Biospectra : ISSN: 0973-7057, Vol. 19(2), September, 2024, pp. 203-208

An International Biannual Refereed Journal of Life Sciences

Plant Science

ISSN : 0973-7057

GC-MS Phytochemical analysis of ethanolic leaf extract of *Croton sperciflorus* Morung of Dalma Range East Singhbhum, Jharkhand.

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Received : 25th June, 2024 ; Revised : 24th July, 2024

DOI:-https://doi.org/10.5281/zenodo.15062042

Abstract- *Croton sperciflorus* Morung is a traditional medicinal plant, which belongs to the family Euphorbiaceae. The main aim of the present investigation is to determine important bioactive compounds found in ethanolic leaf extract of *Croton sperciflorus* Morung of Dalma Range East Singhbhum Jharkhand. GC-MS analysis shows the presence of 47 phytochemicals, among those MOME INSOITROL shows highest peak area of 41.95% followed by Phythol 10.85%, ERGOST-5-E-N-3-OL 3.92%, Squalane 3.5%, 9,12,15-Octadecatrienoic acid, methyl ester (zzz) 3.03%. These phytochemicals are medicinally important bioactive compounds and possess antimicrobial, antifungal, antibacterial and other biological properties.

Key words: GC-MS, Phytochemicals, Antimicrobial, Antifungal, Antibacterial

INTRODUCTION

Croton sperciflorus Morung belongs to family Euphorbiaceae and commonly known as "Ban Tulsi" growing mainly on road side. It is an annual herbaceous weed with aromatic smell growing up to 1 to 2 feet tall. Leaves are alternate but a whorl of three leaves at the point of branching. The leaves are 3 to 5 cm long, lanceolate, both caulin and ramal wavy and toothed. Inflorescence is simple terminal raceme with white flowers. Flowers are unisexual but monoecious. Female flowers are found at the proximal end while male flowers at distal end. Staminate flowers are with 5 sepals and 5 white petals and 12-13 stamen. Pistilate flower is with 5 sepals and 3 carpels. Hypogynous disc of red gland present. Fruit is schizocarpic regma and seed is carunculate.

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Nowadays traditional and complementary medicines are widely used in many regions of the world. Plant products play an important role in preventing and curing human diseases. Traditional medicines are safe and cheap and easily available from surroundings. *Croton sperciflorus* Morung is one of the most important traditional medicinal plants of family Euphorbiaceae. The Powdered leaves are used to control high blood pressure and used as antiseptic and antitode.[1] It Is also used to treat liver and skin diseases, cuts and wounds, venereal sores and cholera.[2]

Croton sperciflorus Morung is rich in bioactive compounds including alkaloids, flavonoids, tannins, steroids.[1] Due to the presence of large no. of bioactive compounds, which are secondary metabolites, medicinal plant possesses properties such as antibacterial,[3] antifungal,[4] antidiabetic,[5] antiinflammatory,[6] antioxidant,[7] anticancer,[2] antimicrobial and antiparasitic[8].

The aim of the present study is to identify the phytochemicals present in the ethanolic leaf extract of *Croton sperciflorus* Morung by GC-MS analysis and their bioactivity by literature survey.

MATERIALS & METHODS

Collection of Plant Material

Fresh and healthy leaves of *Croton sperciflorus* Morung were collected from forest of Dalma range of East Singhbhum, Jharkhand by field trips during September to November. Collected leaves were washed properly under running tap water and also with distilled water. The leaves were than dried in the shade under room temperature and ground through motor and pestle to make powder form. The powdered sample was dissolved in methanol in 1:10 ratio i.e., 10 grams of leaf powder was mixed with 100 ml of methanol.[9] This solution was placed in incubator shaker at 28°C, 80 rpm for 72 hours. Then this solution is filtered with the help of Whatman filter paper no. 1. Filtrate was kept in room temperature in a dark place for 3 to 4 days to evaporate the methanol completely and the dry solid extract is obtained which is called stock.

GC-MS Analysis

From stock 10 mg of extract was taken and mixed with 1000 ml of ethanol. Extracts were mixed properly by using thermomixture and then centrifuged. The palate was removed and the supernatant was transferred to the new centrifuge tube. Then prepared test samples were sent to Advanced Instrumentation Research Facility at Jawaharlal Nehru University New Delhi. GC-MS analysis was done by QP 2010 ultra. Helium was used as carrier gas with flow rate of 16.3 mL/min and column flow rate of 1.21 mL/min. Injection temperature was 260°C and Injection mode was split and column oven temperature was 260°C. The total time taken for this analysis was 40 minutes. For identification of phytoconstituents retention time and molecular weight were compared with the GC-MS spectra database of online Wiley library and NIST (National Institute of Standard Technology).

