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RESEARCH ARTICLE

Antioxidant assay and GC-MS profiling of methanolic fraction of parent plant parts and calli of *Coccinia grandis* L.

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Abstract

Coccinia grandis L., a member of the family Cucurbitaceae, is a naturalized medicinal plant in India, commonly called as "ivy gourd." It is a source of valuable natural compounds useful as anti-fungal, anti-hypersensitive, anti-hyperglycemic, anti-microbial, anti-ulcer, antioxidant, anti-inflammatory (for wound healing), anti-cancer, antipyretic, analgesic and hepatoprotective (against jaundice and hepatitis). Micropropagation can protect such plant wealth from vulnerability. However, the calli need to be tested and compared with parent plant for beneficial phytochemicals. Hence, the present studies were carried out for antioxidant efficacy assays and GC-MS-based phytochemical profiling using *C. grandis* leaf & stem calli, and corresponding explants. GC-MS analysis revealed 86 metabolites in the parent plant (stem, leaves) and callus (stem callus, leaf callus). Among the identified bioactive compounds, 9-Octadecenamide was recorded to be 85% in stem, 57.87% in stem callus and 64.36% in leaf callus and 10-Nonadecanol was 33.42% in leaves. Some valuable bioactive compounds of parent plant parts and callus, useful in the treatment of hepatic problems, could be identified as Hexadecanoic acid-methyl ester, 5-(Hydroxymethyl)-2-(dimethoxy methyl) furan; 1-Eicosanol; Gamma-sitosterol; Olean-12-en-3-ol, acetate, (3-beta)-; Benzene-propanoic acid,3,5-bis(91,1-dimethylethyl)-4-hydro; Heneicosane; Beta-Amyrin; 1-Chloroheptacosane; Neophytadiene; 1-Octadecanol; Canadine; Protopine; Isopropyl linoleate and Behenic alcohol.

Keywords: *Coccinia grandis*, DPPH, FRAP, GC-MS, TP, TF.

Introduction

Coccinia grandis (Cucurbitaceae), commonly known as "ivy gourd," a member of Cucurbitaceae, is a medicinally important herbaceous, dioecious, and perennial plant. Ivy gourd is native to North Central East Africa (Chun 2001) and is also cultivated in the Indo-Malayan region (Muniappan *et al*. 2009). Medicinal plants are a rich source of bioactive medicinal compounds which can be orally transmitted, and they are the richest cache of drugs not only in the traditional system of medicines but also in modern medicines, folk medicines, pharmaceuticals, food supplements, synthetic drugs, and chemical entities (Sateesh *et al*. 2011). Approximately, 80% of the world population in developing countries dwells on herbal and traditional medicines for their primary healthcare needs (WHO, 2002-2005). Their rich plant biodiversity contributes to the rural livelihood as well (Nogueira *et al*. 2018).

In this investigation, an important member of family Cucurbitaceae, *Coccinia grandis*, has been selected for studying the antioxidant efficacy and GC-MS based phytochemical profiling, as the family is known for abundant pharmaceutically important bioactive metabolites including polyphenols and flavonoids (Mohammed *et al*. 2020). *Coccinia grandis* has been one of those important medicinal plants (with *Solanum trilobatum,* and *Justicia gendarussa*) which have been used to treat and prevent several epidemics for a few hundred years (Singh, 2015). Though almost every plant produces medicinal phytochemicals during metabolic processes, such as flavonoids, tannins, alkaloids, saponins, phenols, steroids, and terpenoids having medicinal value (Schlesier *et al*. 2002); yet the type and amount may vary from plant part to plant part and species to species. Almost all parts of the Ivy gourd are used

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for cooking purposes in Asia (Tamilselvan *et al*. 2011). Its leaf and stem have various therapeutic properties and are used for the treatment of various illnesses such as psoriasis, ringworm, gastrointestinal disorders, chronic sinusitis, diabetes, urinary tract infections, and respiratory diseases such as asthma and bronchitis. Its roots are useful in the treatment of skin lesions (Tenia), arthritis, mouth ulcers, wheezing, phlegm, etc. (Nautiyal *et al*. 2018; Pekamwar *et al*. 2013). Approximately, 25 flavonoids and phenolic acids such as gallic acid, vanillic acid, protocatechuic, gentisic acid, salicylic acid, syringic acid, ellagic acid, beta-resorcylic acid, m-coumaric acid, caffeine acid, rutin, kaempferol, apigenin, and quercetin were detected in *Amaranthus gangeticus* (Rakesh *et al*. 2021). Callus cultures have been shown to have a specific role in the *in vitro* production of secondary metabolites (Constable and Barbehenn, 2008) which may be beneficial in preventing the uprooting of plants, promoting conservation and sustainability, both.

