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Original Article

Spectroscopic Analysis of Lagos spinach- seed (*Celosia argentea* Linn.)

Falguni Saraswat[1,*], Mukeshkumar Nariya[1], Vinay J Shukla[2]
[1]Department of Pharmacology, ITRA, Jamnagar, Gujarat, INDIA.
[2]Pharmaceutical Chemistry Lab, ITRA, Jamnagar, Gujarat, INDIA.

ABSTRACT

Background: *Celosia argentea* Linn. *(C. argentea)* commonly known as Cock's Comb. It's a leading leafy vegetable used for treatment of anti-inflammatory, antioxidant and possessing immunostimulating activities. This species contains a surplus of phytochemicals including terpenoids, glycosides, flavonoids and tannins. The full complement of bioactive chemicals has yet to be identified, which is a critical step in explaining its medical use. The *Celosia* genus contains around 30 species, the majority of which have had little phytochemical or pharmacological research done on them. The aim of this study is to get a better chemo-semantics of *Celosia* species, large-scale multi-targeted metabolic profiling and fingerprinting approaches was used. **Methods:** FTIR, GC-MS and LC-MS spectra of extracted components were analyzed with multivariate data from *C. argentea*. The crude extract was examined at different wavelengths from 4000 cm^{-1} and 400 cm^{-1} by using Shimadzu 8400 Spectrophotometry system and the distinctive peaks were detected. For GC-MS analysis, sample was extracted with methanol (as per API procedure), filtered in ashless filter paper and extract was concentrated. The NIST Ver. 2.0 - Year 2005 library was used for chemical detection. **Results:** The FTIR profile showed different peaks ranging from 4000 cm^{-1} and 400 cm^{-1} and spectrum with confirmed presence of alcohols and phenols, amines, alkanes, alkynes aldehydes and ketones, carboxylic acids functional groups in methanol extract of *C. argentea*. Analysis of the mass spectrum GC-MS analysis provides different peaks determining presence of Pentadeconic acid, n-Hexadecoic acid, 9-12-Octadecadienoic acid, Cis-vaccenic acid, Octadecanoic acid. The results of LC-MS analysis provide specific data of bioactive components which may suggest the Butralin, Fulvine, Dihydrocapsaicin, Lisinopril, 9Z-Octadecen-12-yonic acid, N-Hexadecanoylpyrrodine etc. The phytoconstituents may use to treat diseases and further insight in future researches on *Celosia argentea*. **Conclusion:** The molecules reported through above exploration of spectral techniques may justify traditional therapeutic manifestation of *C. argentea* and useful in activity guided isolation.

Keywords: Active components, *Celosia argentea*, FTIR, GC-MS, LC-MS, Phytochemical.

Correspondence

Ms. Falguni Saraswat
Department of Pharmacology, ITRA, Jamnagar, Gujarat, INDIA.
Email id: saraswatfalguni@protonmail.com

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INTRODUCTION

Herbal therapies are widely used in many industrialized countries, with complementary and alternative medicines (CAMs) gaining popularity in the United Kingdom and the rest of Europe, as well as North America and Australia.[1] Herbal medicines are, therefore, often viewed as a balanced and moderate approach to healing and individuals who use them as home remedies and over-the-counter drugs spend huge amount of money (in excess of billions of dollars) on herbal products. This explains in part the reason sales of herbal medicines are booming and represents a substantial proportion of the global drug market.[2]

Herbs are constantly being screened as antidiabetic, anti-inflammatory, antioxidant, anti-bacterial, antiapoptosis, antidiarrhoeal, anthelmintic, antiaging, antimalarial, antispasmodic, hepatoprotective and immuno-stimulating activities.[3] The herbs contain a variety of bioactive components that can be detected by GC or LC-MS (at concentrations of less than 1 mg) and FTIR. FTIR methods are more explored due to its simplicity, cost-effectiveness, and speed of the tests for detecting phytocomponents, they can be employed together or separately.[4-6]