RESULT & DISCUSSION

GC-MS analysis of ethanolic extract of *Croton sperciflorus* Morung revealed the presence of 47 phytochemicals by 47 peaks in chromatogram. The GC-MS chromatogram of 47 compounds is depicted in figure 1. Out of these 47 phytochemicals, MOME INOSITOL is found in higher concentration with highest peak area 41.95% and retention time. 11.899 minutes. Some other major phytochemicals are phytol with 10.85% of peak area. 9,12,15–Octadecatrienoic acid, methyl ester (ZZZ) with 3.03% of peak area, squalene with 3.50% peak area, Ergost-5-EN-3-OL with 3.92% peak area with retention time 27.526 minutes. NERYL LINALOOL ISOMER and fumaric acid, decyl-3-pentyl ester are found in lowest amount with peak area 0.05% and retention time 17.830 minutes and 19.030 minutes respectively. Percentage peak area, retention time, molecular weight, nature and molecular formula are given in table 1. Many bioactive compounds are effective against many diseases. The bioactivity of 10 chemical compounds is identified and mentioned in table 2.

GC-MS analysis of chloroform leaf extract of *Croton bonplandianus* (syn. *Croton sperciflorus*) 11 phytochemicals were reported.[1] All 11 phytochemicals are totally different from compounds identified in present study. Only four phytochemicals were reported in the n-hexane extract of seeds of *C. bonplandianus* by GC-MS.[2] Out of these 4 phytochemicals, 2 compounds are similar to the identified

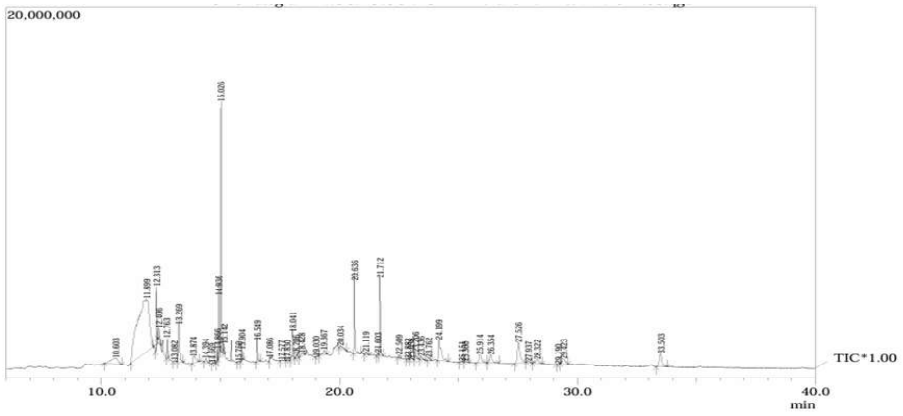

Fig. 1- Chromatogram

compounds of present study i.e., squalene and phytol. Squalene possesses antibacterial, antitumor, cancer preventive properties.[2] Phytol also possess antimicrobial, anticancer and anti-inflammatory properties.[2]

Table 1- Phytochemical compounds identified from medicinal plant *Croton sperciflorus* Morung

Peak#	R.Time	Area	Area%	Name
1	10.603	4009269	2.99	4-Hydroxy-2-methylpyrrolidine-2-carboxylic acid
2	11.899	56156452	41.95	MOME INOSITOL
3	12.313	3642362	2.72	Neophytadiene
4	12.406	1403207	1.05	2,6,8-Trimethylbicyclo [4.2.0] oct-2-ene-1,8-diol
5	12.763	1215208	0.91	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
6	13.082	132019	0.10	3-O-Methyl-d-glucose
7	13.269	4005387	2.99	Hexadecanoic acid, methyl ester
8	13.874	1790985	1.34	n-Hexadecanoic acid
9	14.394	491217	0.37	Palmitic Acid, TMS derivative
10	14.669	138697	0.10	13-HEXYL-OXA-CYCLOTRIDEC-10-EN-2-ONE
11	14.866	549819	0.41	9,12-Octadecadienoic acid, methyl ester
12	14.936	4050557	3.03	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
13	15.026	14526579	10.85	Phytol
14	15.142	351959	0.26	OCTADECANOIC ACID, METHYL ESTER
15	15.750	92489	0.07	Oxalic acid, 2-ethylhexyl nonyl ester
16	15.904	741432	0.55	Phytol, acetate
17	16.549	1435337	1.07	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
18	17.086	163970	0.12	2,4-DIISOPROPENYL-1-METHYL-1-VINYLCYCLOHE
19	17.577	159038	0.12	Dimethyl diallylmalonate
20	17.830	70340	0.05	NERYL LINALOOL ISOMER
21	18.041	2323916	1.74	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
22	18.206	585133	0.44	BENZENEACETIC ACID, A L P H A . -(1-HYDROXYCYC
23	18.428	849047	0.63	4-Hexyl-1-(7-methoxycarbonylheptyl) bicyclo[4.4.0]deca-2
24	19.030	72258	0.05	Fumaric acid, decyl 3-pentyl ester
25	19.367	967710	0.72	9,10,12,13-Tetrabromooctadecanoic acid
26	20.034	716135	0.53	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropy
27	20.636	4691784	3.50	Squalene
28	21.119	358462	0.27	8-Hexadecene, 8,9-diheptyl-
29	21.603	174399	0.13	GERANYL LINALOOL ISOMER B
30	21.712	5328609	3.98	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,
31	22.509	297912	0.22	(6E,10E,14E,18E)-2,6,10,15,19,23-HEXAMETHYL-1,6,1
32	22.882	89518	0.07	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-
33	23.038	153771	0.11	Succinic acid, di(dodec-9-yn-1-yl) ester
34	23.206	995917	0.74	Silane, dimethyl (dimethylpentyloxysilyloxy) tetradecyloxy-
35	23.436	410668	0.31	Nonacosan-14-one
36	23.762	392771	0.29	17-Pentatriacontene
37	24.199	4688453	3.50	2H-1-BENZOPYRAN-6-OL, 3,4-DIHYDRO-2,5,7,8-TET
38	25.151	142388	0.11	SOLANESOL
39	25.300	125984	0.09	Hexacosyl acetate
40	25.914	2054032	1.53	CHOLEST-5-EN-3-OL, 6-NITRO-, ACETATE (ESTER), (
41	26.354	1826453	1.36	Lanost-8-en-3-ol, (3. beta.)-
42	27.526	5243992	3.92	ERGOST-5-EN-3-OL
43	27.937	347282	0.26	Phytol, acetate
44	28.322	1253830	0.94	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,
45	29.190	115094	0.09	Ergost-25-ene-3,6-dione, 5,12-dihydroxy-, (5. alpha., 12.bet
46	29.423	1377072	1.03	24-Norursa-3,12-diene
47	33.503	3170819	2.37	Phytyl decanoate
		133879732	100.00	