Coccinia grandis leaves, stem and root have certain therapeutic properties *viz* antioxidant, anti-fungal, anti-hypersensitive, anti-hyperglycemic, anti-microbial, anti-ulcer, analgesic, anti-inflammatory (for wound healing), anti-cancer and antipyretic, hepatoprotective (against jaundice and hepatitis), etc. (Nautiyal *et al*. 2018; Pekamwar *et al*. 2013; Kumar *et al*. 2015; Tamilselvan *et al*. 2011), due to the presence of specific phytoconstituents. The present work, therefore, has been aimed at phytochemical profiling for antioxidant and other potential activities of the parent plant parts as well as of the callus.

Material and Methods

Collection of plant material

Healthy plant parts of *Coccinia grandis* (L.) Voigt. (Cucurbitaceae) were collected from Chaudhary Charan Singh University, Meerut, District-Meerut, Uttar Pradesh in July 2022. The plant specimen was identified by the Botanical Survey of India, Calcutta (Brochure no. CNH/Tech. II/2022/63, BSI India) fig.1a, b, c, and d. *Coccinia grandis* (L) Voigt. callus was raised from healthy plant parts on MS medium supplemented with 1.0mg/L NAA + 0.5mg/L Kn (from leaf explants) and on MS supplemented with 1.0 mg/L 2,4-D + 0.5 mg/L Kn (from stem explants) at 25 to 28°C temperature, and 50-65 % RH under 12 hours PAR (Photosynthetically Active Radiation).

Sample preparation

Fresh young leaf, stem, micropropagated plantlets (leaf, stem), and corresponding calli were dried in the oven for one week and then ground into fine powder. 1.0 gram of fine powder was extracted in methanol (250 mL) using a Soxhlet extraction unit for 48 hours and was filtered through Whatman no-1 filter paper, followed by concentration on water bath evaporator and was used directly for the estimation of total flavonoids, phenolics, antioxidant potential through biochemical assays and GC-MS analysis.

Figure 1: a. mother plant, b. micropropagated plant c. leaf callus and d. stem callus

Determination of total Phenolic and flavonoid content

In vitro and *in vivo* total phenolics were estimated according to Bray and Thorpe (1954) and flavonoid content according to Zhishen *et al*. (1999) using gallic acid (1 mg/mL) and rutin (1mg/mL) as standard, respectively.

DPPH radical scavenging activity

The ability of methanolic extracts of *Coccinia grandis* (mother plant, callus, and micro-propagated plant) to scavenge the free radicals was estimated through DPPH radical activity. An aliquot of 10µL of different sample concentrations was added to a volume of 2.5 mL from DPPH methanolic solutions (60µM). The reaction mixtures were well shaken and incubated for 12 hours at room temperature in dark and absorbance was recorded at 515 nm using UV-2600#SHIMADZU spectrophotometer. The negative controls were prepared in ethanol and methanol and read against DPPH at 294 ± 0.2nm. Ascorbic acid and Catechol were used as the positive controls. The IC_{50} values (half maximal inhibitory concentration) were determined from the sample calculation of inhibition using the various concentrations of extracts (Re-Pellegrini *et al*. 1999).

Ferric-reducing antioxidant Potential (FRAP) assay

FRAP reagent was freshly prepared by mixing 30mM acetate buffer (pH-3.6), 0.031g TPTZ (2,4,6-Tri(2-pyridyl)-S-triazine) in 10 mL of 40mM HCl and 20mM $FeCl_3.6H_2O$ in 10:1:1 ratio, respectively. To 3.99 mL FRAP reagent 10µl test extract was added. After 10 min. the absorbance was recorded spectrophotometrically (UV-2600#SHIMADZU) at 593nm

against the blank (3.99mL FRAP+10µl methanol). To quantify the concentration of antioxidants having ferric-TPTZ reducing ability, a calibration curve was drawn using vitamin C as a reference (Polovnikova and Voskresenskaya 2008).

Gas chromatography-Mass spectroscopy analysis (GC-MS analysis)

Callus and plant part extracts were analysed by QP-2010 Ultra GC-MS (#Shimadzu, Kyoto, Japan). Rxi-5-SIL MS capillary column (30m × 0.25mm id ×0.25µm) was used for the separation of compounds. Helium (>99.99%) was used as carrier gas with 39.9cm/second of linear velocity. The GC-MS-QP-2010 ultra system comprised the auto-injector (AOCX-20i) and headspace sampler (AOC-20s), a mass selective detector having a temperature of 230°C and interface temperature was 270°C (rectified to 260°C), possessing a split injection mode. 50°C temperature was initially applied for 3 minutes and further programmed to increase to 280°C at a ramp rate of 15°C/minute. The total flow programme was 16.3 mL/minute, with a column flow of 1.21 mL/minute. The analysis was done at the University Science Instrumentation Centre, Jawaharlal Nehru University, Delhi. All the data of GC-MS analysis from explants, calli, and micropropagated plant genotypes based on the mass spectrum were interpreted by the National Institute of Standards and Technology, US (NIST, US). The resultant compounds' names, molecular weight, structure, and function were analyzed through NIST, Pub Chem library, and WILEY8.

