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GCMS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS AND SCREENING FOR ANTI-ANAEMIC ACTIVITY OF THE METHANOLIC EXTRACT OF *BETA VULGARIS*

Farha Fatma*[1] and Dr. Arun Kumar[2]

[*1]Research Scholar, University Department of Botany, Ranchi University, Ranchi.
[2]Associate Professor and Head, University Department of Botany.
Dean, Faculty of Sciences, Ranchi University, Ranchi, Jharkhand, India- 834008.

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Corresponding Author: Farha Fatma
Research Scholar, University Department of Botany, Ranchi University, Ranchi.
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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 *B. vulgaris* for their anti-anaemic activity in Ranchi district of Jharkhand. The bioactive compounds present in methanolic extract, identified by GCMS chromatogram showed 37 peaks indicating presence of 37 bioactive compounds providing significant potential as medicinal plant. Major bioactive compounds found in GCMS of *B. vulgaris* methanolic extract are found to be Pyridine,3-(1-methyl-2-pyrrolidinyl)-(S) (79.62%) followed by 1-H-indene, 1-hexadecyl-2,3-dihydro (2.61%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (2.57%) and other remaining compounds ranging from 2.18% to 0.11%. 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 medicines. 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, *Beta vulgaris*, herbal powder, haemoglobin, anaemia.

INTRODUCTION

The herbal plants biodiversity and cultural biodiversity of India is highly rich that contributes to traditional system of medicine. Many plant derived drugs employed in modern medicine were discovered through ethno botanical investigations. Traditional medicine is important as it provides health services to 75-80% of world's population. They provide us with key chemical structure for the development of new phytomedicine.[1]

Beta vulgaris (beetroot) plant belongs to Amaranthaceae family, they are important vegetables consumed around the world. Vitamins and minerals found in beetroot are most likely active constituents amenable for its impact.[2-4] Beetroot is a commonly consumed vegetable showing a rich source of antioxidants. It contains multiple biologically active phytochemicals including flavonoids, polyphenols, therapeutic enzymes, ascorbic acid, dehydroascorbic acid (DHAA) and inorganic nitrate.[5-7] It provides valuable nutrients like potassium, calcium, magnesium, sodium, iron, zinc, phosphorus, copper and manganese.[8]

Anaemia, one of the oldest, most common and widespread blood disorder, is a public health problem in both developing and developed countries.[9] 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. Phytochemical constituents of methanolic extract of *Beta vulgaris* was determined using Gas Chromatography Mass Spectrometry (GCMS) technique to identify the presence of bioactive components.

GCMS analysis of *B. vulgaris* methanolic extract showed 37 peaks confirming presence of 37 compounds. The result proved ability of this plant to improve blood haemoglobin level and therby curing anaemia.

MATERIAL AND METHOD

The present work centers on phytochemical analysis of *B. vulgaris* 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 *Beta vulgaris.*

Collection and identification of plant material

Fresh root of *B. vulgaris* 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 samples was performed in Advanced Instrumentation Research Facility, Jawaharlal Nehru University, Delhi.

Identification of phytocomponents

Identification of the active components in the extract 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

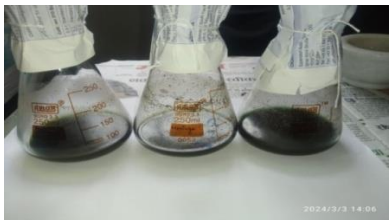
Herbal powder was formulated from *B. vulgaris* and was given to anaemia patients. They were given for a period of 15 days. Before and after haemoglobin level was checked using a haemocytometer.

- 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.

***Beta vulgaris* powder**

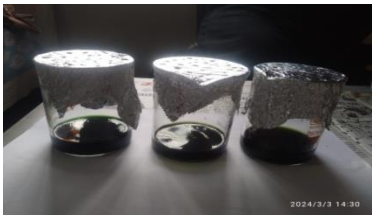
i) Fresh beetroots were bought from market.
ii) It was washed and peeled off.
iii) Thin slices were made and chopped into small pieces.
iv) It was dried for two to three days.
v) Now fine powder was made using blender.
vi) This powder is packaged and used as per requierement.

