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Alginate encapsulation in *Glycyrrhiza glabra* L. with phyto-chemical profiling of root extracts of *in vitro* converted plants using GC-MS analysis

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ABSTRACT

Objective: To investigate the conversion potential of alginate encapsulated nodes of *Glycyrrhiza glabra* with phyto-chemical evaluation of root extract of field transferred plants.

Methods: The excised axenic nodal segments were encapsulated in alginate matrix planted on Murashige and Skoog (1962) medium with different supplementation and formulations of PGRs. The two year old field transferred plants were evaluated for phyto-compounds analysis using GC-MS technique.

Results: Varied responses were observed during the study, maximum conversion 95.83% ± 2.40% was obtained in these encapsulates when planted on MS medium containing 2.5 μM Kinetin and 0.5 μM α-Naphthalene acetic acid, which eventually developed into complete plantlets in a single step. Further, GC-MS analysis showed the presence of different phyto-compounds in the methanolic root extracts of *in vitro* converted plants. The results obtained revealed the presence of about 47 phyto-compounds along with various potential bioactive compounds useful for industrial and pharmaceutical purposes.

Conclusions: This study investigates high frequency regeneration and conversion of *Glycyrrhiza glabra* in a single step in short time. Also, the *in vitro* raised plants are analysed for bioactive compounds after field transfer, which shows the presence of numerous compounds useful for commercial and pharmacological purposes.

1. Introduction

Glycyrrhiza glabra (*G. glabra*) L. is an immensely important plant belonging to the family Leguminosae. In commerce and market, it is well known as licorice. The plant is known for its high economic value and numerous therapeutic applications. The plant is of great interest because of its rhizome or stoloniferous roots which contain numerous important bioactive compounds. The two main classes of bioactive compounds are saponins and flavonoids, which contains glycyrrhizin [1–3] and glabridin or glabrene [4,5] respectively, as the major constituents. The commercial source of licorice is subjected to hand harvesting from wild which may target its sustainability and lead to genetic erosion of the species [6]. Such an inconsiderate mode of harvesting also causes damage and degradation to the natural growth area and soil [7] and restricts further production of plants in their natural habitat. In conventional practice, it is propagated through the cuttings of underground roots and rhizomes which is a time-consuming process [8].

Synthetic seed based micropropagation system is a fast and robust method for the regeneration of complete plantlets in one step, limiting the use of economically important part of the plant as propagule. During initial encapsulation studies, somatic embryos (SE) were employed for synthetic seed preparation [9]. But there are numerous plant species including *G. glabra* where differentiation of SE is still a challenge. The first non-embryogenic tissue encapsulation was materialised for *Morus indica* [10], which set free the concept of only SE encapsulation, and the encapsulation of different vegetative propagules like shoot tips, nodes, axillary buds, shoot buds, bulblets, calli etc., were also started [11,12]. The previous studies conducted in

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Salvia splendens [13], *Physalis peruviana* [14] *Chrysanthemum* [15], *Manihot esculenta* [16] *etc*. on encapsulation of various non-embryogenic explants have proved to be worthwhile in producing complete plantlets in a single step.

The present study aimed to understand the optimal media composition for (i) high incidence synseed conversion in *G. glabra* through nodal segment encapsulation (ii) to study the phyto-chemical profiling of methanolic root extract of *in vitro* converted plants.

2. Materials and methods

2.1. Encapsulation source

The axenic nodal segments were harvested from the proliferating cultures of *G. glabra* maintained in MS medium containing BA (5.0 μM) + mT (2.5 μM) as described by Akhtar and Shahzad [17] and encapsulated in sodium alginate to prepare the calcium-alginate beads.

2.2. Encapsulation of nodes and synseed preparation

The encapsulation matrix was prepared using 3% sodium alginate (Sigma–Aldrich, India) in double distilled water (DDW) without any PGR or sucrose supplementation and 100 mM $CaCl_2 \cdot 2H_2O$ (Qualigens Fine Chemicals, Mumbai, India) was used for complexion into beads, as per the earlier reported protocols [18–20].

Under the laminar air flow, using a sterile glass pipette, complexion into beads were made when matrix containing single axenic nodes were dropped in 100 mM $CaCl_2 \cdot 2H_2O$ solution and were retained there for (7–10) min, with gentle stirring for proper polymerization and formation of isodiametric beads. The beads were subsequently collected and washed with sterilized half-strength MS nutrient solution followed by 3–4 times washing with sterilized DDW. The excess moisture from the beads was removed by transferring them onto the petriplates containing sterilized Whatman filter paper.