Data Analysis

All the research data are expressed as mean ± standard deviation, and all experiments were carried out in triplicate. All data was statistically analyzed by SPSS 25 (*P* value ≤ 0.05) for Windows using ANOVA and post-hoc LSD and Duncan.

Results and Discussion

Variation in total phenolic and flavonoid content

The synthesis of bioactive compounds, especially phenolic and flavonoid content is principally affected by various environmental factors such as temperature, light, nutrients, soil, and water (Orduna 2010), which is well evidenced in our study. Estimated levels of TPC (Total Phenolic Content) and TFC (Total Flavonoid Content) of *Coccinia grandis* plant, callus, and micropropagated plant, having distinct growing conditions, are presented in figure 2 a, b. The TPC of stem (0.0905 ± 0.0006 mg GAE equivalent/gram of extract) of *Coccinia grandis* (CS) was significantly above the calli (CL-C, CS-C) and micropropagated plant parts (MCL, MCS). From the estimation, it can be inferred that CS, MCL, and MCS could be more positive antioxidants than other tissues studied (CL, CL-C, and CS-C). *Coccinia grandis* is rich in polyphenols (Poommaree *et al*. 2021), is further confirmed

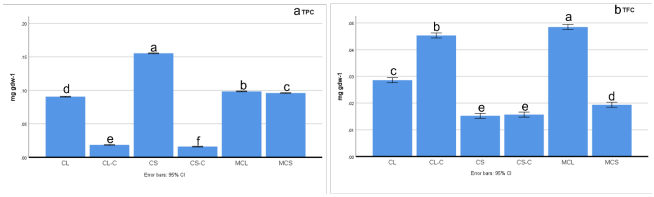

Figure 2: a, b represents the total phenolic and total flavonoid content (Duncan's formula)

by the GC-MS report. Polyphenols and flavonoids are good proton donors and electron acceptor antioxidant metabolites. The MCL of micropropagated *Coccinia grandis* plant was recorded to show maximum concentration of TFC (0.0485 ± 0.0003 mg RUT equivalent per gram dry weight of extract) followed by CL-C (leaf callus, 0.0453 ± 0.0014 mg RUT equivalent per gram dry weight of extract), while the lowest total flavonoid content was found in CS (0.0153 ± 0.0003 mg RUT equivalent per gram dry weight of extract) and CS-C (0.0157 ± 0.0005 mg RUT equivalent per gram dry weight of extract) (Figure. 2 b.). Our investigations show that MCL and CL-C contained more TFC, whereas CS, MCL, MCS showed more total phenolic content, compared to the other tissues. Thus, indicating leaf callus to undertake flavonoid pathway for antioxidant potential and mother plant or micropropagated plant to be more inclined to undertake phenolic pathway for displaying their antioxidant potential.

Differences in free radicals' antioxidant potential

Based on a single test model antioxidant potential of the plant extracts is not convenient to conclude because there is no well-developed and standardized protocol designed up to know that can provide an effect of antioxidant activity in the test sample (Alam *et al*. 2013, Sadeer *et al*. 2020). Antioxidant activity is a complex procedure, usually happening through various mechanisms and is also affected by various factors, which cannot be completely explained with one single method. Hence, it is important to perform more than one type of antioxidant capacity measurement to understand the several mechanisms of antioxidant activity (Schlesier *et al*. 2002, Wong *et al*. 2006). Previous research revealed that for the antioxidant activity of phytoconstituents in the targeted sample, a multi-*in vitro* antioxidant assay that measures the hydrogen or electron atom transfer from antioxidant phytocompounds must be used (Munteanu and Apetrei 2021). Granato *et al*. (2018) reported the quantification of total flavonoid, phenolic and antioxidant activity assays as a valuable approach to evaluate the health perspective for quality control of food material and natural yields. Therefore, in the present investigation, antioxidant potential in mother plant parts (leaf, stem), regenerant plant parts (leaf, stem), and calli (leaf callus and stem callus) of *Coccinia grandis* were evaluated using DPPH and FRAP assays.