Celosia argentea Linn. is an herbaceous plant which belongs to the family Amaranthacaceae is known as *Mayurshikha* in Ayurveda and one of the leading leaf vegetables in south-western Nigeria.[7] The seeds contain 11.6-17% of protein and 6.4-10.9% of fatty oil. Flowers are used in menorrhagia, blood disorders whereas seeds and roots yield triterpenoid, saponins. An alcoholic extract of seeds possess significant diuretic activity.[8] Till date, No Spectroscopic analysis has been done on *Celosia argentea* seed powder. Therefore, the present study was carried out to characterize the bioactive components present in methanolic extract of seed powder *C. argentea* using FTIR, GC-MS and LC-MS spectroscopic methods.

MATERIALS AND METHODS

Plant Material and Preparation of Powder Extract

Seeds of *C. argentea* were collected in the month of November and December naturally from Bangalore, Karnataka (3200ft.,12.9°N,77.5°E). The collected plants were washed, open-air-dried under the shade, pulverized in to a moderately coarse powder. Powdered sample was extracted with methanol using soxhlet apparatus for 48 hr. Extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract contains both polar and non-polar phytocomponents.

FTIR Spectroscopic Analysis

FTIR analysis was performed using *C. argentea* seed powder sample subjected to Shimadzu 8400 Spectrophotometry system. Spectroscopy grade KBr was used to grind the samples, and a pellet of uniform mixture was formed using a hydraulic press. The pellet's maximal opacity was

ensured, and IR spectra were obtained between 4000 cm^{-1} and 400 cm^{-1}. The FTIR's peak values were recorded. For spectrum confirmation, each and every analysis was done twice.

GC-MS Analysis

GC MS analysis was carried out on a GC-MS (Shimadzu, model GC-MS-QP-2010 ultra). Sample and gas chromatograph interfaced to a mass spectrometers (GC-MS) employed the following conditions: Column Restek Rxi-1ms silica capillary column (0.25mm ID ×0.25μ M d*f*, composed of 100% Dimethyl poly siloxane) at max. temperature 350°C, operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume as per requirement (Split ratio of 10:1). Sample was prepared according to ratio 10%w/v. Injector temperature was 250°C with split injection mode and ion-source temperature was 200°C. The Scan speed was 3333 with M/Z range from 40 to 999 and MS detector voltage supply of 1Kv. Total GC running time was 25min.

Identification of Components

By comparing the average peak area of each component to the total areas, the relative percentage quantity of each component was computed. The NIST (National Institute of Standards and Technology) Ver.2.0-Year 2005 library was used for the detection. The NIST database, which contains over 62,000 patterns, was used to interpret the GC-MS results. The unknown component's spectra were compared to the known component's spectrum contained in the NIST collection. The components of the test materials were identified by their name and molecular weight.

LC-MS Analysis

LC-MS analysis was carried out on a 1290 Infinity UHPLC System, 1260 infinity Nano HPLC with Chipcube, 6550 iFunnel QTOFs and Liquid chromatograph interfaced to a mass spectrometer (LC-MS) instrument of mass range 50-3200amu with mass resolution 40000FWHM, accuracy of less than 1ppm employing the following Liquid chromatograph conditions: Column of Hypersil GOLD C_{18} 100 x 2.1mm-3 MICRONat 25°C with injection volume of 5 μl, mobile phase of Positive ionization mode (A = 0.1% Formic acid in Water, B =Acetonitril)with flow of 0.6 ml / min in Gradient (B= 5% at 0 min, B = 95% at 12 min, B = 95% at 20 min; B = 5% at 22 min, B = 5% at 25 min) in running time of 25min.

QTOF condition Auto Ms/Ms mode conditions: Ion Mode of Dual AJS ESI with drying gas temperature (250°C) and flow (13 L/min); Vaporize / sheath gas Temperature (350°C). The chamber of 4.23 μA with capillary volume of 0.051 μA, Nebulizer at 35 psig; Fixed Collision Energies (10, 20, 30, 40, 50 V); Precursor per cycle Max 5; Precursor Threshold of 400 counts with Scan speed of 25000 counts/spectrum.

