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Gomathi S
PG and Research, Department of Botany, Government Arts College (Autonomous), Karur, Tamil Nadu, India

Velayutham P
Principal,
Krishna College of Arts and Science, Kolluthannipatti Karur, Tamil Nadu, India

Karthi C
PG and Research, Department of Botany, Government Arts College (Autonomous), Karur, Tamil Nadu, India

Santhoshkumar S
PG and Research, Department of Botany, Government Arts College (Autonomous), Karur, Tamil Nadu, India

Correspondence
Velayutham P
Principal,
Krishna College of Arts and Science, Kolluthannipatti Karur, Tamil Nadu, India

In vitro callus induction and phytochemical screening of *Corbichonia decumbens* (Forssk.) Exell through GC MS analysis

Gomathi S, Velayutham P, Karthi C and Santhoshkumar S

Abstract
Corbichonia decumbens (Forssk.) Exell a member of Aizoaceae is an important medicinal herb. The present study aims to develop an efficient protocol for callus induction from the leaf explants of *C. decumbens* and assessment of phytochemical compounds in the methanolic extracts of callus. The leaf explants were cultured on MS medium supplemented with different concentrations of auxins (2,4-D and NAA) alone or in combination with BAP. Among the tested, higher frequency (96.6%) of callus induction was observed on medium containing 5 µM 2,4-D plus 2 µM BAP. The well-developed callus was pulverised and extracted with methanol for GC -MS analysis. The results revealed that 31 phytochemical compounds from methanol extracts. The major compounds were 1-Pentadecene (15.35%); 1-Heptadecene (12.98%); 2-Pyrrolidinone (10.04%); 8-Octadecanone (9.75%); 1-Nonadecene (8.00%); 8-Pentadecanone (7.77%).

Keywords: *Corbichonia decumbens*, leaf, auxins, 2,4-D, BAP, callus, GC MS

1. Introduction
Corbichonia decumbens (Forssk.) Exell (syn. *Origia decumbens* Forssk.) belongs to Azioaceae and is an immensely important medicinal plant. It is also known as stone plant or carpet weed. It is a prostrate, glabrous, succulent, annual herb and is widely distributed and naturalized in Africa, West Asia, India and West Pakistan. It is enumerated in the Red list of South African medicinal plants under least concern [1]. It produces large number of secondary metabolites, terpenoids, alkaloids, tannins, flavonoids, glycosides, steroids, phenols, etc. [2]. These important secondary phytocompounds are isolated by extraction from whole plant or specific tissues. The plant is used as a tonic, gonorrhoea and kidney stone problems [3]. Pharmacologically the plant is reported to possess antimicrobial [2], antinociception [4] anti-inflammatory [5], antiulcer [6] and antioxidant activities [7].
Plants obtained through conventional methods are observed to be inadequate as a sole source of raw materials. *In vitro* rapid production of plants/cultures not only complements or adds options of resources, but will also protect natural important flora from over exploitation. It offers other important advantages such as continuous availability of biochemicals, optimized production of compounds or even with enhanced yield. Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to produce desirable medicinal compounds from plants [8].
Gas Chromatography-Mass Spectroscopy is the most commonly used technique for identification and quantification of compounds in extracted samples. Based on the literature survey, limited work has been done in *in vitro* regeneration study [9]. However, GC MS study has been conducted for phytochemical profiling of stem and root of *C. decumbens* [10]. Since there is no work has been conducted from callus of *C. decumbens*, it is worthwhile to screen the phytocompounds from methanolic extract of *C. decumbens* callus by GC MS analytic technique. To the best of our knowledge this is the first report in *C. decumbens* on the assessment of phytochemical compounds from callus.

2. Materials and Methods
2.1. Plant material and Establishment of explants
Leaf segments of *C. decumbens* were collected from the campus of Government Arts College, Karur, Tamilnadu, India. The plant was identified with the help of the Flora of Presidency of Madras. Leaf segments were thoroughly washed under running tap water for 30 min and then rinsed in a detergent solution for 5 min. Subsequently, they were surface sterilized with 70% alcohol for 30 sec, and finally washed with 0.1% (w/v) $HgCl_2$ solution for 2-3min, followed by

3 to 5 rinses with sterile distilled water after every treatment to remove all traces of chemicals under laminar air flow chamber. Disinfected explants were aseptically cut into 1-2 cm segments and were carefully inoculated onto the MS culture media.