DPPH and FRAP scavenging activities

The antioxidant activity of *Coccinia grandis* (Mother plant, callus, and micropropagated plant) was assessed for complementary tests. In the present investigation with *Coccinia grandis*, the leaf callus (CL-C) and MCS showed higher IC_{50} values in methanolic extracts than CL, CS, and CS-C. This can be a good alternative source with antioxidant potential for combating several inflammatory diseases, diabetes, and hepatic diseases. This is a very small amount as compared to our results in CL, CL-C, CS, CS-C, MCL, and MCS (Fig.3 a, b). All plant and callus extracts were able to reduce the stable purple-colored radical DPPH into yellow-colored DPPH-H. The methanolic extracts obtained from the mother plant, callus, and regenerated plant of *C. grandis* exhibited strong free radical scavenging activity, the strongest, with an IC_{50} value, being in methanolic extracts of CL-C (20.5 ± 0.011 µg/mL) and MCS (22.99 ± 0.08 µg/mL).The lowest capacity to reduce DPPH was observed in the methanolic extract of CS-C (30.7 ± 0.33 µg/mL). Methanolic extracts of all samples were less effective than the synthetic antioxidant vitamin-C (1.52 ± 0.008 µg/mL and 1.01 ± 0.01µg/mL) and Catechol (2.14 ± 0.012 µg/mL and 2.52 ± 0.015 µg/mL) (Fig. 3a). Poommaree *et al*. (2021) reported that in IC_{50} the value of *Coccinia* leaf extract was 4.85 mg/mL or equaled 98.6 µmolTrolox equivalents per gram of extract. *Coccinia grandis* leaf extract was useful in the treatment of keratinocytes and human fibroblasts from hydrogen peroxide-induced oxidative stress and the survival rate of cells increased by more than 20% during the whole experimental study. FRAP assay measures the reducing potential of antioxidant activity producing a blue colored Fe^{2+} TPTZ complex (Ferrous tripyridyltriazine from ferric complex) under a non-physiological pH of approximately 3.6. FRAP is the strongest antioxidant assay for evaluation of the antioxidant compounds of dietary phenols and polyphenols. In an acidic environment, potential discount can be suppressed via protonation with polyphenolic compounds, however, in basic media proton dissociation from phenolic compounds takes place, which can boom the potency to reduce a pattern. In the present study, the highest FRAP activity in methanolic extracts of CS-C, MCS (0.012 ± 0.001mg equivalents of Ascorbic acid/g dry weight of plant material) was recorded (Fig.3b). Surveswaran *et al*. (2007) screened 133 medicinal plants from India with the use of numerous strategies and mentioned an assay value ranging from 0.16 to 124.05 µmol Trolox Equivalent Antioxidant Capacity/gram.

Pharmaceutically important bioactive compounds detected in calli (leaf, stem) and parental (leaf, stem) genotypes through Gas chromatography-Mass spectroscopy

The genotype (leaf, stem) of *Coccinia grandis* and calli were extracted with methanol for analysis of bioactive

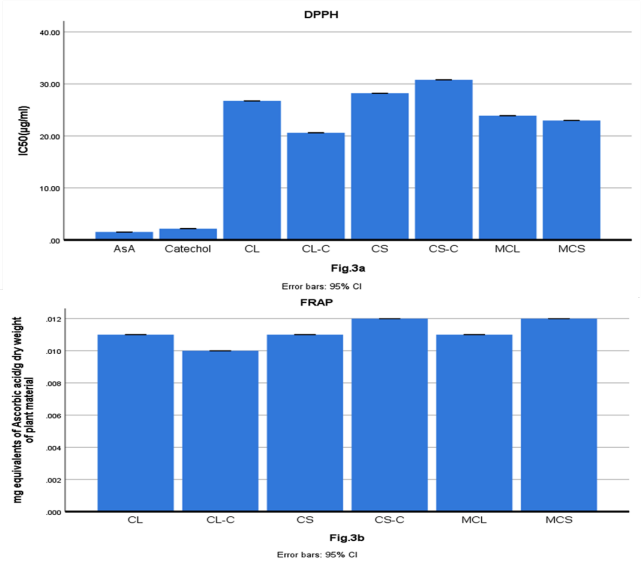

Figure 3 a: IC_{50} value calculated from DPPH assay of methanolic extracts of *Coccinia leaf* (CL), *Coccinia stem* (CS) explants, micropropagate leaf, stem (MCL and MCS) and callus derived from respective explants of *C. grandis* and Figure 3 b; mg-eq Ascorbic acid per gram dry weight of plant material estimated for FRAP assay of methanolic extracts of Coccinia leaf (CL), *Coccinia* stem (CS) explants, micropropagate leaf, stem (MCL and MCS) and callus derived from respective explants of *C. grandis* (leaf callus (CLC) and stem callus (CSC)