LC-MS Compound Detection

Metabolite detection in non-target metabolic tests is largely based on search based on manual confirmation. First, the m / z molecular value of interest is searched against databases. Metabolites with molecular weights within the specified tolerance range in the amount of question m / z are available in the information as storage ID. Thereafter, the true combinations of these placement indicators was subjected to tandem MS (MS / MS) studies alongside the sample. By comparing the MS / MS spectra with the time of storage of true chemicals and interest molecules in the sample, the identity of the molecules can be verified.[9] However, the identification of putative from multi-based search is rarely different, due to the presence of isomers and the relative accuracy of multiple spectrometers. In some cases, a single ion molecule can have more than 100 placement detection, making manual verification expensive and difficult. As a result, this method works only for a limited number of molecules.[10]

RESULTS

The results of FTIR peak values and functional groups, intensity and assignment were represented in Table 1. The FTIR spectrum profile was illustrated in the Figure 1. FTIR spectrum confirms presence of alcohols and phenols, amines, alkanes, alkynes aldehydes and ketones, carboxylic acids functional groups. Hence, the methanol extract of *C. argentea* subjected to FTIR analysis for the identification of chemical constituents which have rigidity under molecules present in *C. argentea* analysis was performed on methanolic extract of *C. argentea* seed powder. Analysis of the mass spectrum GC-MS was carried out utilising the National Institute of Standards and Technology (NIST) database, which has over 62,000 patterns. The components of the test materials were identified by their molecular formula, mass, retention time (RT), name of compound, molecular weight, area. The methanolic extract of *C. argentea* yielded at least five chemicals, according to GC chromatogram data (Figure 2 and Table 2). GC chromatogram data peaks was interpreted with unknown spectra from NIST library collection and compared with Hits score, RT data of MS peak data (Figures 2.1- 2.5).

LC-MS analysis was performed on methanolic extract of *C. argentea* seed powder showed total 49 active principles with their molecular

Table 1: FTIR peak values and functional groups of *C. argentea* Linn.

Peak values	Intensity	Assignment	Functional groups
3302.24	str	C-H	Alkynes
2924.18	str	CH_3, CH_2&CH 2 or 3 bands	Alkanes
2854.74	str	CH3, CH_2&CH 2 or 3 bands	Alkanes
1728.28	str	C=O(saturated aldehyde)	Aldehyde and Ketones
1666.55	var	C=O (symmetry reduces intensity)	Alkenes
1550.82	med-str	NH_2 scissoring (1° amines)	Amines
1450.52	str	α – CH_2 bending	Aldehyde and Ketones
1365.65	med	CH_2 & CH_3 deformation	Alkanes
	med	O-H bending (in-plane)	Alcohols and Phenols
1219.05	med-str	O-C (sometimes 2 peaks)	Carboxylic acid and Derivatives
1057.03	med	C-N	Amines
648.10	str	C-H deformation	Alkynes
509.22	-	-out of plane-	-

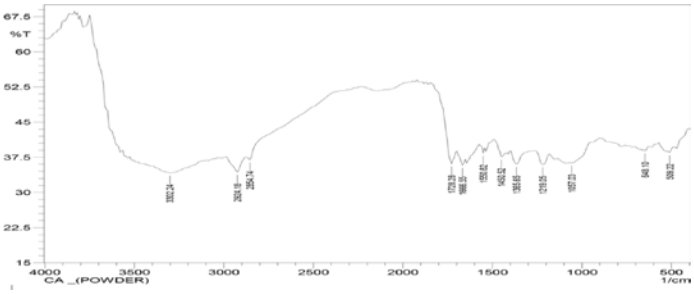

Figure 1: FTIR spectrum of *C. argentea* Linn.