2.3. Re-growth media and culture condition

The MS basal medium [21] supplied with 3% (w/v) sucrose (Fisher Scientific) was used without any cytokinin supplementation or with varying concentrations (1.0, 2.5 and 5.0 μM) of different cytokinins *viz*. BA, Kn, mT and TDZ. The medium was adjusted to a pH of 5.8 using 1N NaOH or 1N HCl, gelled with 0.8% agar–agar prior to sterilization by autoclaving at 121 °C and 15 psi for 17 min. The wide mouth flasks (100 mL, Borosil, India) containing 50 mL MS medium each placed with six encapsulated nodes (24 replicates), were incubated in culture room at (24 ± 2) °C temperature under 16/8 h photoperiod in cool fluorescent light of 50 $\mu mol^{-1} \cdot s^{-1}$ under 55% ± 5% relative humidity. After 4 weeks, data for % re-growth from the beads and % conversion into plantlets were recorded.

2.4. Low temperature (4 °C) storage

The effect of low temperature (4 °C) storage on the viability of encapsulated nodes and conversion was evaluated for different intervals (0, 2, 4, 6 and 8 weeks). The alginate beads containing single nodes were contained in sterile beakers wetted thoroughly with DDW, cautiously sealed with para-film to confirm the total aseptic storage condition. At the completion of each storage period, the synseeds were first washed with sterile DDW (2–3 times) and cultured onto the optimal media combination *i.e.* MS medium containing Kn (2.5 μM) + NAA (0.5 μM) or Kn (2.5 μM) + IAA (0.5 μM) following 4 week incubation under described conditions.

2.5. Acclimatization

The converted plantlets with well-developed shoot and root systems were freed from gel matrix by washing under flowing tap water and carefully transferred to the sterilised Soilrite™ (soil: sand: peat moss) mixture contained in the thermocol cups. The transferred plantlets were covered with polythene bags for maintaining humidity, and fostered with quarter-strength MS organic solution. The plantlets were incubated in culture room at (24 ± 2) °C temperature following a gradual removal of poly-bags to acclimatize the plantlets.

2.6. GC-MS analysis

The gas chromatography and mass spectrometry (GC-MS) analysis of the methanolic root extract of two year old *G. glabra* plants was accomplished using a GC-MS (Hewlettt-Packard 6890/5973) operating at 1 000 eV, equipped with Agilent 7890A/5975CGC HP-5.

The harvested roots were washed, cut into pieces and air dried. The dried pieces were crushed into fine powder using mortar and pestle. An amount of 1 g powder was dissolved in *n*-hexane (100 mL) and incubated for (3–4) h at 100 rpm for (3–4) d, and an extraction solvent methanol (10 mL) was used and solution was incubated in water bath (200 °C). After 24 h, volume was maintained with the solvent and the process was repeated twice. Using a syringe filter (0.22 μm), the extract was filtered and phyto-compound profiling was achieved.

2.7. Statistical analysis

The experiment was conducted with 24 replicates for each treatment, repeated thrice. The data analysis was done using SPSS ver. 16.0 (SPSS Inc., Chicago USA). The significance of difference among means was carried out using Duncan's test at 5% level. Results were expressed as mean ± SE.

3. Results

In the present study, DDW without any PGR or carbohydrate source was used for the preparation of encapsulated matrix. The synseeds when planted on control medium *i.e.* MS medium without any PGR, showed 45.83% ± 4.80% re-growth, which indicated regenerative ability of the encapsulated propagule under the defined composition of the matrix, however, no simultaneous roots were produced. As depicted in Table 1, a range of single PGRs at different concentrations were screened. In an incubation time of (5–7) d, sprouting of beads was observed in all the media tested. Within 4 week of culture time, maximum re-growth of 88.88 ± 1.38 was

Table 1
Effect of single PGR treatments augmented in MS medium in regrowth and conversion of plantlets from encapsulated axenic nodes of *G. glabra* L. (4 week).