2.2. Culture medium and culture condition

The basal culture medium used throughout the experiments consisted of MS [11] salts and B5 [12] vitamins with 3% (w/v) sucrose and 0.8% (w/v) agar. The surface sterilized leaf explants were cultured on MS medium supplemented with different concentrations of auxins (2,4-D & NAA) ranging from 5-25 µM alone or in combination with cytokinins (BAP) ranging from 1-5 µM for callus induction. The pH of the medium was adjusted to 5.8 ± 0.1 with 0.1 N NaOH or 0.1 N HCl before autoclaving at 121 °C and 15 lbs for 20 min.

All cultures were maintained at 25 ± 2 °C under 16 hrs photoperiod at a photosynthetic flux of 12.6 µmol m^{-2} s^{-1}, which was provided by cool white fluorescent lamps with 55-60% relative humidity. All experiments were carried out with 10 replicates and each experiment repeated thrice.

2.3. Preparation of the extracts

The callus of *C. decumbens* was shade dried at room temperature. The dried callus was powdered by mechanical grinder. About 10 g of powdered sample was extracted with methanol by using soxhlet apparatus. The extraction was continued until complete extraction and the solvent was removed at the reduced pressure with the help of vacuum evaporator to yield a viscous dark brown colour residue of methanolic extract. This methanolic extract was subjected to GC-MS analysis.

2.4. Gas Chromatography - Mass Spectrum analysis

Methanolic extract of *in vitro* callus of *C. decumbens* was performed on a PerkinElmer Clarus 600 GC System, fitted with a Rtx-5MS capillary column (30 m X 0.25 mm inner diameter, X 0.25 um film thickness; maximum temperature, 350°C), coupled to a Perkin Elmer Clarus 600 °C MS. Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 ml/min. The injection, transfer line and ion source temperatures were all 290 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from auto tune. The oven temperature was programmed from 60 °C (hold for 2 min) to 280 °C at a rate of 3 °C/min. The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 µl) were taken in a syringe and injected into injector with a split ratio 30:1. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The percentage composition of the crude extract constituents was expressed as a percentage by peak area.

The identification and characterization of chemical compounds of crude extracts was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries (NIST Library). The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

3. Results and Discussion

3.1. Callus induction and Proliferation

In the present study, *in vitro* callus induction from leaf explants of *C. decumbens* was developed. Leaf explants were cultured on MS medium supplemented with auxins 2,4-D and NAA, ranging from 5 - 25 µM alone or in combination with low concentration of BAP ranging from 1-5 µM (Table 1). The effects of different concentrations of 2, 4-D and NAA were tested individually and the higher frequency of callus induction (83.3%) was observed on MS medium fortified with 5 µM 2,4-D followed by 15 µM NAA (73.3 %). However, increased concentration of auxins significantly reduce the callus induction. A wide range of variations were observed in percentage of callus induction and nature of callus. The morphology of the callus varies according to the type and concentrations of growth regulators used in the medium either alone or in combination (Table 1, Fig. 1). The colour of the callus such as white, whitish green, green and greenish white was noted in various concentrations of plant growth regulators (PGRs). The medium containing lower concentrations of 2,4-D 5 µM and 10 µM produced a white coloured callus (Fig. 1). Among the tested PGR, 2,4-D was found to be more effective for callus induction than NAA in accordance with the earlier reports on *Physalis pubescens* [8], *Biophytum sensitivum* [13], *Withania somnifera* [14] and *Spermacoce hispida* [15].