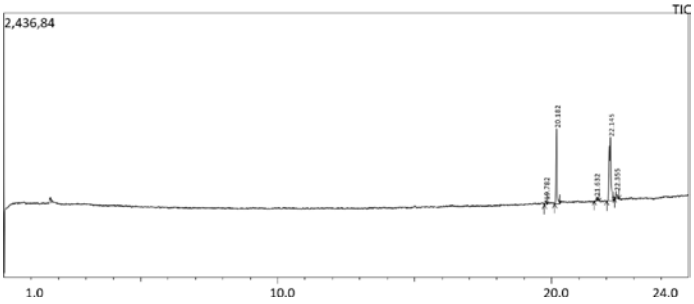

Figure 2: GC Chromatogram of *C. argentea*.

Table 2: GC-MS analysis of methanolic extract of *C. argentea*.

Real Time	Name of compound	Mol. Formula	Mol. Weight	Area
19.782	Pentadeconic acid	$C_{17}H_{32}O_2$	270	111718
20.182	n-Hexadecoic acid	$C_{16}H_{32}O_2$	256	1613879
21.632	9,12-Octadecadienoic acid	$C_{19}H_{34}O_2$	294	96736
22.145	Cis-vaccenic acid	$C_{18}H_{34}O_2$	282	2755141
22.355	Octadecanoic acid	$C_{18}H_{36}O_2$	284	151313

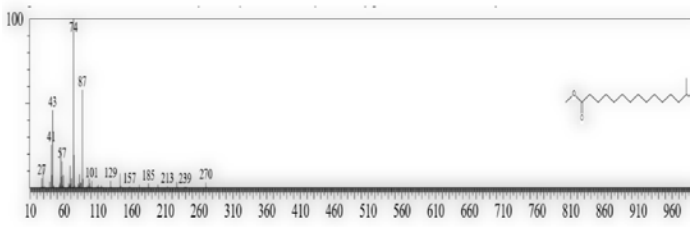

Figure 2.1: MS peak and structure of Pentadeconic acid.

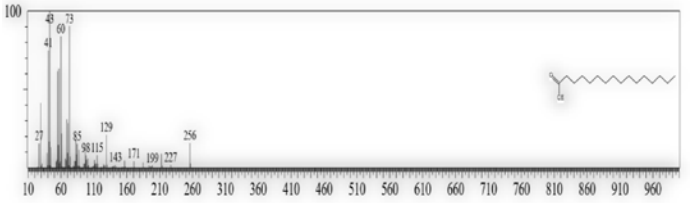

Figure 2.2: MS peak and structure of n-Hexadecoic acid.

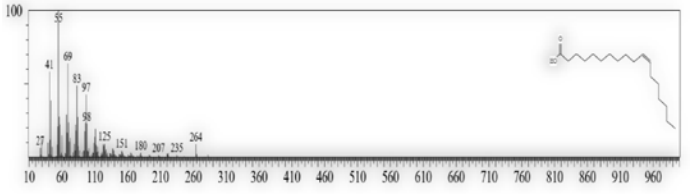

Figure 2.3: MS peak and structure of 9,12-Octadecadienoic acid.

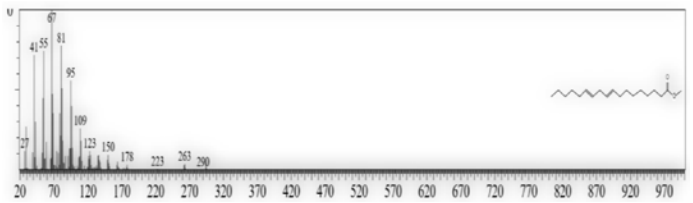

Figure 2.4: MS peak and structure of Cis-Vaccenic acid.

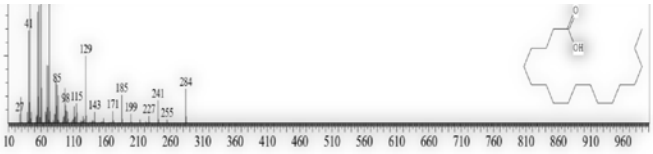

Figure 2.5: MS peak and structure of Octadecanoic acid.