(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) Field of *B. vulgaris*

(b) Washing of *B. vulgaris*

(c) Peeling off

(d) Peeled off

(e) Sliced pieces of *B. vulgaris*

(f) Cut into small pieces

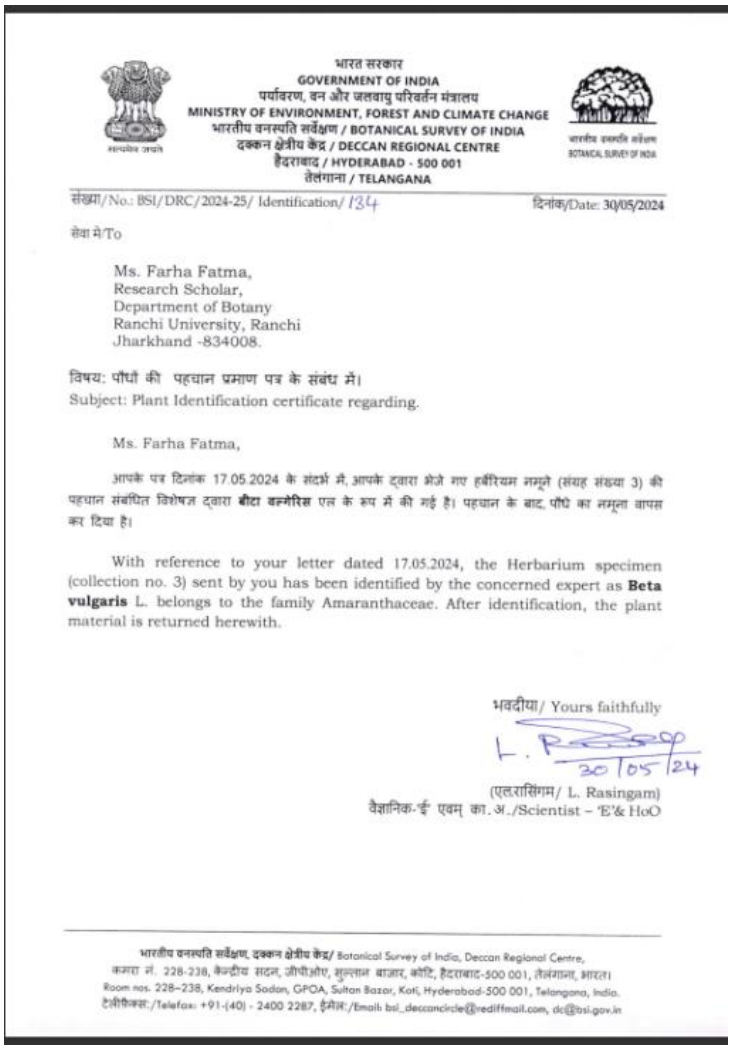
(g) Drying

(h) Powder

Fig. 2: Herbal powder formation from *Beta vulgaris*.

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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GOVERNMENT OF INDIA
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MINISTRY OF ENVIRONMENT, FOREST AND CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण / BOTANICAL SURVEY OF INDIA
दक्कन क्षेत्रीय केंद्र / DECCAN REGIONAL CENTRE
हैदराबाद / HYDERABAD - 500 001
तेलंगाना / TELANGANA

संख्या/No.: BSI/DRC/2024-25/ Identification/ 134 दिनांक/Date: 30/05/2024

सेवा में/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 (संग्रह संख्या 3) की पहचान संबंधित विशेषज्ञ द्वारा **बीटा वल्गेरिस** एल 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. 3) sent by you has been identified by the concerned expert as **Beta vulgaris** L. belongs to the family Amaranthaceae. 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, जीपीओ, 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: Identification certificate of *B. vulgaris* from BSI, Hyderabad.

RESULT AND DISCUSSION

In present study, bioactive compounds present in methanolic extract of *B. vulgaris* has been identified by GCMS technique. The mass spectra of the phytocomponents in *A. dubius* was compared with that in the NIST library and WILEY library database supporting characterisataion and identification of bioactive compounds. GCMS chromatogram showed 37 peaks indicating presence of 37 phytochemical constituents.