Table 1: Effect of plant growth regulators on callus induction and proliferation from the leaf of *Corbichonia decumbens*

Concentrations of PGR (µM)			Percentage of Response	Nature of Callus
2, 4-D	NAA	BAP		
5	---	---	83.3	white friable calli
10	---	---	76.6	white friable calli
15	---	---	70.0	yellowish friable calli
20	---	---	61.6	pale yellowish friable calli
25	---	---	65.0	white friable calli
5	---	1	88.3	light greenish friable calli
5	---	2	96.6	greeenish friable calli
5	---	3	85.0	yellowish green compact calli
5	---	4	71.6	yellowish compact calli
5	---	5	63.3	pale green friable calli
---	5	---	61.6	yellowish green friable calli
---	10	---	68.3	yellowish friable calli
---	15	---	73.3	pale yellowish friable calli
---	20	---	60.0	yellowish compact calli
---	25	---	58.3	light greenish friable calli
---	15	1	80.0	yellowish green friable calli
---	15	2	91.6	yellowish green compact calli
---	15	3	83.3	greeenish friable calli
---	15	4	76.6	greenish compact calli
---	15	5	65.0	yellowish friable calli

Values are Mean of 3 replicates recorded after 45 days of culture.

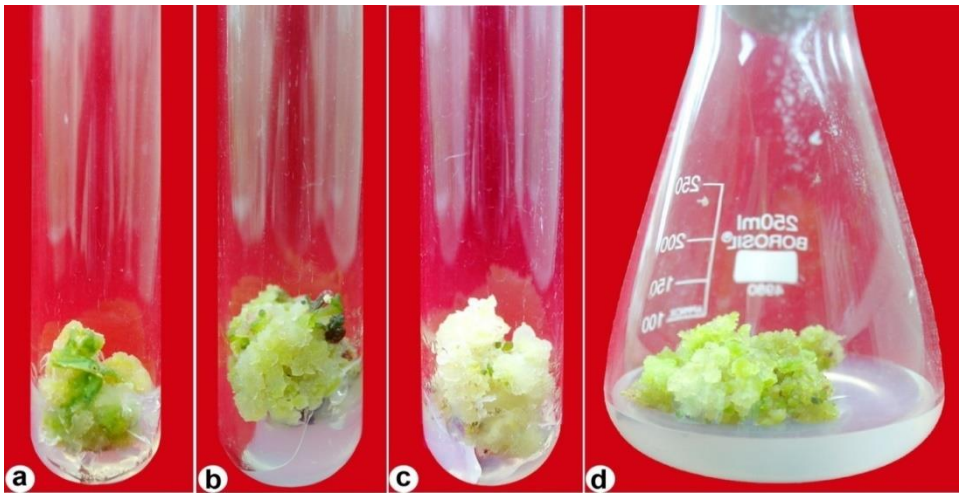

Fig 1: Callus culture from the leaf explant of *Corbichonia decumbens*. a. initiation of callus from the leaf; b and c. callus growth in15 days; d. callus proliferation in 30 days.

The optimum concentration of 2,4-D (5 µM) or NAA (15 µM) was tested with BAP (1-5 µM) for callus induction and proliferation from leaf explants of *C. decumbens* [Table 1]. Among the different combinations tested, the highest per cent (96.6 %) of callus induction was observed on MS medium fortified with 5 µM 2,4-D and 2 µM BAP [Table 1]. The combination effect of auxins and cytokinins played a vital role in callus induction and proliferation. The combination of 2,4-D and BAP was more effective for callus induction than that of NAA and BAP. Similar to our observation, callus proliferation on 2,4-D and BAP containing medium has also been reported in *Capsicum annuum* [16], *Simmondsia chinensis* [17], *Ceropegia bulbosa* [18] and *Nilgirianthus ciliatus* [19]. However, NAA and BAP combination also yielded significant callus proliferation in *Sesamum indicum* [20], *Orthosiphon aristatus* [21], *Asteracantha longifolia* [22] and *Brucea mollis* [23].

3.2. GC-MS analysis

GC-MS chromatogram of methanolic callus extract of *C. decumbens* showed 6 major peaks and several minor peaks (Fig. 2). As many as 31 phytochemicals corresponding to these peaks were presented in Table 2.