Table 3: LC-MS analysis of methanolic extract of *C. argentea* seed powder.

Name of compounds	Formula	Mass	RT	Score
5-Methyl-2-phenyl-2-hexenal	$C_{13}H_{16}O$	188.1186	6.762	80.6
Arnebinol	$C_{16}H_{20}O_2$	244.1475	6.784	89.44
Istamycin C0	$C_{16}H_{34}N_4O_4$	346.2563	7.837	85.16
Adrenic Acid	$C_{22}H_{36}O_2$	332.2763	9.077	57.16
ent-16-Kauren-19-ol acetate	$C_{22}H_{34}O_2$	330.2605	10.175	58.6
3'-Methoxy-[6]-Gingerdiol3,5-diacetate	$C_{22}H_{34}O_6$	394.2348	10.897	93.77
Macrophorin C	$C_{22}H_{32}O_5$	376.2232	12.33	84.35
17-Propyl-5alpha-androst-2-en-17beta-ol	$C_{22}H_{36}O$	316.280	13.274	61.12
Methyl-3b,24-dihydroxy-11,13(18)-oleanadien-30-oate	$C_{31}H_{48}O_4$	484.3661	14.109	47.64
3beta-Fluoro-5alpha-androstan-17beta-ol	$C_{19}H_{31}FO$	294.2378	14.332	74.88
Ajugasterone C	$C_{27}H_{44}O_7$	480.3098	14.878	60.03
Minaprine	$C_{17}H_{22}N_4O$	298.1787	17.664	89.53
(3xi,6E)-1,7-Diphenyl-6-hepten-3-ol	$C_{19}H_{22}O$	266.1719	17.263	40.87
Granisetron	$C_{18}H_{24}N_4O$	312.1947	18.535	92.16
Ibogaine	$C_{20}H_{26}N_2O$	310.2003	18.947	44.19
Cannabinol	$C_{21}H_{26}O_2$	310.1942	19.268	58.49
Butralin	$C_{14}H_{21}N_3O_4$	295.1524	4.364	97.7
Fulvine	$C_{16}H_{23}NO_5$	309.1568	4.592	97.03
Norharman	$C_{11}H_8N_2$	168.0682	4.826	85.8
Condurango glycoside A	$C_{53}H_{78}O_{17}$	986.5068	5.699	51.52
CDP-DG(18:1(9Z)/20:4(5Z,8Z,11Z,14Z))	$C_{50}H_{83}N_3O_{15}P_2$	1026.5384	6.227	50.0
Annofoline	$C_{16}H_{25}NO_2$	263.1877	6.784	95.72
Almitrine	$C_{26}H_{29}F_2N_7$	477.2468	6.855	83.53
Dihydrocapsaicin	$C_{18}H_{29}NO_3$	307.214	8.002	98.02
N-Jasmonoylisoleucine	$C_{18}H_{29}NO_4$	323.2074	7.225	65.05
Lisinopril	$C_{21}H_{31}N_3O_5$	405.2258	7.563	98.17
Penbutolol	$C_{18}H_{29}NO_2$	291.2179	8.147	67.8
Dyclonine	$C_{18}H_{27}NO2$	289.2032	8.284	79.74
Dihydrodeoxystreptomycin	$C_{21}H_{41}N_7O_{11}$	567.2887	9.075	90.35
N-Formyl-norleucyl-leucyl-phenylalanyl-methylester	$C_{23}H_{35}N_3O_5$	433.2576	9.162	84.89
19-Noretiocholanolone	$C_{18}H_{28}O_2$	276.2082	14.621	82.56
Panaquinquecol 1	$C_{18}H_{28}O_3$	292.2027	11.243	81.32
9Z-Octadecen-12-ynoic acid	$C_{18}H_{30}O_2$	278.2236	12.09	96.14
Petroselinic acid	$C_{18}H_{34}O_2$	282.255	14.089	95.97
Sclareol	$C_{20}H_{36}O_2$	308.2705	14.337	95.59
dl-alpha-Tocopherol nicotinate	$C_{35}H_{53}NO_3$	535.4	15	81.04
23-Acetoxysoladulcidine	$C_{29}H_{47}NO_4$	473.3509	15.607	83.73
Mycinamicin VII	$C_{29}H_{47}NO_7$	521.3473	16.301	50.9
Myxalamid C	$C_{24}H_{37}NO_3$	387.2761	17.446	94.43
N-Hexadecanoylpyrrolidine	$C_{20}H_{39}NO$	309.3023	19.485	96.44