B. vulgaris methanolic extract showed 37 peaks indicating presence of 37 bioactive compounds providing significant potential as medicinal plant. Major bioactive compounds found in GCMS of *B. vulgaris* methanolic extract are found to be Pyridine,3-(1-methyl-2-pyrrolidinyl)-(S) (79.62%) followed by 1-H-indene, 1-hexadecyl-2,3-dihydro (2.61%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (2.57%) and other remaining compounds ranging from 2.18% to 0.11%.

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

SampleInformation

Analyzedby	: $Admn.$
Analyzed	:4/22/202412:25:28PM
SampleType	: $Organic$
SampleName	:FF-BV(M)
MethodFile	: D:\GCMS\GCMS METHOD\Organic\Extract.qgm

ChromatogramD:\GCMS\GC-MSDATA\Odisa\Amitpatnaik\22.4.2024\FF-BV(M).qgd

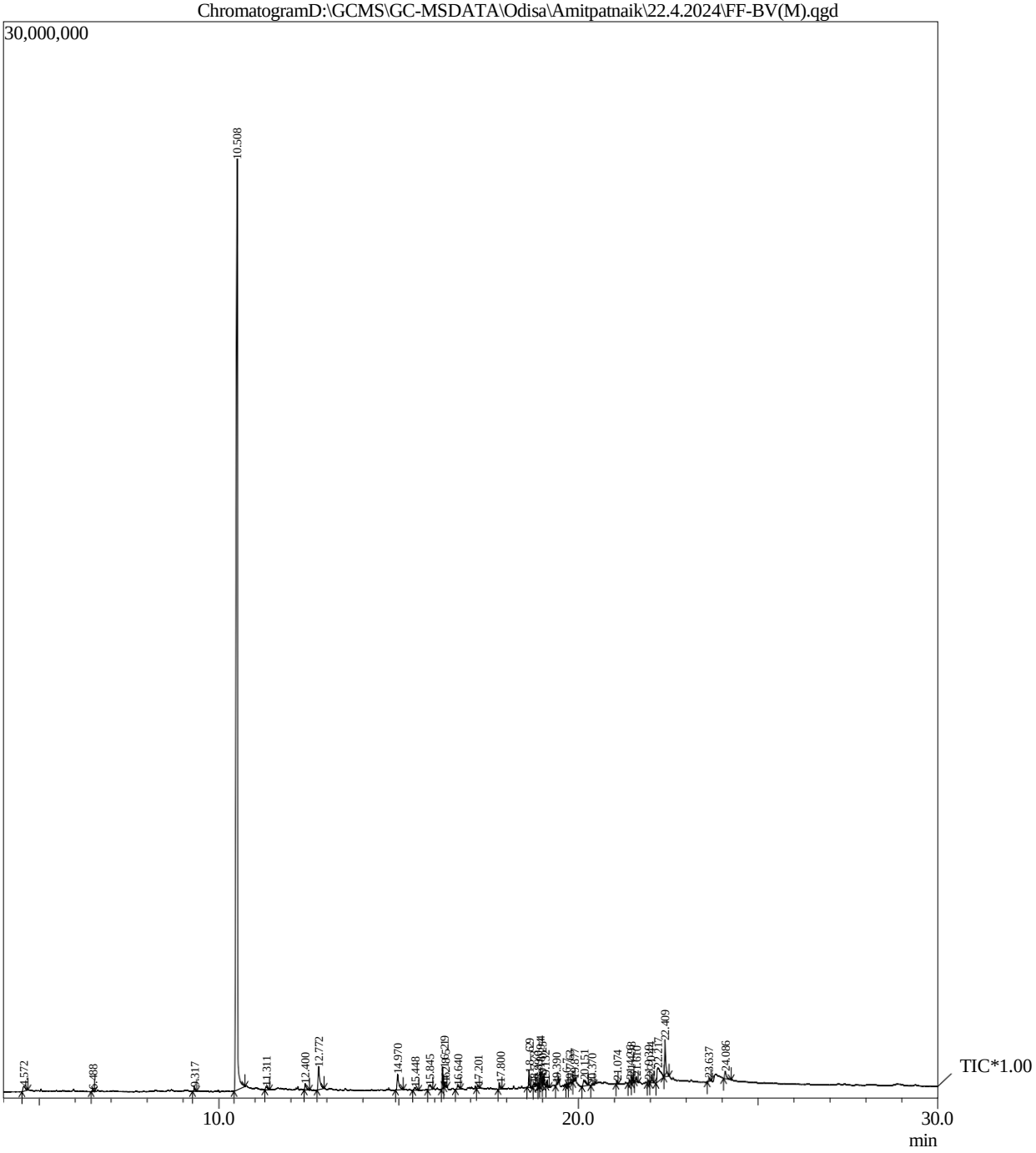

Table 2: Peak Report TIC.

Peak#	R. Time	Area	Area%	Name
1	4.572	621622	0.62	PENTANOICACID,3-METHYL-
2	6.488	114502	0.11	3-OCTANOL, 2-METHYL-
3	9.317	205343	0.21	Sulfurousacid,hexyloctylester
4	10.508	79687953	79.62	PYRIDINE,3-(1-METHYL-2-PYRROLIDINYL)-,(S)-
5	11.311	329505	0.33	2,2'-BI-1H-PYRROLE,1,1'-DIMETHYL-
6	12.400	490029	0.49	Anabasine
7	12.772	2181636	2.18	1,2,3,6-Tetrahydro-2,3'-bipyridine
8	14.970	1448904	1.45	Cotinine
9	15.448	319077	0.32	2(4H)-BENZOFURANONE,5,6,7,7A-TETRAHYDRO-6-
10	15.845	603230	0.60	2,3-Quinoxalinedione,1,4-dihydro-6-methyl-
11	16.219	896707	0.90	Neophytadiene
12	16.285	197066	0.20	2-Pentadecanone,6,10,14-trimethyl-
13	16.640	392125	0.39	N'-Propylnornicotine