PGR	μM	Shoot regrowth (%) from beads	Callus frequency	% Conversion of beads into plantlets	Number of roots produced per bead
Control	0.0	45.83 ± 4.80^{d}	–	–	–
BA	1.0	68.05 ± 2.77^{c}	+	–	–
	2.5	68.05 ± 1.39^{c}	+	–	–
	5.0	87.49 ± 4.81^{a}	++	–	–
Kn	1.0	–	–	70.83 ± 2.40^{b}	2.94 ± 0.25^{b}
	2.5	–	–	84.14 ± 3.64^{a}	3.49 ± 0.11^{b}
	5.0	83.33 ± 2.40^{a}	+	–	–
mT	1.0	72.22 ± 1.39^{bc}	+	–	–
	2.5	72.22 ± 2.78^{bc}	+	–	–
	5.0	88.88 ± 1.38^{a}	++	–	–
TDZ	1.0	–	–	80.55 ± 3.67^{a}	4.30 ± 0.20^{a}
	2.5	79.16 ± 4.81^{ab}	++	–	–
	5.0	83.33 ± 2.40^{a}	+++	–	–

Beads prepared with 3% sodium alginate in 100 mM $CaCl_2 \cdot 2H_2O$. Data represents the mean ± SE of 24 replicates per treatment in three repeated experiments. Means within the same column followed by the different letters are significantly different according to the Duncan's multiple range tests at 5% level.

achieved in MS + mT (5.0 μM) but no roots were formed as callus formation from the basal region of propagule was evidenced. However, the lower concentrations of PGRs *i.e.* Kn (1.0 μM) and (2.5 μM) and TDZ (1.0 μM) resulted in conversion of synseeds into plantlets and maximum conversion *i.e.* 84.14% ± 3.64% was evidenced in MS + Kn (2.5 μM).

Further enhancement in the re-growth or conversion frequency was achieved with the addition of auxins (NAA, IAA and IBA) in the optimal concentration of cytokinins (Table 2). Maximum conversion frequency in this case was obtained in MS + Kn (2.5 μM) + NAA (0.5 μM), showing 95.83% ± 2.40% conversion and forming 7.26 ± 0.11 mean number of roots/bead, while other combinations resulted in re-growth of beads but prevalence of callus hindered rooting, thereby, limited the conversion frequency. The shoot and root length of plantlets recovered from synseeds after 6 weeks of culture has been depicted in Table 3.

In the present study, different storage periods (0, 2, 4 and 6 weeks) were also investigated for the viability of synseeds and their conversion when planted on optimal medium compositions *i.e.* MS + Kn (2.5 μM) + NAA (0.5 μM) and MS + Kn (2.5 μM) + IAA (0.5 μM). In both cases, a decline in conversion frequency was noticed after each storage-duration. The observations suggest that the germplasm of *G. glabra* can be best stored for 4 weeks of encapsulation at 4 °C, thereafter, a sharp decline (Table 4) in conversion frequency was caused.

Table 3
Effect of medium composition on shoot and root length of plantlets recovered from converted beads after 6 week of incubation.

Medium composition	Mean shoot length (cm)	Mean root length (cm)
MS + Kn (1.0 μM)	6.00 ± 0.04^{c}	5.17 ± 0.03^{b}
MS + Kn (2.5 μM)	6.61 ± 0.10^{b}	5.36 ± 0.05^{ab}
MS + TDZ (1.0 μM)	5.74 ± 0.12^{c}	2.86 ± 0.14^{d}
MS + Kn (2.5 μM) + NAA (0.5 μM)	7.64 ± 0.17^{a}	5.51 ± 0.08^{a}
MS + Kn (2.5 μM) + IAA (0.5 μM)	7.35 ± 0.06^{a}	3.91 ± 0.03^{c}

Beads prepared with 3% sodium alginate in 100 mM $CaCl_2 \cdot 2H_2O$. Data represents the Mean ± SE of 24 replicates per treatment in three repeated experiments. Means within the same column followed by the different letters are significantly different according to the Duncan's multiple range tests at 5% level.

Table 2
Effect of optimum composition of BA, Kn and mT in combination with auxins augmented in MS medium in regrowth and conversion of encapsulated axenic nodes (4 week).

PGRs (μM)				Regrowth from beads (%)	Callus frequency	Conversion into plantlets (%)	Roots produced per bead (*n*)
Cytokinins	NAA	IAA	IBA				
BA (5.0 μM)	0.5	–	–	80.55 ± 1.39^{bc}	++	–	–
	–	0.5	–	83.33 ± 0.00^{bc}	++	–	–
	–	–	0.5	79.16 ± 2.40^{c}	+++	–	–
Kn (2.5 μM)	0.5	–	–	–	–	95.83 ± 2.40^{a}	7.26 ± 0.11^{a}
		0.5	–	–	–	83.33 ± 2.40^{b}	5.00 ± 0.19^{b}
	–	–	0.5	91.66 ± 0.00^{a}	++	–	–
mT (5.0 μM)	0.5	–	–	87.49 ± 4.81^{ab}	++	–	–
	–	0.5	–	86.11 ± 1.39^{abc}	++	–	–
	–	–	0.5	56.94 ± 5.00^{d}	+++	–	–

Beads prepared with 3% sodium alginate in 100 mM $CaCl_2 \cdot 2H_2O$. Data represents the Mean ± SE of 24 replicates per treatment in three repeated experiments. Means within the same column followed by the different letters are significantly different according to the Duncan's multiple range tests at 5% level.