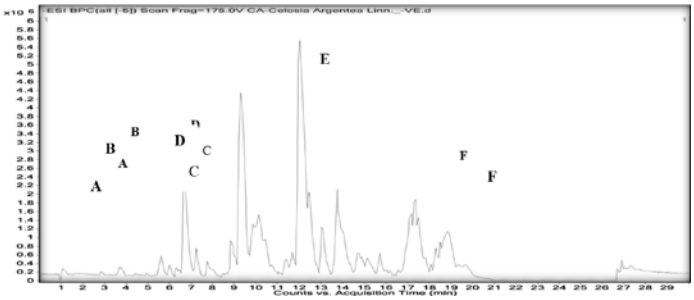

Figure 3: LC Chromatogram of C. *argentea*.
(A= Butralin; B= Fulvine; C=Dihydrocapsaicin; D=Lisinopril; E=9Z-Octadecen-12-yonic acid; F=N-Hexadecanoylpyrrodine)

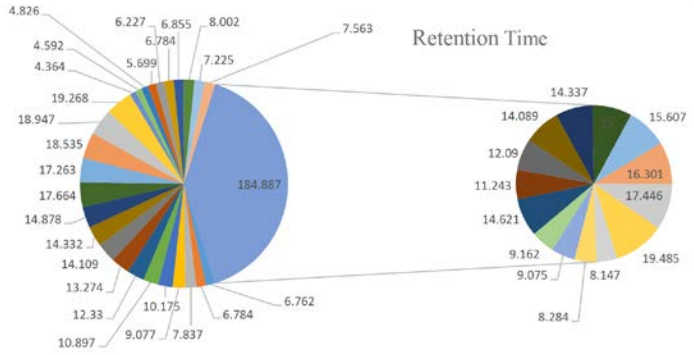

Figure 4: LC-MS (Compiled Retention Time data provided by SAIF Bombay) of *C. argentea*.

formula, mass, retention time (R*t*), area, score are as (Table 3 and Figure 3). Combined data of LC-MS (RT) of *C. argentea* Figure 4.

DISCUSSION

Nature is a one-of-a-kind source of phytochemically diverse structures, many of which have interesting biological activity and therapeutic characteristics. An active search for new lead compounds for the development of novel pharmacological therapies is particularly crucial in the context of the worldwide spread of diseases such as AIDS, chronic diseases, and a variety of malignancies. It is difficult to establish definite functionality and structure–activity correlations regarding the effects of phytochemicals in biological systems activity with the current information presented in this research.[11] This is because to the abundance of phytochemicals with similar chemical structures, as well as the intricacy of physiological responses. Furthermore, based on the number of phytochemicals discovered thus far, nature must have many more in store. Many more phytochemicals should be identified as synthesis methodology improves and more sophisticated isolation and analytical techniques are developed.[12] According to the literature, *Celosia argentea* possess a variety of unique qualities, including antidiaherrol, anti-inflammatory, antioxidant, anti-diabetic, and anti-hepatotoxic. *C. argentea* has extensive medicinal significance in folk use, in addition to the records in published publications. Saponin, cyclic-peptides, phenols, fatty acids, amino acids, and minerals are found in *C. argentea*, with saponin being the primary pharmacological active component. Saponin may be a good target for further research because to its bioactivities.[13] Spectroscopic techniques should be used to identify more promising bioactive chemical classes or individual compounds for further drug-disease correlation.