compounds through GC-MS. All the compounds were identified based on their RI (Retention index), molecular formula, and NIST (National Institute of Standard and Technology) library. The GC-MS screening of methanolic extract of calli and parental genotype displayed 86 phyto components. The volatile phyto- components were classified into the following classes such as alcohols, terpenoids, fatty acids, hydrocarbons, and ester. Some valuable bioactive compounds were identified that played a great role in treatment of ailments. Interestingly, few similar compounds were detected in all genotypes but they have low and high area % that may be important points for tissue culture (Table 1). The major bioactive compound in all the genotypes is 9-octadecenamide which has a maximum area% in CS (85.05%) and lowered in CS-C (57.87%), and CL-C (64.36%) (Table 1). Hameed *et al*. (2016) reported 9-octadecenamide as bioactive compound in the methanolic extract of *Cinnamon* bark that shows antibacterial and antifungal activity against *Klebsiella pneumoniae* and *Aspergillus flavus*. Kim *et al*. (2020) reported that 9-octadecenamide is a volatile compound derived from pumpkin pie and golden ring plants that show strong antioxidant activity. 9-octadecenamide (oleamide)recorded in our studies is reported to be a strong hypolipidemic substitute that can decrease serum TC, TG, LDL-C, and hepatic TG (Ming-Ching *et al*. 2010). Ethyl alpha-d-glucopyranoside is another major bioactive compound of *Coccinia* callus (CS-C, CL-C) that is used in cosmetic and functional food materials, and it shows remarkable toxicity (Yoshikawa *et al*. 1994). Velayutham and Karthi (2015)

Table 1: Phytochemical compounds identified by GC-MS from methanolic extracts

S. No.	Name of the compounds	Chemical structure	Retention time (RT)	Molecular mass (mg/mol)	Area chromatogram (% content)			
					Leaf	stem	Leaf callus	Stem callus
CS 1.	1-(4-isopropylphenyl)-2-methylpropyl acetate	$C_{15}H_{22}O_2$	11.863	234	1.76			
2.	3-Buten2one, 4(2,5,6,6tetramethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	11.913	206	0.21			
3.	1-(4-isopropylphenyl)-2-methylpropyl aceta	$C_{15}H_{22}O_2$	11.956	234	0.36			
4.	Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5,5,8,8-hexamethyl-, (1.al	$C_{15}H_{26}$	12.248	206	0.29			
5.	Neophytadiene	$C_{20}H_{38}$	13.685	278	0.29			
6.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	14.614	270	0.29			
7.	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	16.248	294	0.29			
8.	9,12,15-octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	16.311	292	1.04			
9.	Phytol	$C_{20}H_{40}O$	16.408	296	1.15			
10.	Monomethyl monobutyl «capped» tetraeth	C13H28O5	16.773	264	1.15			
11.	Octacosanol	$C_{28}H_{58}O$	19.694	410	0.79			
12.	9-Octadecenamide	$C_{18}H_{35}NO$	21.900	281	85.05	0.25		
13.	Farnesol 1	$C_{15}H_{26}O$	22.925	222	0.24			
14.	MMU	C6H13N3O2	23.263		0.42			
15.	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	23.365	281	0.73			
16.	5,5,7,7-Tetraethylundecane	$C_{19}H_{40}$	24.061	268	0.40			
17.	Behenic alcohol	$C_{22}H_{46}O$	24.144	326	0.43			
18.	26,27-dinorergosta-5,23-dien-3-ol, (3.beta.)-	$C_{26}H_{42}O$	24.144	370	0.87			
19.	Acetic acid 4,4,10,13,14-pe ntamethyl-17-(2-met,	$C_{33}H_{56}O_4$	26.413	535	3.11			
CS-C 20	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	$C_8H_{12}O_4$	7.133	172		4.04		
21.	Ethyl-alpha-d-glucopyranoside	$C_8H_{16}O_6$	11.784	208		24.35		
22.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	13.120	270		0.37		
23.	Alphad6,3furanose, methyl-beta-d-gluco	C7H12O6	15.482			5.72		
24.	Monomethyl monobutyl» capped» tetraeth	$C_{13}H_{28}O_5$	16.777	264		1.04		
25.	Hexadecanal	$C_{16}H_{32}O$	19.196	240		0.38		
26.	Octacosanol, Olean-12-en-3-ol, acetate, (3-beta.)-	$C_{28}H_{58}O$	19.694	410		1.17		
27.	9-Octadecenamid-E	$C_{18}H_{35}NO$	21.896	210		57.87		
28.	Squalene	$C_{30}H_{50}$	22.048	410		0.74		
29.	Farnesol-1	$C_{15}H_{26}O$	22.923	222		0.22		
30	9-Octadecenamide	$C_{18}H_{35}NO$	23.367	281		0.25		

