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GCMS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS AND SCREENING FOR ANTI-ANAEMIC ACTIVITY OF THE METHANOLIC EXTRACT OF *AMARANTHUS CAUDATUS* 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 *A. caudatus* leaves for their anti-anaemic activity in Ranchi district of Jharkhand. The bioactive compounds present in methanolic leaves extract, identified by GCMS chromatogram showed 55 peaks indicating the presence of 55 bioactive compounds. The plant contains large amount of secondary metabolites having wide range of biological activities on physiological system. 2-Ethylbutyric acid, eicosyl ester (9.20%) was found to be major compound and fifty four other major compounds such as 4 H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (8.51 %), n-Hexadecanoic acid (8.47%), Phytol (7.28%), 1,2,3,-Propanetriol (6.70%), Chondrillasterol (6.51%) and remaining compounds with peak area ranging from 5.26 % to 0.21% were found to be present. Herbal medicines were 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, *Amaranthus caudatus*, herbal powder, haemoglobin, anaemia.

INTRODUCTION

Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Humans have been using plants to cure diseases. According to WHO, at least 80% people in developing countries depend on herbal plants.[1-2]

Plant derived medicines are widely used because they are safer than synthetic alternatives as well as easily available and cheaper.[3] *Amarnthus caudatus* is a fast growing herb. The leaves are highly nutritious and main nutrients present in leaves are carbohydrates, proteins, vitamins and minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese.

Anaemia, one of the oldest, most common and widespread blood disorder, is a public health problem in both developing and developed countries.[4] 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 *Amaranthus caudatus* was determined using Gas Chromatography Mass Spectrometry (GCMS) technique to identify the presence of bioactive components.

GCMS analysis of *A. caudatus* methanolic extract showed 55peaks confirming presence of 55 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 *A. caudatus* 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 *Amaranthus caudatus*. Many scientists have been worked on phytochemical investigations.[5-7]

Collection and identification of plant material

Fresh leaves of *A. caudatus* 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.

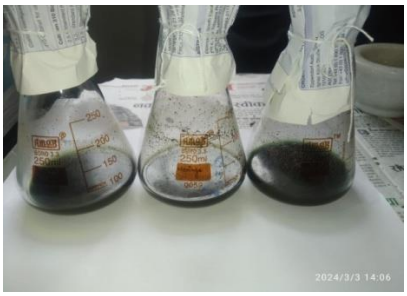
Herbal powder formulation and haemoglobin level monitoring

Herbal powder was formulated from *A. caudatus* 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.

Amaranthus caudatus powder -

i) Fresh *Amaranthus caudatus* leaves were collected.
ii) It was washed using tap water and then with distilled water.
iii) Now leaves were shade dried for two to three days.
iv) Fine powder was made using blender.
v) This powder is packed into packets or bottles for storage purpose.
vi) This is used as per requirement.

(a) Weighing of sample

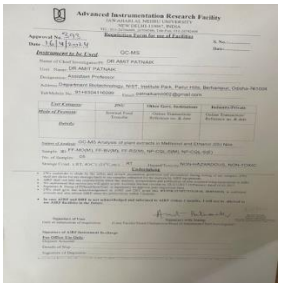


(b) Extract preparation

(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 *A. caudatus* **(b) Plant of *A. caudatus*** **(c) Washed leaves**

(d) Drying of leaves **(e) Dried leaves** **(f) Powder formation**

Fig. 2: Herbal powder formation from *A. caudatus.*

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

Haemoglobin level check

Before and after consumption of *A. caudatus* 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

RESULT AND DISCUSSION

In present study, bioactive compounds present in methanolic extract of *A. caudatus* has been identified by GCMS technique. The mass spectra of the phytocomponents in *A. caudatus* was compared with that in the NIST library and WILEY library database supporting characterisataion and identification of bioactive compounds. GCMS chromatogram showed 55 peaks indicating presence of 55 phytochemical constituents.

GCMS chromatogram of *A. caudatus* leaves methanolic extract showed 55 peaks which indicated presence of 55 compounds. The plant contains large amount of secondary metabolites having wide range of biological activities on physiological system. 2-Ethylbutyric acid, eicosyl ester (9.20%) was found to be major compound and fifty four other major compounds such as 4 H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (8.51 %), n-Hexadecanoic acid (8.47%), Phytol (7.28%), 1,2,3,-Propanetriol (6.70%), Chondrillasterol (6.51%) and remaining compounds with peak area ranging from 5.26 % to 0.21% were found to be present.