14	17.201	224143	0.22	7-Oxabicyclo[4.1.0]heptan-3-ol,6-(3-hydroxy-1-butenyl)-1
15	17.800	319777	0.32	HEXADECANOICACID,ETHYLESTER
16	18.629	819223	0.82	NEROLIDOL-EPOXYACETATE
17	18.773	224219	0.22	KAURAN-18-AL,17-(ACETYLOXY)-,(4.BETA.)-
18	18.890	125296	0.13	SCLAREOLIDE
19	18.944	969544	0.97	Phytol
20	19.025	428956	0.43	NEROLIDOL-EPOXYACETATE
21	19.132	223131	0.22	PHENANTHRENE,9-DODECYLTETRADECAHYDRO-
22	19.390	105054	0.10	5-HEXENYLPENTANOATE
23	19.673	109593	0.11	HEPTADECANOICACID,ETHYL ESTER
24	19.763	212521	0.21	2H-1-Benzopyran-2-one,3,4-dihydro-6-methyl-
25	19.877	144527	0.14	DUVATRIENDIOL
26	20.151	717659	0.72	1-Heptatriacotanol
27	20.370	142295	0.14	CYCLOPENTANOL,1,2-DIMETHYL-3-(1-METHYLET
28	21.074	140036	0.14	4,8,12,16-Tetramethylheptadecan-4-olide
29	21.426	203662	0.20	Nerolidolisobutyrate
30	21.498	408511	0.41	3-Buten-2-one,4-(3-hydroxy-6,6-dimethyl-2-methylenecyc
31	21.610	329073	0.33	3-Buten-2-one,4-(3-hydroxy-6,6-dimethyl-2-methylenecyc
32	21.939	181856	0.18	Widdrolhydroxyether
33	22.044	482860	0.48	octadecanoicacid,3-oxo-,ethylester
34	22.217	2614952	2.61	1H-Indene,1-hexadecyl-2,3-dihydro-
35	22.409	2509264	2.51	Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl)ethyleste
36	23.637	239310	0.24	1,3,5-Trisilacyclohexane
37	24.086	720548	0.72	Octadecanoicacid,2,3-dihydroxypropylester
		100083709	100.00	

CONCLUSION

The edible plant species of *B. vulgaris* 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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