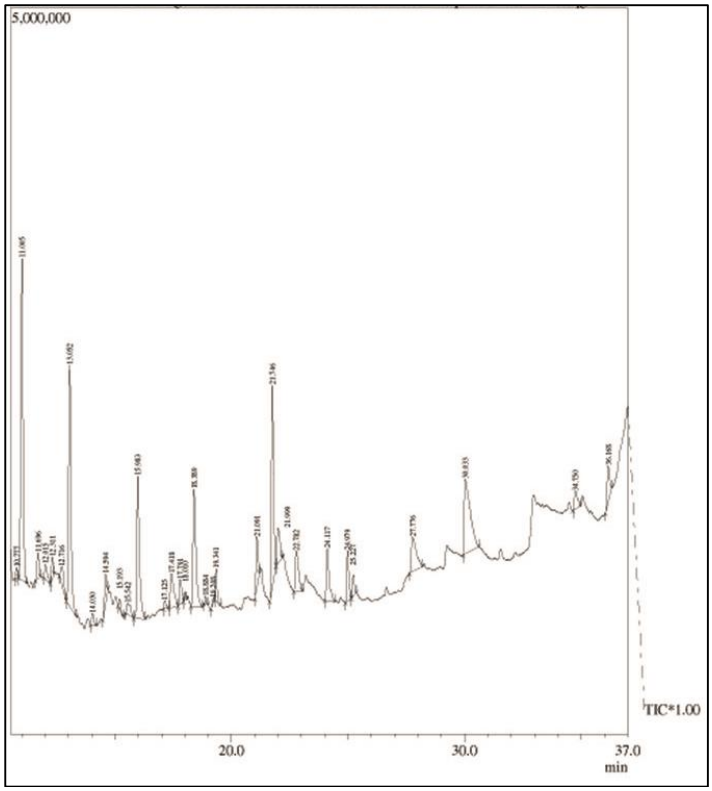

Fig 2: GC- MS spectrum of methanolic callus extracts of *Corbichonia decumbens*

The major constituents (>4%) were found to be 1-Pentadecene (15.35%); 1-Heptadecene (12.98%); 2-Pyrrolidinone (10.04%); 8-Octadecanone (9.75%); 1-Nonadecene (8.00%); and 8-Pentadecanone (7.77%) along with other minor compounds. These compounds were found to be present in small quantities (<4%). The highest peak area (%) of 15.35 was obtained by 1-Pentadecene (RT 12.45) and lowest peak area of 0.38 was obtained by Benzocyclodecene, tetradecahydro- (RT 15.19).

Table 2: GC-MS profile of methanolic callus extracts of *Corbichonia decumbens*

S. No	RT	Area %	Name of compound	Molecular formula	Molecular weight
1	10.78	0.43	Pentafluoropropionic acid, octadecyl ester	$C21H_{37}F_5O_2$	416
2	11.01	15.35	1-Pentadecene	$C_{15}H_{30}$	210
3	11.7	1.14	Cyclohexane, decyl-	$C_{16}H_{32}$	224
4	12.02	0.84	Nonadecyl pentafluoropropionate	$C_{22}H_{39}F_5O_2$	430
5	12.31	0.86	Tritetracontane	$C_{43}H_{88}$	604
6	12.72	1.22	1-Hexadecene	$C_{16}H_{32}$	224
7	13.05	12.98	1-Heptadecene	$C_{17}H_{34}$	238
8	14.03	0.59	Dodecylcyclohexane	$C_{18}H_{36}$	252
9	14.59	1.47	Tetrahydro-4H-pyran-4-ol	$C_5H_{10}O_2$	102
10	15.19	0.38	Benzocyclodecene, tetradecahydro-	$C_{14}H_{26}$	194
11	15.54	0.96	1-Undecene, 9-methyl-	$C_{12}H_{24}$	168
12	15.98	8	1-Nonadecene	$C_{19}H_{38}$	266
13	17.13	0.35	Cyclohexane, eicosyl-	$C_{26}H_{52}$	364
14	17.42	2.83	Benzeneacetaldehyde	C_8H_8O	120
15	17.79	1.46	Pentacosanoic acid, methyl ester	$C_{26}H_{52}O_2$	396
16	18.01	0.25	Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester	$C_{22}H_{44}O_2$	340
17	18.39	7.77	8-Pentadecanone	$C_{15}H_{30}O$	226
18	18.88	0.25	Pentadecane, 1-methoxy-13-methyl-	$C_{17}H_{36}O$	256
19	19.21	0.22	Benzene, nitro-	$C_6H_5NO_2$	123
20	19.34	1.11	1-Octacosanol	$C_{28}H_{58}O$	410
21	21.09	2.56	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
22	21.75	9.75	8-Octadecanone	$C_{18}H_{36}O$	268
23	22	2.31	Butyrolactone	$C_4H_6O_2$	86
24	22.78	3	Phenol	C_6H_6O	94
25	24.12	3.31	Phenol, 2,4-bis(1,1-Dimethylethyl)-	$C_{14}H_{22}O$	206
26	24.98	2.33	10-Nonadecanone	$C_{19}H_{38}O$	282
27	25.23	0.86	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
28	27.78	3.94	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150
29	30.03	10.04	2-Pyrrolidinone	C_4H_7NO	85
30	34.75	1.34	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334
31	36.17	2.12	Dibutyl phthalate	$C_{16}H_{22}O_4$	278