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

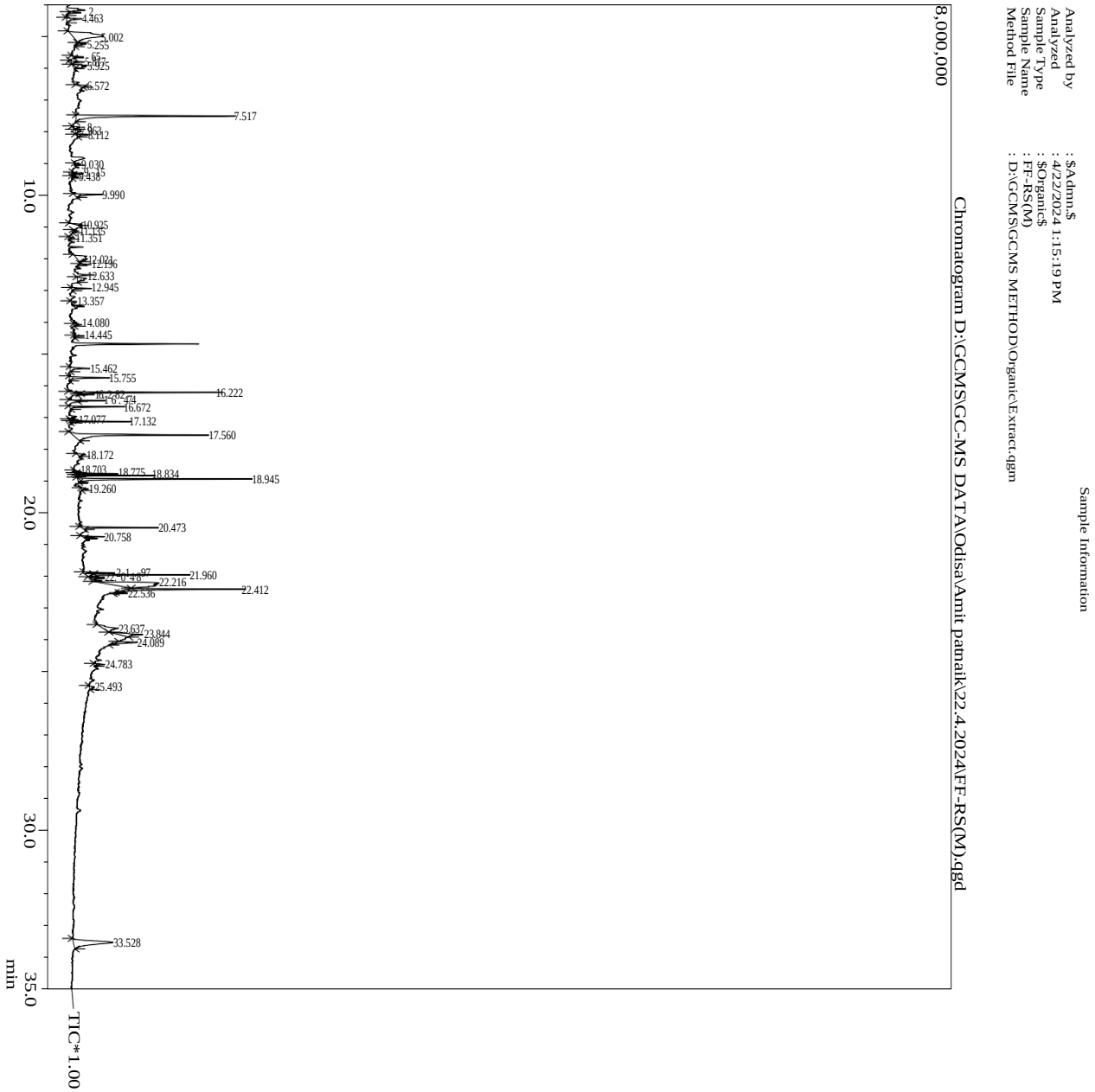


Table 2: Peak Report TIC.