31.	1-Eicosanol	$C_{20}H_{42}O$	24.151	298		0.23		
32.	Gamma.-Sitosterol	$C_{29}H_{50}O$	26.406	414		0.66		
33.	Olean-12-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	26.984]	468		1.28		
34.	Lup-20(29)-ene-3, 28-diol, (3.beta.)-	$C_{32}H_{52}O_2$	28.201	468		0.55		
CL 35.	Dodecane	$C_{12}H_{26}$	5.435	170			0.79	
36.	Pentadecane	$C_{15}H_{32}$	8.408	212			1.72	
37.	Decane, 3,8-dimethyl-	$C_{12}H_{26}$	10.648	170			0.26	
38.	Cyclohexanone-3-ethyl-	$C_8H_{14}O$	10.927	126			0.21	
39.	Hexadecane	$C_{16}H_{34}$	11.012	226			1.03	
40.	1-Chloroheptacosane	$C_{27}H_{55}Cl$	12.974	414			0.38	
41.	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	13.292	296			0.32	
42.	Neophytadiene	$C_{20}H_{38}$	13.689	278	0.29		0.78	
43.	7,9Ditertbutyl1oxaspiro (4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	14.547	276			0.22	
44.	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	14.620	326			0.23	
45.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro	$C_{18}H_{28}O_3$	14.687	292			0.93	
46.	1-OCTADECANOL	$C_{18}H_{38}O$	16.195	270			0.48	
47.	9,12-octadecadienoic acid (z,z)-, methyl este	$C_{19}H_{34}O_2$	16.253	294			0.19	
48.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	16.316	292			1.51	
49.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[	$C_{20}H_{40}O$	16.409	296			7.01	
50.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	19.433	213			0.89	
51.	10-Nonadecanol	$C_{19}H_{40}O$	22.700	284			1.55	
52.	Canadine	$C_{20}H_{21}NO_4$	23.630	339			0.51	
53.	3,4-dimethoxy-6-methyl-5,7,8,15-tetrahydrob	$C_{21}H_{24}ClNO_5$	23.748	405			4.04	
54.	Protopine	$C_{20}H_{19}NO_5$	23.839	353			3.07	
55.	erythro-9,10-Dibromopentacosane	$C_{25}H_{50}Br_2$	24.048	508			0.40	
56.	10-Nonadecanol	$C_{19}H_{40}O$	24.153	284			33.42	
57.	1-Eicosanol	$C_{20}H_{42}O$	26.287	298			8.66	
58.	.gamma.-Sitosterol	$C_{29}H_{50}O$	26.414	414			7.33	
59.	erythro-9,10-Dibromopentacosane	$C_{25}H_{50}Br_2$	29.146	508			0.90	
60.	Phytyltetradecanoate	$C_{34}H_{66}O_2$	29.353	506			6.12	
61.	Hexadecanoic acid, octadecyl ester	$C_{34}H_{68}O_2$	29.628	508			0.78	
62.	Isopropyl linoleate	$C_{21}H_{38}O_2$	33.029	322			5.63	
63.	9,10,12,13-Tetra Bromo-octadecanoic acid	$C_{21}H_{36}O_2$	33.322	320			10.63	
CL-C 64.	Dodecane	$C_{12}H_{26}$	5.441	216				1.96
65.	Decane,3,8dimethyl	$C_{12}H_{26}$	7.990	170				0.48
66.	Tetradecane	$C_{14}H_{30}$	8.411	198				4.85

67.	Pentadecane, 3-methyl	$C_{16}H_{34}$	10.64	264	0.80
68.	Hexadecane	$C_{16}H_{34}$	11.011	226	3.04
69.	Ethyl.alpha.dglucopyranoside	$C_8H_{16}O_6$	11.875	208	9.03
70.	Heptadecane, 3-methyl	$C_{18}H_{38}$	12.976	254	0.92
71.	Hexadecane	$C_{16}H_{34}$	13.294	226	1.19
72.	Phosphonic acid, dioctadecyl ester	$C_{36}H_{75}O_3P$	13.420	587	0.36
73.	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	14.004	278	0.78
74.	3-Sec-butyl-4-(2,2,3,3-tetramethylcyclopro	$C_{15}H_{24}O_2$	14.544		0.32
75.	decanoic acid, methyl ester	$C_{11}H_{22}O_2$	14.620	186	0.28
76.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro	$C_{18}H_{28}O_3$	14.689	292	4.00
77.	Heneicosane	$C_{21}H_{44}$	15.333	296	0.50
78.	Octane, 2-cyclohexyl-	$C_{14}H_{28}$	15.512	196	0.26
79.	MONOMETHYLMONOBUTYL «CAPPED» TETRAETH	$C_{13}H_{28}O_5$	16.788	264	1.67
80.	1,3-Propanediol, dodecyl ethyl ether	$C_{17}H_{36}O_2$	16.942	272	0.23
81.	1-Eicosanol	$C_{20}H_{42}O$	19.699	298	0.89
82.	9-Octadecenamide	$C_{18}H_{35}NO$	21.896	281	64.36
83.	Squalene	$C_{30}H_{50}$	22.050	410	0.31
84.	Cholestan-3-one, 4,4-dimethyl-, oxime, (5.alp	$C_{29}H_{52}O$	22.923	416	0.22
85.	Beta-Amyrin	$C_{30}H_{50}O$	26.983	426	2.29
86.	5,11,17,23-Tetratert-butylpentacyclo[19.3.1.1	$C_{44}H_{56}O_4$	29.696	668	1.26

