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HUMAN PATHOGENIC ANTIMICROBIAL ACTIVITY AND GC-MS ANALYSIS OF CARALLUMA TRUNCATO-CORONATA (SEDGW.) GRAVELY & MAYUR

P Ravikumar
Post Graduate and Research Department of Botany, DST-FIST Sponsored
Government Arts College (Autonomous) Coimbatore 641018 India.

***Abstract*— Caralluma truncato-coronata (Sedgw.) Gravely & Mayur is one of the endangered and rare genera of the Dogbane family, Apocynaceae was extracted by ethanolic Soxhlet method and was phytochemically screened by GC-MS. Among the compounds screened, the bioactivity and the name of the compound viz., Styrene, Deferiprone, Amyl acetate, Cetyl alcohol, Docosanol, Octadecene, Stearic acid, Phytol, Diacetylverrucarol, an amine alkene, a terpenoid and a cyano compound were identified. This report is the first of its kind to analyse the ethanolic constituents of C.truncato-coronata using GC-MS.The results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of C.truncato-coronata for various ailments by traditional practioners. The antimicrobial susceptibility testing on the human pathogenic bacteria and fungi on Agar Well diffusion Method in MHA significantly recorded the maximum inhibitory zone in Candida tropicalis and Enterococcus faecalis.The isolation of individual phytochemical constituents may proceed to find a novel drug.**

***Key words*—. Caralluma truncato-coronata, Gravely&Mayuris, etc**

I.INTRODUCTION

Caralluma truncato-coronata (sedgew.) Gravely&Mayuris a genus of flowering plants in the dogbane family Apocynaceae, well known as Famine Food, Appetite Suppressant and Thirst quencher1 consisting of about 120 species. Ethno botanically it is Generally Recognized As Safe (GRAS) status for use as a neutraceutical to combat the most serious public health concern, obesity. Many species of Caralluma are commonly used as traditional medicine for the treatment of rheumatism, diabetes, leprosy, paralysis, and inflammation and have antimalarial, antitrypanosomal, anti-ulcer, antioxidant, antinociceptive, and antiproliferative activities2. The phytochemicals in fruits, vegetables Spices and traditional herbal medicinal plants have been found a vital protective role against many human chronic diseases including cancer and cardiovascular diseases. Phytocomponents including pregnane glycosides, flavonoid glycoside, flavones, magastigmane glycosides, pregnane steroids, steroidal glycosides, saturated and unsaturated hydrocarbons, aromatic and non-aromatic volatile compounds, and β-sitosterol are reported to be radical Scavengers and inhibitors of Lipid Peroxidation3. Availability of pregnane glycosides in Caralluma is an indication of the appetite-suppressant property of this genus. This coupled with the GRAS status of the extract of C. fimbriata has opened the possibility of developing an anti-obesity/appetite-suppressant product from other species of Caralluma4. Due to uniqueness of curing different ailments the present investigation was executed to determine the possible phytochemical components of the Ethonolic extract of Caralluma truncato-coronata using GC-MS and its human pathogenic antibacterial and antifungal activity.

II.MATERIALS AND METHODS

The plant material used was collected from the natural habitats of Madukarai, Coimbatore, Tamil Nadu, India and identified and authenticated by Botanical Survey of India (BSI), Coimbatore, India as Caralluma truncato-coronata (Sedgw.) Gravely & Mayur (Certificate No. BSI/SRC/5/23/2012-13/Tech.1375).The voucher specimen was deposited in the Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous) Coimbatore-641018.

III.EXTRACTION OF DRIED PLANT

The fresh whole plants were carefully washed with tap water to remove soil particles and adhered debris, rinsed with distilled water, and air-dried for 1 hour; it was cut into 1x1mm pieces and dried in shade for two weeks. The bone dried material were ground into powder, sieved in the British Standard Sieve Number 10 and stored in the desiccators until use.

IV.PREPARATION OF EXTRACT

The shade dried and powdered C.truncato-coronata was extracted with ethanol by Soxhlet. The extracts were rotary evaporated at maximum temperature of 45°C and stored in a vial for further GC-MS analysis, in the Chemistry Division, The South Indian Textile Research Association, Coimbatore 641014. GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass-spectrometer (GC-MS) instrument was used, employing the following conditions: Column DB35-MS standard non-polar column with a specifications of 30 mts, 0.25mm with film 0.25uM. Helium(99.999%)was used as a carrier gas at a constant flow of 1ml/min and an injection volume of 1 ul was employed (split ratio of 10:1) injector temperature 250C:ion-source temperature 280C.The oven temperature was programmed from 70C(isothermal for 2 min),to 260Cat 10C/Min. Mass spectra were taken at 70eV with a scan interval of 0.5 seconds and fragments from 45 to 450Da.Total GC running time was 39.03Mins.The Caralluma extract was dissolved in ethanol and filtered with polymeric solid phase extraction (SPE) column and analysed in GC-MS for different components.

