treating various ailments.[9,8] *Moringa oleifera* is a fast growing evergreen tree with many health benefits. This plant is reported to be used in phytomedicine as anti-oxidant, anti microbial, anti ulcer, anti inflammatory and as a hypercholesteromic agent.

Anaemia, one of the oldest, most common and widespread blood disorder, is a public health problem in both developing and developed countries.[2] Anaemia is a nutrition deficiency associated with malnutrition. It is a clinical condition that is characterized by reduction in haemoglobin concentration with or without a reduction in red blood cell count.

The present work reveals phytochemical constituents present in ethnomedicinal plants used in the cure of anaemia in different blocks of Ranchi district of Jharkhand state, India. This area i.e. Ranchi district of Jharkhand has a rich heritage of medicinal plants. Ethnic people cure various human diseases by using medicinal plants. These people depend on medicinal plants for medicine as alternative of allopathic medicine for curing various ailments/ diseases. The indigenous people are well acquainted with medicinal plants of the area.[3-8] Phytochemical constituents of methanolic extract of *Moringa oleifera* was determined using Gas Chromatography Mass Spectrometry (GCMS) technique to identify the presence of bioactive components.

GCMS analysis of *M. oleifera* methanolic extract showed 42 peaks confirming presence of 42 compounds. The result proved ability of this plant to improve blood haemoglobin level and therby curing anaemia.

MATERIAL AND METHOD

The present research work centers on phytochemical analysis of *M. oleifera* that is used to cure anaemia by indigenous people of Ranchi district of Jharkhand. Gas chromatography mass spectrometry (GCMS) was carried out for the identification of phytochemicals present in methanolic extract of *Moringa oleifera.*

Collection and identification of plant material

Fresh leaves of *M. oleifera* was collected from Ranchi, Jharkhand, India. The plants was identified and authenticated by Botanical Survey of India (BSI), Hyderabad.

Preparation of plant extract

It was washed gently with distilled water to eliminate contaminants. It was shade dried and coarsely crushed. Now plant extract was prepared by suspensing 5 gm of powdered plant sample in 50 ml of methanol. The extraction was allowed to stand for 72 hours at room temperature. The extract was filtered using Whatmann filter paper in a beaker. Now beaker was covered with aluminium foil and pores were created on foil and left for a week. After one week this was transferred into sterile bottle and sent for GCMS analysis.

Gas Chromatogrpahy Mass Spectroscopy analysis

The methanolic extract obtained from sample was subjected to Gas Chromatography Mass Spectroscopy for the determination of bioactive compounds. GCMS analysis of plant sample was performed in Advanced Instrumentation Research Facility, Jawaharlal Nehru University, Delhi.

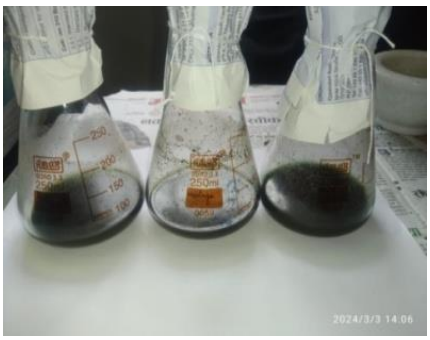
Identification of phytocomponents

Identification of the active components in the extracts was performed by comparison of their retention indices, peak area percentage and mass spectra pattern with those stored on the National Institute of Standards and Technology (NIST) digital library data, Wiley library and also with published literature. NIST14.LIB and WILEY8.lib library sources were used for matching the identified components from the plant material.

Herbal powder formulation and haemoglobin level monitoring (*Moringa oleifera* powder)

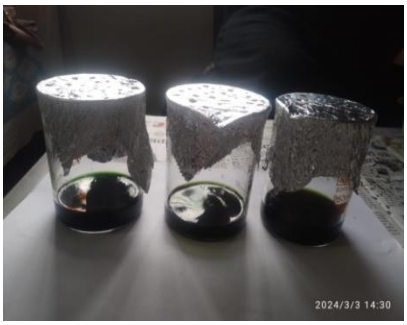
i) *Moringa* leaves were collected.
ii) It was washed with tap water and again with distilled water.
iii) Leaves were shade dried for two days.
iv) Fine powder was made using blender.
v) This was packaged and used as per requirement.
vi) From 100 gm of *Moringa* leaves, 10 gm of powder is produced.

(a) Weighing of sample



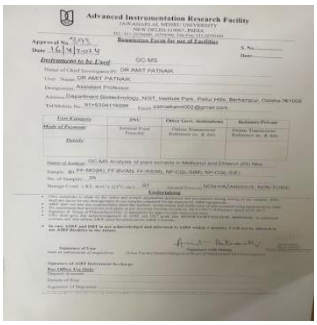
(b) Extract preparation

(c) Filtering sample



(d) Extract preparation

(e) Sample sent for GCMS



(f) AIRF approval for GCMS

Fig. 1: Sample preparation for GCMS analysis.