Table 2. Biological activity of identified phytochemicals from medicinal plant *Croton sperciflorus* Morung

S. No.	Name of the Compounds	M.F.	M. W.	Chemical Nature	Biological Activity
1	MOME INOSITOL	$C_7H_{14}O_6$	194	Polysaccharide	Anti-cirrhotic, lipotrophic, anti-alpecic Cholesterolytic and have sweetining properties.[10,11]
2	Neophytadiene	$C_{20}H_{38}$	278	Diterpene	Anti-bacterial, anti-inflammentary, anti-oxidant,[12,13] anti-microbial, analgesic, anti-pyretic[14]
3	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Fatty acid ester	Anti-bacterial, anti-oxidant, anti-tumor, immunostimutant.[15] Hypochlosterolemic, lubricant, anti-alopecic[16] anti-microbial, anti-oxidant[17]
4	n-Hexadecanoic acid	$C_{16}H_{34}O_2$	256	Fatty acid	Anti-inflammatory, ant-oxidant, anti-androgenic, hypocolesterolenia[18]
5	Octadecadienoic acid, Methyl ester	$C_{19}H_{38}O_2$	298		Anti-fungal and anti-oxidant[19]
6	9,12-Octadecadienoic acid,Methyl ester	$C_{19}H_{34}O_2$	294	Fatty acid, methyl ester	Anti-histamenic, hepatoprotective, hypocholesterolemic, anti-eczemic.[20] anti-inflammatory, anti-arthritic, hepatoprotective, anti-androgenic nematicide, anti-acne, anti-corroneri and insectifuse[21]
7	9,12,15 Octadecatrienoc acid, Methyl ester (ZZZ)	$C_{19}H_{32}O_2$	292	Fatty acid, methyl ester	Anti-inflammatory, anti-histamic, anti-eczemic, anti-acne[5]
8	Phytol	$C_{20}H_{40}O$	296	Triterpne	Anti-microbial, anti-cancer, diuretic, anti-inflametery[2,5]
9	Squalene	$C_{30}H_{50}O$	410	Terpenoid	Anti-bacterial, anti-oxidant, anti-tumor, cancer preventive, immunostimulant, chamopreventive[15,22,23]
10	Ergost 5-en-3-ol	$C_{28}H_{48}O$	400	Sterol	Liver disease, jaundice, anti-oxidant, anti-cancerous[12] anti-microbial activity, hypocholesterolemic[14]

CONCLUSION

The GC-MS analysis indicate that the plant *Croton sperciflorus* Morung contains number of phytochemicals. Many bioactive phytochemicals have biological medicinal properties, which made them compelling to be used in the field of medicines as well as traditional medicine. Many phytochemicals possess antimicrobial, antibacterial, antifungal, anti-inflammatory, anticancer, antioxidant and other properties. So, this supports the use of plant for the remedy of variety of diseases. These properties made this plant much more viable in the field of medicine. Many phytoactive compounds were still to be identified for other influential biological properties where as some bioactive compounds were yet to be discovered.

ACKNOWLEDGEMENT

Authors are thankful to AIRF, JNU, New Delhi for GC-MS analysis and Dr (Prof.) Kunul Kandir, Head of the Department of Botany, Ranchi University, Ranchi, for providing lab facilities to conduct research work. Authors are also thankful to Dr. Amit Patnayak, Department of Botany, Ranchi University, for his coopertaion and suggestions in this work.

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