FTIR Based on the peak value in the region of infrared radiation, the FTIR spectrum was utilized to determine the functional group of the active components. Above data give us prediction of compound as FTIR represents data of 12 compounds with their peak value, intensity, assignment of bonds present and functional group present. The use of FTIR spectroscopy to detect biomolecular composition has been proven to be a reliable and sensitive approach. Whereas GC-MS and LC-MS analysis of compounds 5 and 40 respectively with detailed structure, name of compound, area, molecular formula etc. which can be specifically used towards predication of a moiety active against specific disease.

CONCLUSION

According to the findings, the increased extraction capacity of methanol could have resulted in the production of a large number of active substances responsible for a wide range of biological functions. Methods are sequential pattern to predict and confirm the components present were FTIR places key role in identification of functional group with described range which helps further specifically differentiate the specific bioactive component present in compound through LCMS interpretation. Whereas GCMS describes us the data listing Fatty acids present in *C. argentea*. In the present study, the molecules reported through above exploration of spectral techniques may justify traditional therapeutic manifestation of *C. argentea* and useful in activity guided isolation. Selection of compound is dependent to chemoinformatic outlet of sequential study of this study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

FTIR: Fourier transform infrared; **GCMS:** Gas chromatography mass spectroscopy; **LCMS:** Liquid chromatography followed by mass spectroscopy; **NIST:** National institute of standard and technology; **MS:** Mass spectroscopy

REFRENCES

1. Anastasi J. The use of complementary and alternative medicine by the American public. Institute of medicine; 2005.
2. World Health Organization. WHO Monogr Sel Med Plants. 1999.
3. Karthiyayini R, Nithiya. Pharmacognostic and preliminary phytochemical studies of *Celosia argentea*, L. Leaf. Int J Pharmacogn Phytochem Res. 2015;7(2):237-9.
4. Liebler DC, Burr JA, Philips L, Ham AJL. Gas chromatography-mass spectrometry analysis of Vitamin E and its oxidation products. Anal Biochem. 1996;236(1):27-34. doi: 10.1006/abio.1996.0127, PMID 8619492.
5. Aysal P, Ambrus AD, Lehotay SJ, Cannavan A. Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. J Environ Sci Health B. 2007;42(5):481-90. doi: 10.1080/19312450701392490, PMID 17562455.
6. Ibrahim M, Aal MAE. Spectroscopic study of the interaction of heavy metals with organic acids. Int J Environ Pollut. 2008;35(1). doi: 10.1504/IJEP.2008.021134.
7. Kanu CL, Owoeye O, Imosemi IO, Malomo AO. A review of the multifaceted usefulness of *Celosia argentea* Linn. Eur J Pharm Med Res. 2017;4(10):72-9.
8. Khare CP. Indian medicinal plants—an illustrated dictionary, New Delhi: First Indian reprint. Vol. 1. Springer (India) Pvt. Ltd.; 2007. p. 134.
9. Cui Q, Lewis IA, Hegeman AD, Anderson ME, Li J, Schulte CF, *et al*. Metabolite

identification via the madison metabolomics consortium database. Nat Biotechnol. 2008;26(2):162-4. doi: 10.1038/nbt0208-162, PMID 18259166.
10. Smith CA, O'Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, *et al.* Metlin: A metabolite mass spectral database. Ther Drug Monit. 2005;27(6):747-51. doi: 10.1097/01.ftd.0000179845.53213.39, PMID 16404815.
11. Harvey A. Strategies for discovering drugs from previously unexplored natural products. Drug Discov Today. 2000;5(7):294-300. doi: 10.1016/s1359-6446(00)01511-7, PMID 10856912.
12. Hamburger M, Hostettmann K. 7. Bioactivity in plants: The link between phytochemistry and medicine. Phytochemistry. 1991;30(12):3864-74. doi: 10.1016/0031-9422(91)83425-K.
13. Du Z, Qu X. Analysis on Fatty Acids in *Celosia argentea* L. Seed Oil by Gas Chromatography. Journal of Jiangxi Normal University (natural Science). 2002 Jan 1;26(2):110-2.

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