(a) *M. oleifera* tree

(b) Collection of leaves

(c) Washing of leaves

(d) Drying

(e) Powder formation

Fig. 2: Herbal powder formation from *Moringa oleifera.*

Herbal powder was formulated from *M. oleifera* and was given to anaemia patients. They were given for a period of 15 days.

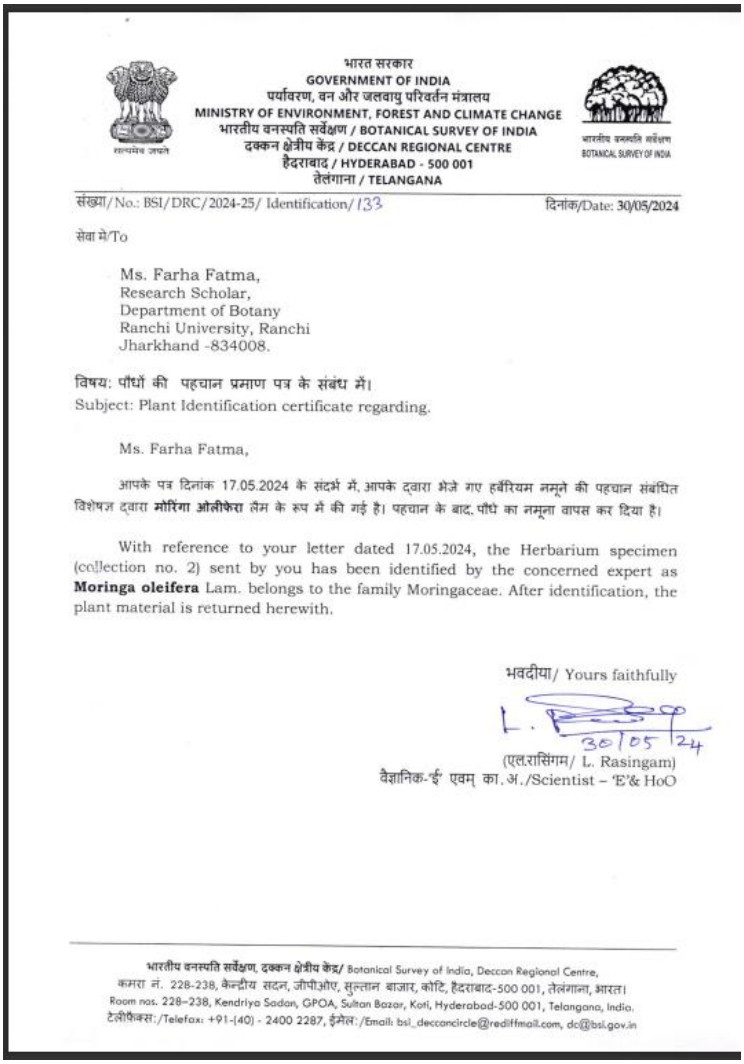
Haemoglobin level check

Before and after consumption of *M. oleifera* herbal powder, haemoglobin level was checked using a haemocytometer.

- Haemocytometer device was bought from market. It is having stripes, pricking needle and a charger.
- It was charged first.
- The code was set.
- Stripe was inserted and patient's finger tip was pricked.
- Few drops of blood was placed on stripe.
- With 30-60 seconds the reading was observed on screen.

Table 1: Haemoglobin level before and after consumption of herbal powder.

S. No.	Name	Pre test haemoglobin level (g/dl)	Post test haemoglobin level (g/dl)
1.	Manir Khan	8.4	10.2
2.	Manjhari	8.8	10.2
3.	Munni	10.1	11.2
4.	Shamina	11.0	12.3
5.	Konu	12.2	13.1
6.	Ruqaiya	7.6	9.6



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तेलंगाना / TELANGANA

संख्या/No.: BSI/DRC/2024-25/ Identification/133 दिनांक/Date: 30/05/2024

सेवा मे/To

Ms. Farha Fatma,
Research Scholar,
Department of Botany
Ranchi University, Ranchi
Jharkhand -834008.

विषय: पौधों की पहचान प्रमाण पत्र के संबंध में।
Subject: Plant Identification certificate regarding.