V.IDENTIFICATION OF COMPONENTS

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard Technology Version 2011.The spectrum of the unknown component was compared with the spectrum of the known components stored in the MS Data Library. The name, molecular weight and structure of the test material were ascertained.

Antimicrobial Susceptibility Testing5 by Agar Well Diffusion Method in Muller Hinton Agar in 90mm plate (MHA) was tested in Bioline Laboratory, Coimbatore-641002.

VI.RESULTS

Phytocomponents in Ethonolic extract of Caralluma truncato-coronata by GC-MS

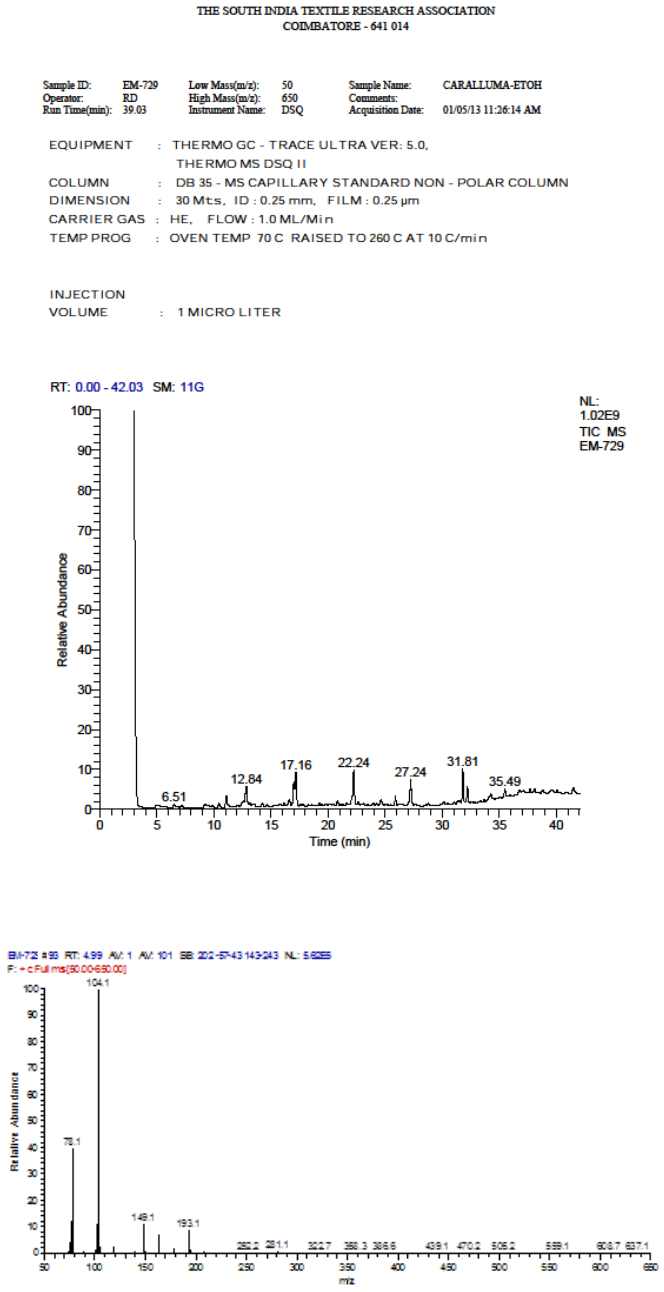

Figure 1

The ethanolic extract of Caralluma truncato-coronata was subjected to GC-MS analysis and the original full Mass Spectrogram, Library Search Results and the Library Search Graphics Table were presented in Figure1,with the Compound name, Retention Time, Molecular Formula, Molecular Weight and the Area % In Table 1.The striking results revealed that the presence of 1-Octadecene (11.91%), 1-Hexadecanol (8.91%), Phytol Isomer (8.22%), Acetic acid phenyl ester (3.68%), Squalene (3.44%), 1,3-diformyl-2-chloro-5-isoproylbenzene(2.55%), Cholesta-8-14-dien-3-ol,4,4-dimethyl-(3a,5a) Acetate (2.41%), (2S,4R,5S)-2-ethyl-5methoxycarbonyl-4-methyl-5-hexanolide (2.18%).