In vitro production of secondary metabolite is used to reduce harvesting of plants from natural habitats. There are great interests for therapeutic uses of secondary metabolites in *C. decumbens*. All other therapeutically used plants came from field collections, which means that their supply was limited and uncertain [24]. Metabolic contents varied significantly (P<0.05) between the plants according to the type and concentration of cytokinins as well as explant type used in callus-based regeneration [25]. *In vitro* callus induction and production of their secondary metabolites were reported on *Valeriana officinalis* [25], *Valeriana glechomifolia* [26], *Solanum trilobatum* [27] and *Harpagophytum procumbens* [28].

Table 3: Biological activities of identified phytocompounds from the methanolic callus extracts of *Corbichonia decumbens*

Sl. No	Name of Compound	Biological activities
1	1-Pentadecene	Antitumor
2	1-Hexadecene	Antimicrobial and antioxidant
3	1-Nonadecene	Antibacterial and antifungal
4	8-Pentadecanone	Anticancer
5	1-Octacosanol	Antiviral activity
6	Hexadecanoic acid, methyl ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide,Lubricant, Antiandrogenic, Hemolytic 5-Alpha reductase inhibitor
7	8-Octadecanone	Antifungal
8	Butyrolactone	Antimicrobial; antidiabetic; antioxidant
9	Phenol	Antibacterial; Antioxidant; Antipyruvetic; Antiseptic; Antiviral; Cancer-Preventive; Carcinogenic; Emetic; Fungicide; Pesticide
10	Phenol, 2,4-bis(1,1-dimethylethyl)-	Antioxidant, Antibacterial
11	10-Nonadecanone	Anticancer
12	2-Methoxy-4-vinylphenol	FLavor; Perfumery
13	2-Pyrrolidinone	Antioxidant; Anticancer
14	1,2-Benzenedicarboxylic acid, butyl octyl ester	Antimicrobial, Antifouling
15	Dibutyl phthalate	Antimicrobial activity

The identified compounds possess many biological properties (Table 3), for instance, 1-Pentadecene possesses anticancer property; 2-Pyrrolidinone possesses anticancer and antioxidant [29]; 8-Octadecanone has been reported to be antifungal [30]. The 1-Nonadecene was also reported as antifungal and antibacterial compounds. Singh *et al*. (2014) reported that the methanolic extract of callus of *Naringi crenulata* possessed various phytocompounds [31]. In the present study also the methanol extracts of callus of *C. decumbens* revealed the presence of various compounds. Rameshkumar *et al*. (2018) revealed that the higher amount of squalene content and various phytoconstituents were

presented in the callus of *Nilgirianthus ciliatus* than wild plant [19]. Yang *et al*. (2019) demonstrated that callus culture of *Helicteres angustifolia* yield a higher content of phenolic compounds, flavonoids, terpenoids, saponins and triterpenoids [32].

The results showed that the biochemical potential of leaf derived callus with high amounts of bioactive compounds which can be utilized for commercial and medicinal preparations.

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