Ms. Farha Fatma,

आपके पत्र दिनांक 17.05.2024 के संदर्भ में, आपके द्वारा भेजे गए हर्बेरियम नमूने की पहचान संबंधित विशेषज्ञ द्वारा **मोरिंगा ओलीफेरा** लैम के रूप में की गई है। पहचान के बाद, पौधे का नमूना वापस कर दिया है।

With reference to your letter dated 17.05.2024, the Herbarium specimen (collection no. 2) sent by you has been identified by the concerned expert as **Moringa oleifera** Lam. belongs to the family Moringaceae. After identification, the plant material is returned herewith.

भवदीया/ Yours faithfully
30/05/24
(एल.रासिंगम/ L. Rasingam)
वैज्ञानिक-'ई' एवम् का.अ./Scientist – 'E'& HoO

भारतीय वनस्पति सर्वेक्षण, दक्कन क्षेत्रीय केंद्र/ Botanical Survey of India, Deccan Regional Centre,
कमरा नं. 228-238, केन्द्रीय सदन, जीपीओए, सुल्तान बाजार, कोटि, हैदराबाद-500 001, तेलंगाना, भारत।
Room nos. 228–238, Kendriya Sadan, GPOA, Sultan Bazar, Koti, Hyderabad-500 001, Telangana, India.
टेलीफैक्स:/Telefax: +91-(40) - 2400 2287, ईमेल:/Email: bsi_deccancircle@rediffmail.com, dc@bsi.gov.in

Fig. 3: Plant identification certificate from BSI, Hyderabad.

RESULT AND DISCUSSION

In present study, bioactive compounds present in methanolic extract of *M. oleifera* has been identified by GCMS technique. The mass spectra of the phytocomponents in *M. oleifera* was compared with that in the NIST library and WILEY library database supporting characterisataion and identification of bioactive compounds. GCMS chromatogram showed 42 peaks indicating presence of 42 phytochemical constituents.

GCMS chromatogram of *M. oleifera* leaves methanolic extract showed 42 peaks confirming presence of 42 phytochemical constituents. *M. oleifera* contains bioactive compounds with medicinal properties as confirmed by GCMS. It revealed 1,2,3-Propanetriol be the major phytocompound present in *Moringa* followed by 2-Ethylbutyric acid, nonyl ester (9.44%), Phytol (6.89%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethy) ethyl ester (6.32%), 2-Ethylbutyric acid, eicosyl ester (5.16%) and other remaining compounds ranging from 4.76% to 0.21%.

The findings revealed that there was a significant improvement in level of haemoglobin after administration of the herbal powder.