S.NO	RT	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	PEAK AREA%
1	4.99	Styrene	C_8H_8	104	1.09
2	6.51	Triethoxysilanola	$C_6H_{16}O_4Si$	180	1.00
3	9.22	4-Hydroxy-5,6-dimethylpyridin-2(1H)-one (Deferiprone)	$C_7H_9NO_2$	139	1.59
4	10.45	4'-Methylpent-3'-enyl Phenylglyoxylate	$C_{14}H_{16}O_3$	232	1.34
5	11.09	Acetic acid, pentyl ester	$C_7H_{14}O_2$	130	3.68
6	12.84	1-Hexadecanol	C16H34O	242	8.91
7	13.50	Behenic alcohol	$C_{22}H_{46}O$	326	0.97
8	14.23	2,2-Dideuteroootadecanal	$C_{18}H_{34}D_2O$	268	1.11
9	20.85	3,4-Ethylenedioxythiophene-2-carboxaldehyde	$C_7H_6O_3S$	170	1.30
10	22.24	1-Octadecene	$C_{18}H_{36}$	252	11.91
11	23.97	2,2-Dimethyl-2,3-dihydrocyclopentano[b]indanone-1,4(4H)-dione	$C_{14}H_{12}O_2$	212	1.36
12	24.97	1,3-diformyl-2-chloro-5-isopropylbenzene	$C_{11}H_{11}O_2$	210	2.55
13	25.90	(2S,4R,5S)-2-Ethyl-5-methoxycarbonyl-4-methyl-5-hexanolide	$C_9H_{14}O_4$	186	2.18
14	28.77	Hexadecanoic acid, 2-methyl methyl ester	$C_{18}H_{36}O_2$	284	1.35
15	30.14	2-Ally-4-chloro-1,3-dimethoxy-5-(methoxymethyl)benzene	$C_{13}H_{17}ClO_3$	256	1.65
16	31.81	Phytol isomer	$C_{20}H_{40}O$	296	8.22
17	34.28	2,6,10,14,18,22-etracosahexaene, 2,6,10,15,19,23-hexamethyl(Squalen)	$C_{30}H_{50}$	410	3.44
18	35.49	3-Pyrrolidin-2-yl-propionic acid	$C_7H_{13}NO_2$	143	2.61
19	36.79	Isochiapin B	$C_{19}H_{26}O_6$	350	1.53
20	37.14	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)	$C_{34}H_{64}O_2$	504	1.45
21	38.10	Dimethoxyglyceroldocosy lether	$C_{27}H_{56}O_5$	46	1.78
22	38.81	Tetrakis(Dimethylsilylcarbodiimide)	$C_{12}H_{24}N_8Si_4$	392	1.01
23	39.56	1-Phenyl-1,1-dioxoethylene-4-peracetoxy-4-cyano-octane(Ethaneperoxoic aacid)	$C_{19}H_{25}NO_5$	347	1.75
24	41.50	Cholesta-8,14-dien-3-ol, 4,4-dimethyl-, (3á,5à)-Acetate	$C_{29}H_{48}O$	412	2.41

Table-1

The Hit spectrum and the Spectrum profile of GC-MS confirmed the presence of these components with their respective Retention Time. The individual fragmentation patterns of the components were illustrated in Figure -2.

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Compound Structure **Hit Spectrum**