reported that ethyl-alpha-d-glucopyranoside also shows antioxidant, antibacterial, hypolipidemic, alpha-amylase inhibitor, and anticonvulsant activity.

9-Octadecenamide; Farnesol-1; Acetic acid 4,4,10,13,14-pentamethyl-17-(2-met, Ethyl alpha-d-glucopyranoside; Hexadecanoic acid; methyl ester; 5-(Hydroxymethyl)-2-(dimethoxymethyl)furan; 1-Eicosanol; Gamma-sitosterol; Olean-12-en-3-ol,acetate, (3-beta)-/ Beta-Amyrin acetate; Dodecane; Tetradecane; Benzene-propanoic acid,3,5-bis(91,1-dimethylethyl)-4-hydro; Heneicosane; Beta-Amyrin; 1-Chloroheptacosane; Neophytadiene; 1-Octadecanol; Canadine; Protopine; Isopropyl linoleate; 9,10,12,13-Tetrabromooctadecanoic acid; Behenic alcohol, recorded in the present studies, are most active bioactive compounds which may be used in the drug development (Table 1). Lup-20(29)-ene-3, 28-diol, (3-beta) can defend against anti-cancer, antimicrobial and antihyperglycemic disorders. It is also known as an antioxidant (Liu *et al*. 2021). Lup-20(29)-ene-3, 28-diol, (3-beta) is absent in CS, CL, CL-C except CS-C. Triterpenes are known as cyclic and acyclic compounds, cyclic Triterpenes can be classified as monocyclic, bicyclic, tricyclic, tetracyclic, and pentacyclic compounds. Pentacyclic Triterpenes Lup-20(29)-ene-3,28-diol, (3-beta) has the following potent biological actions such as anti-viral, anti-diabetic, antioxidant, anti-inflammatory, and anti-cancer (Nogueira *et al*. 2018).The chromatogram of CS, CS-C, CL, and CL-C are shown in figure 4 a, b, c and d. Beta-Amyrin is found only in CL-C genotypes; it has a high potential to overcome the hepatic problem and anti-depressive, gastro-protective pentacyclic triterpene (Njerua *et al*. 2013). Canadine and protopine are the most valuable bioactive alkaloid compounds that show several biological activities such as cardiovascular, psychiatric and nervous system disorders (Estela *et al*. 2008). Isopropyl linoleate;9,10,12,13-Tetrabromooctadecanoic acid; Behenic alcohol; 1-Chloroheptacosane; Neophytadiene; 1-Octadecanol; Acetic acid 4,4,10,13,14-pentamethyl-17-(2-met, Ethyl alpha-d-glucopyranoside; Hexadecanoic acid; methyl ester, 5-(Hydroxymethyl)-2-(dimethoxymethyl) furan; 1-Eicosanol; Gamma-sitosterol; Olean-12-en-3-ol,acetate, (3-beta)-/ Beta-Amyrin acetate; Dodecane; Tetradecane; Benzene-propanoic acid,3,5-bis(91,1-dimethylethyl)-4-hydro; Heneicosane are biologically active compounds that are useful as food ingredients, in pharmaceutical industries,