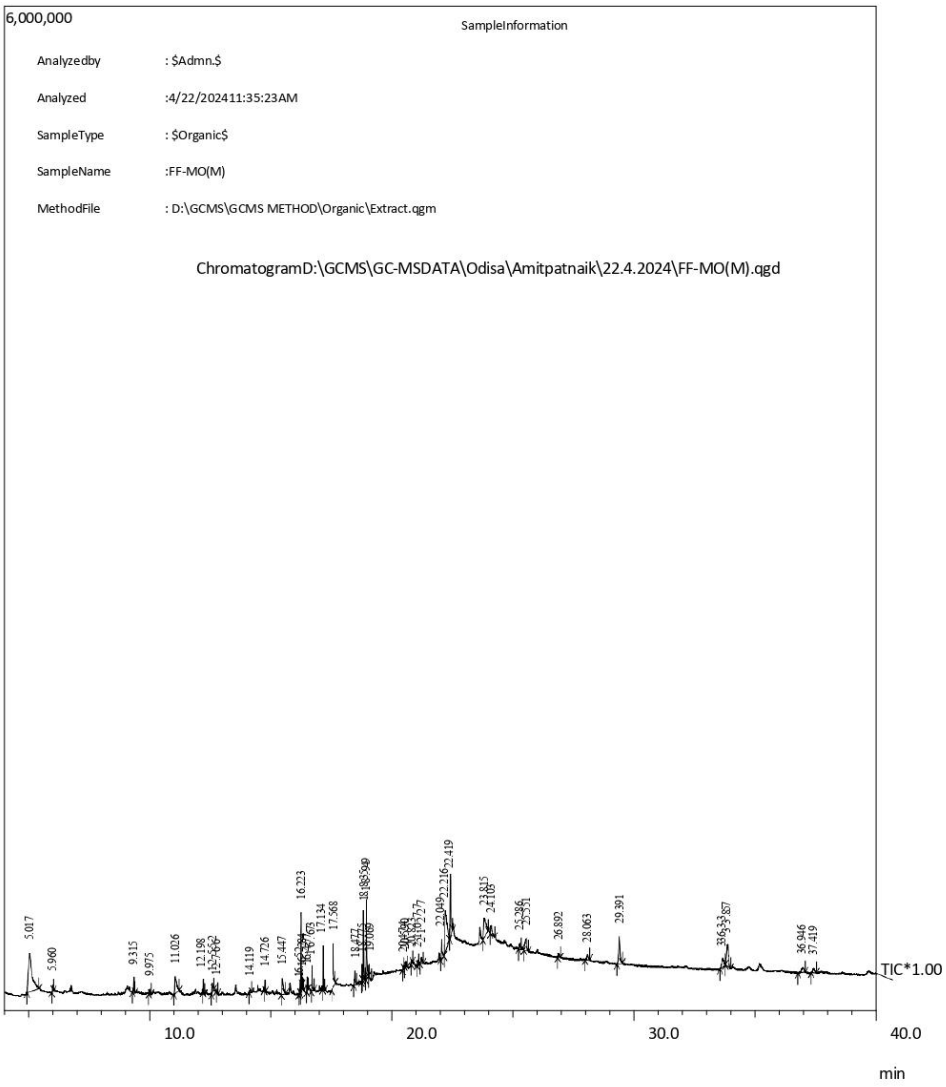

Table 2: Peak Report TIC.

Peak#	R. Time	Area	Area%	Name
1	5.017	2506688	15.92	1,2,3-PROPANETRIOL
2	5.960	71504	0.45	Hexane,3,3-dimethyl-
3	9.315	162975	1.04	Nonadecane
4	9.975	40137	0.25	Hexane,3,3-dimethyl-
5	11.026	603313	3.83	2-PROPENOICACID,3-PHENYL-,METHYLESTER
6	12.198	127110	0.81	Dodecane,4,6-dimethyl-
7	12.552	164484	1.04	.BETA.-D-GLUCOPYRANOSE,1,6-ANHYDRO-
8	12.766	47075	0.30	Octane,2-methyl-
9	14.119	47221	0.30	6-Methylheptyl3-methylbutanoate
10	14.726	92119	0.59	2-Methyltetracosane
11	15.447	366598	2.33	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-
12	16.152	41258	0.26	Cyclopropane, 1-methyl-1-(1-methylethyl)-2-nonyl-
13	16.223	738038	4.69	Neophytadiene
14	16.284	81121	0.52	1-Dodecanol,3,7,11-trimethyl-
15	16.477	126826	0.81	Neophytadiene
16	16.673	278738	1.77	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
17	17.134	403556	2.56	Hexadecanoicacid,methylester
18	17.568	696842	4.43	n-Hexadecanoicacid
19	18.477	178713	1.13	4-Oxazolecarboxylicacid,4,5-dihydro-2-phenyl-,1-methyl
20	18.775	142571	0.91	9,12-Octadecadienoicacid,methylester

21	18.835	652225	4.14	9,12,15-Octadecatrienoicacid,methylester,(Z,Z,Z)-
22	18.949	1084290	6.89	Phytol
23	19.069	102059	0.65	OCTADECANOICACID,METHYLESTER
24	20.474	39847	0.25	Carbonicacid,2-dimethylaminoethylneopentylester
25	20.590	75647	0.48	HEXADECANOICACID,1-(HYDROXYMETHYL)-1,2-
26	20.823	32992	0.21	Bicyclo[6.2.0]decan-9-one,10,10-dichloro-
27	21.077	45538	0.29	4,8,12,16-Tetramethylheptadecan-4-olide
28	21.227	135775	0.86	BICYCLO[2.2.1]HEPTAN-2-ONE,4,7,7-TRIMETHYL-,
29	22.049	313220	1.99	octadecanoicacid,3-oxo-,ethylester
30	22.216	1485996	9.44	2-Ethylbutyricacid,nonylester
31	22.419	995293	6.32	Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl)ethyleste
32	23.815	812182	5.16	2-Ethylbutyricacid,eicosylester
33	24.103	225641	1.43	Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl)ethyleste
34	25.286	135356	0.86	.alpha.-TocospiroB
35	25.551	356583	2.26	.alpha.-TocospiroA
36	26.892	71569	0.45	14,15,16-Trinor-8.xi.-labdan-6.beta.-ol,8,13-epoxy-
37	28.063	105774	0.67	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltr
38	29.391	749510	4.76	VitaminE
39	33.633	334775	2.13	.gamma.-Sitosterol
40	33.857	718484	4.56	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6
41	36.946	183319	1.16	2,4A,8,8-TETRAMETHYL-DECAHYDRO-CYCLOPROP
42	37.419	173107	1.10	Cholest-4-en-3-one
		15746069	100.00	

CONCLUSION

The edible plant species of *M. oleifera* had a rich amount of valuable ingredients with medicinal potentials that are beneficial for health. Phytoconstituents of methanolic extracts were successfully screened using standard procedure.

Therefore further research work is recommended to establish which components in terms of management of anaemia and its application in curing other diseases like hypertension, heart diseases etc. which could be of high economic value.

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