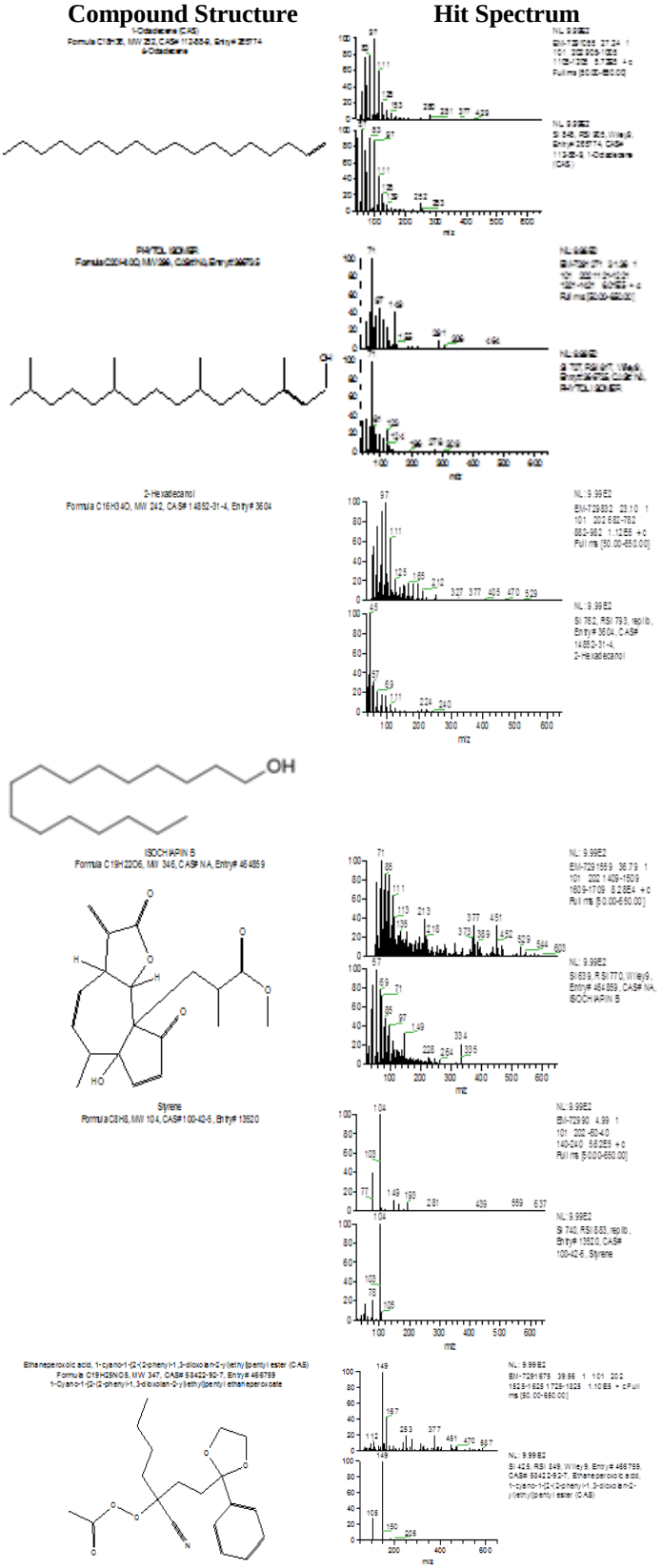

Figure 2 THE SOUTH INDIA TEXTILE RESEARCH ASSOCIATION

The Antimicrobial Susceptibility Testing by Agar Well Diffusion Method in Muller Hinton Agar in 90mm plate of the Human Pathogenic Test Organism is depicted in Fig 3

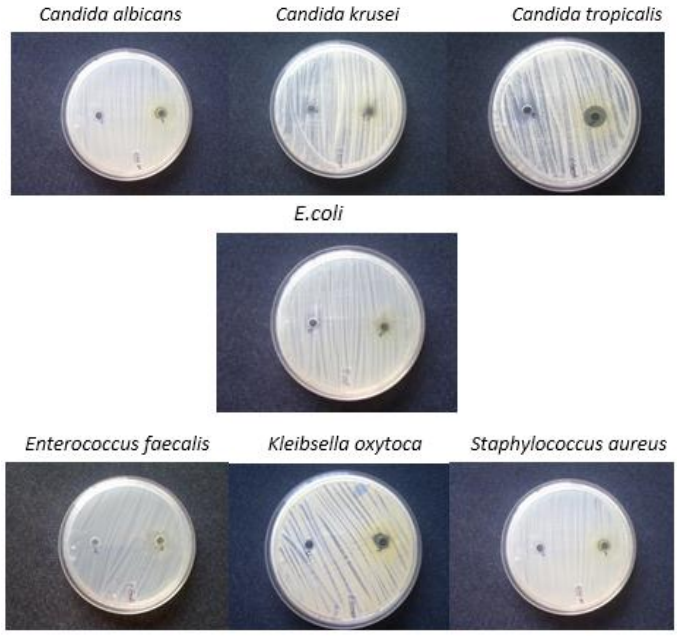

Figure 3

Test Organism	Control	Sample – 1 (mm)
Klebsiella oxytoca	6.0	8.0
Candida albicans	6.0	9.0
Candida krusei	6.0	7.0
Candida tropicalis	6.0	14.0
Staphylococcus aureus	6.0	9.0
Escherichia coli	6.0	7.0
Enterococcus faecalis	6.0	11.0

Zone of Inhibition Report (mm)

The ethanolic extracts of C.truncato-coronata were tested for their antibacterial and antifungal efficacies against both Gram-positive and Gram-negative bacteria, including some clinically-important Risk group 2 human pathogens, possessed a broad spectrum antibacterial and antifungal activity against a variety of both Gram-negative and Gram-positive human pathogenic organisms with a zone of inhibition from 7 to 14 mm. Ethanolic extract exhibited the most pronounced antibacterial effectiveness comparable to standard reference streptomycin, with more potency against Gram-positive than Gram-negative bacteria. These results revealed the potential of ethanolic extract as a suitable antibacterial lead compound that might be used for further development of other derivatives to increase the antimicrobial efficacy.