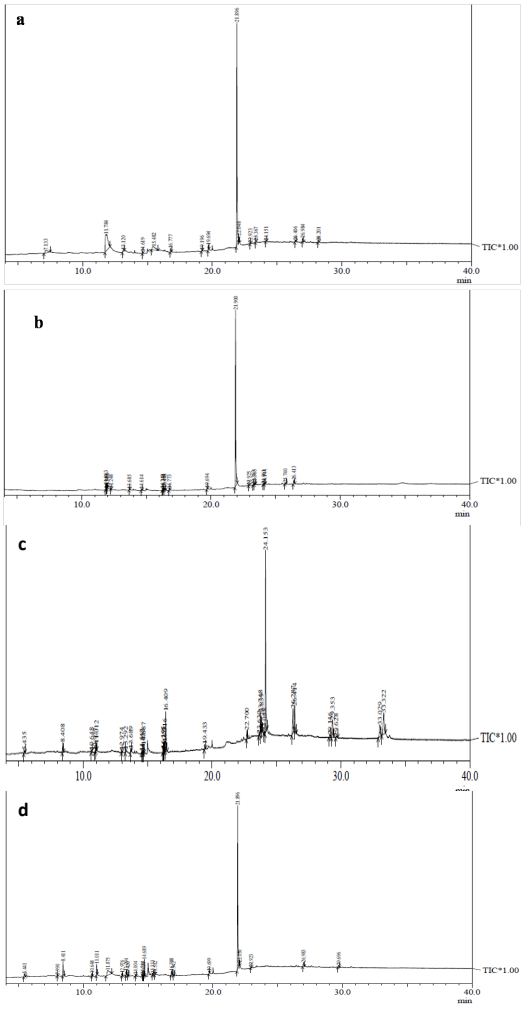

Figure 4: a, b chromatogram of CS (*Coccinia* stem), CS-C, (*Coccinia* stem callus) and c, d chromatogram of CL, (*Coccinia* leaf) CL-C (*Coccinia* leaf callus)

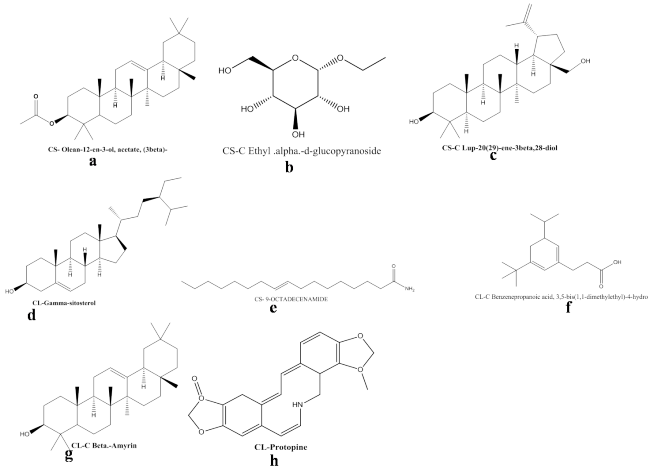

Figure 5: Some identified valuable bioactive compounds structure of CL, CS, CL-C and CS-C : (a) CS- Olean-12-en-3-ol, acetate, (3beta)- (b) CS-C Ethyl alpha-d-glucopyranoside (c) CS-C Lup-20(29)-ene-3beta,28-diol (d) CL-Gamma-sitosterol (e) CS- 9-Octadecenamide (f) CL-C Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro (g) CL-C Beta.-Amyrin (h) CL-Protopine

and for new drug development (Table 1). (Fig.5 shows all valuable bioactive compounds that are present in CL, CL-C, CS and CS-C).

Conclusion

Coccinia grandis (Ivy gourd plant) is cooked as a vegetable in India and Southeast Asia. The parent plant, regenerated plant, and calli have high nutritive and antioxidant plant metabolite content which may potentially prevent several diseases. Ivy gourd has poor seed germination, low fruit settings, and seed set. Micropropagation influenced the synthesis of flavonoids and phenolic compounds and their antioxidant properties in Ivy gourd. Micropropagated plant and callus of *Coccinia grandis* have high flavonoid and antioxidant activity. Thus, CL-C, and MCL have high flavonoid content, and the IC_{50} value of DPPH was highest in the leaf , CL-C, MCL, and MCS in methanolic extracts (where free radicals are produced abundantly), while FRAP activity was maximum in stem tissue

CS-C and MCS of *Coccinia grandis*. 5-(Hydroxymethyl)-2-(dimethoxymethyl) furan; 1-Eicosanol; Gamma-sitosterol; Olean-12-en-3-ol, acetate, (3-beta)-; Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro; Heneicosane; Beta-Amyrin 1-Chloroheptacosane Neophytadiene; 1-Octadecanol; Canadine; Protopine; Isopropyl linoleate; squalene and Behenic alcohol are valuable bioactive compounds recorded in *Coccinia grandis* plant parts (leaf and stem) and corresponding calli (leaf callus and stem callus) which are used in standard medicine as hepatic toners. *In vitro* and *in vivo* screenings of *Coccinia grandis* brought out their activity as antioxidant, as potential nutrient, hepatic toner, and may support new drug formulation.

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