Peak#	R.Time	Area	Area%	Name
1	4.261	227299	0.55	PIPERIDINE, 4-METHYL-
2	4.463	421828	1.01	2-Methylpiperidine
3	5.002	2792858	6.70	1,2,3-PROPANETRIOL
4	5.255	254774	0.61	3,5-Dihydroxycyclohexanamine
5	5.657	173167	0.42	1-Butanamine, 2-methyl-N-(2-methylbutylidene)-
6	5.817	256764	0.62	3-METHYLBUTYL-(3-METHYLBUTYLIDENE)AMINE
7	5.925	586213	1.41	Benzeneacetic acid 1-methylethyl ester
8	6.572	196751	0.47	1,3,5-Triazine-2,4,6-triamine
9	7.517	3546598	8.51	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
10	7.870	152961	0.37	2-(4-Amino-5-thioxo-4,5-dihydro-[1,2,4]triazol-1-yl)-N-(5
11	7.963	237470	0.57	2-furfuryl 2-oxo-3-butyl disulfide
12	8.112	253859	0.61	trans-4,5-Epoxynonane
13	9.030	89112	0.21	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-
14	9.315	134905	0.32	Nonane, 5-butyl-
15	9.438	82777	0.20	2,2,3,3,4,4 HEXADEUTERO OCTADECANAL
16	9.990	633222	1.52	2-METHOXY-4-VINYLPHENOL
17	10.925	136196	0.33	5-OXO-PYRROLIDINE-2-CARBOXYLIC ACID METHY
18	11.135	106234	0.25	Furan-2-carbohydrazide, N2-(3-indolylmethylene)-
19	11.351	100592	0.24	N-Phenethyl-2-methylbutylidenimine
20	12.021	822003	1.97	GUANOSINE
21	12.196	186823	0.45	TETRADECANE
22	12.633	413179	0.99	D-Allose
23	12.945	337816	0.81	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl
24	13.357	104510	0.25	Fumaric acid, ethyl 2-methylallyl ester
25	14.080	150327	0.36	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-
26	14.445	112781	0.27	1,4-Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol
27	15.462	469941	1.13	TETRADECANOIC ACID
28	15.755	836387	2.01	2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO-6-
29	16.222	2026208	4.86	Neophytadiene
30	16.282	196071	0.47	2-Pentadecanone, 6,10,14-trimethyl-
31	16.474	388322	0.93	Neophytadiene
32	16.672	840547	2.02	Neophytadiene
33	17.077	101970	0.24	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
34	17.132	770135	1.85	HEXADECANOIC ACID, METHYL ESTER
35	17.560	3529010	8.47	n-Hexadecanoic acid
36	18.172	210439	0.50	9H-Pyrido[3,4-b]indole
37	18.703	97279	0.23	Pentyl octadecyl ether
38	18.775	557253	1.34	9,12-Octadecadienoic acid, methyl ester
39	18.834	1066882	2.56	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
40	18.945	3033914	7.28	Phytol
41	19.260	147517	0.35	cis,cis,cis-7,10,13-Hexadecatrienal
42	20.473	1155192	2.77	Octanoic acid, 2-dimethylaminoethyl ester
43	20.758	290236	0.70	2-Pyrrolidinone, 1-[2-(4-piperidinyl)ethyl]-
44	21.897	333070	0.80	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
45	21.960	1469139	3.52	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
46	22.048	199203	0.48	octadecanoic acid, 3-oxo-, ethyl ester
47	22.216	3835477	9.20	2-Ethylbutyric acid, eicosyl ester
48	22.412	2190756	5.26	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl est
49	22.536	115002	0.28	Di-n-octyl phthalate
50	23.637	808478	1.94	Cyclohexaneacetic acid, .alpha.-methyl-.alpha.-propyl-, me
51	23.844	858365	2.06	Pentadecafluorooctanoic acid, pentadecyl ester
52	24.089	552630	1.33	Octadecanoic acid, 2,3-dihydroxypropyl ester
53	24.783	207844	0.50	Linolool oxide, TMS derivative
54	25.493	171299	0.41	.alpha.-Tocospiro B
55	33.528	2713144	6.51	Chondrillasterol
		41682729	100.00	

CONCLUSION

The edible plant species of *A.caudatus* 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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