VII. DISCUSSION

The biochemical profile of C.truncato-coronata were characterised using GC-MS. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyse the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyse the ethanolic constituents of C.truncato-coronata using GC-MS. In addition to this, the

results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. GC-MS analysis of ethnologic extract showed the existence of various compounds with different chemical structures are medicinally valuable and possess various significant pharmaceutical applications. Styrene, volatile fluid terpenes aromatic hydrocarbons are the primary constituents of essential oils6 Hexadecanol7 fatty alcohol used as an Opacifier, emulsifier and lubricant, Phytol isomer 8, 9, acyclic diterpene alcohol precursor for vitamin E and K1, Squalene, a triterpene functioning as an immunological adjuvant and chemo preventive substance against cancer10, Deferipron 11 chaletes iron, Amyl acetae12, an organic compound and ester used in the preparation of Penicillin, Behenyl alcohol13 a saturated fatty acid alcohol as an emollient, emulsifier and an Antiviral agent. The presence of various bioactive compounds confirms the application of C.truncato-coronatafor various ailments by traditional practicenor and the isolation of individual phytochemical constituents proceeded to find novel drugs.

VIII.CONCLUSION

From the present GC-MS analysis of ethonolic extract of Caralluma trancato-coronata reveals that it is highly valuable in medicinal usage for the treatment various human ailments and is a potential plant for these phytochemicals.

REFERENCES

[1] Genus: Caralluma R. Br.". Germplasm Resources Information Network. United States Department of Agriculture. 2004-04-15. Retrieved 2010-11-03.

[2] Neuwinger HD, African Ethnobotany: Poisons and Drugs, Chapman & Hall, NewYork,1994,238-239.

[3] Rebecca Kuriyan, Tonyraj, Srinivas SK, Effect of Caralluma fimbriata extract on appetite, food intake and anthropometry in adult Indian men and women. Appetite, 48, 2007, 338-344.

[4] Dutt,et al.,(2012) Pharmacological review of Caralluma R.Br with special reference to appetite suppression and anti-obesity. J Med Food. 2012 Feb:15(2):10819

[5] National Committee for Clinical Laboratory Standards. Performance Standards for antimicrobial susceptibility testing. 8th Informational Supplement. M100 S12. National Committee for Clinical Laboratory Standards, 2002. Villanova, Pa.

[6] Daines, A.M. et al. 2003. The synthesis of naturally occurring Vitamin K and Vitamin K analogues. Current Organic Chemistry 7, 1625-1634.

[7] Asokan, Subashini; Krueger, Karl M; Alkhawaldeh, Ammar; Carreon, Alessandra R; Mu, Zuze; Colvin, Vicki L; Mantzaris, Nikos V; Wong, Michael S (2005). "The use of heat transfer fluids in the synthesis of high-quality CdSe quantum dots, core/shell quantum dots, and quantum rods". Nanotechnology16 (10): 2000–11.

[8] Netscher, T. 2007. Synthesis of Vitamin E. Vitamins & Hormones. 76, 155-202.

[9] Daines, A.M. et al. 2003. The synthesis of naturally occurring Vitamin K and Vitamin K analogues. Current Organic Chemistry 7, 1625-1634.

[10] Smith, Theresa J (2000). "Squalene: potential chemopreventive agent". Expert Opinion on Investigational Drugs9 (8): 1841–8.

[11] Savulescu J (2004). "Thalassaemia major: the murky story of deferiprone". BMJ328 (7436): 358–9.

[12] Smolinske, Susan C (1992).Handbook of Food, Drug, and Cosmetic Excipients.CRC Press. pp. 75–76.

[13] Katz DH, Marcelletti JF, Khalil MH, Pope LE, Katz LR (December 1991). "Antiviral activity of 1-docosanol, an inhibitor of lipid-enveloped viruses including herpes simplex". Proc. Natl. Acad. Sci. U.S.A.88 (23): 10825–9.