Table 4

Effect of storage at 4 °C on synseed conversion into plantlets after 4 week of culture.

Period of storage (weeks)	% Conversion of beads	
	MS + Kn (2.5 μM) + NAA (0.5 μM)	MS + Kn (2.5 μM) + IAA (0.5 μM)
0	95.83 ± 2.40[a]	83.33 ± 2.40[a]
2	84.71 ± 3.67[b]	72.22 ± 1.39[b]
4	66.66 ± 2.40[c]	49.99 ± 2.40[c]
6	36.10 ± 3.67[d]	33.33 ± 2.40[d]
8	0.00 ± 0.00[e]	0.00 ± 0.00[e]

Beads prepared with 3% sodium alginate in 100 mM $CaCl_2 \cdot 2H_2O$. Data represents the Mean ± SE of 24 replicates per treatment. Means within the same column followed by the different letters are significantly different according to the Duncan's multiple range tests at 5% level.

Acclimatization of the obtained plantlets was done in Soilrite and after 4 weeks of incubation under controlled conditions of culture room, the plantlets were transferred to the pots containing soil and manure. About 90% of plants survived acclimatization and grew healthy in due course of time.

The roots of two year old synseed converted plants of *G. glabra* were subjected to GC-MS analysis for the evaluation of metabolic compound content. The methanolic extract of dried-powdered roots showed the presence of 47 phyto-compounds (Figure 1, Table 5) screened under different retention times (RT). The chemical constituents obtained were categorised under different classes of compounds viz. polysaccharides, fatty acids and their ester forms, phytosterols, vitamins *etc*. Based on % area, the bio-activity of major compounds has been listed in Table 6 [22–26].

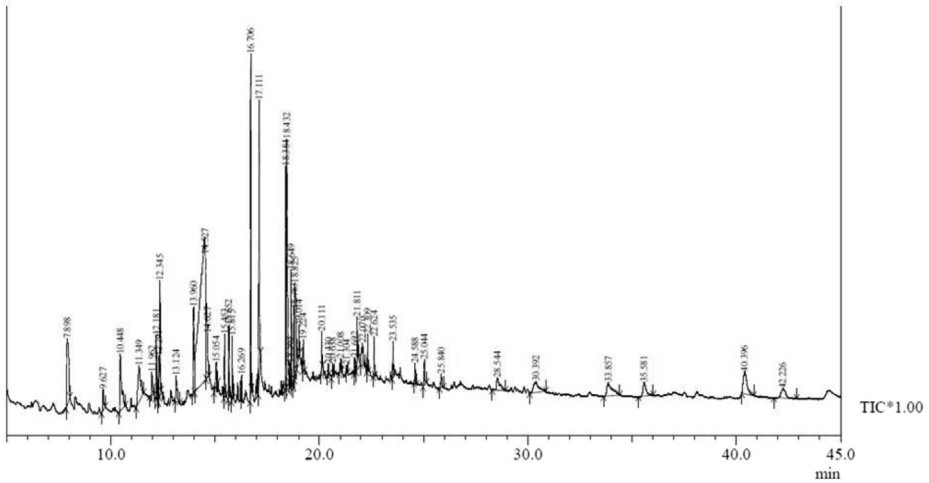

Figure 1. GC-MS chromatogram of methanolic root extract of two year old plants of *G. glabra* revealing peaks for plethora of phyto-compounds.

Table 5

The list of bioactive compounds obtained through GC-MS analysis isolated from methanolic roots extracts of two-year-old *G. glabra*.

Peak	R. Time	Area	Area %	Name
1	7.898	4386151	3.91	Ethyl pipecolinate
2	9.627	824567	0.73	2-methoxy-4-vinylphenol
3	10.448	3312915	2.95	5-oxo-pyrrolidine-2-carboxylic acid methyl
4	11.349	3357771	2.99	Cyclopentanol
5	11.962	626586	0.56	Anabasine
6	12.181	1253744	1.12	Phenol, 2,4-bis(1,1-dimethylethyl)-
7	12.291	909809	0.81	2,2′-Isopropylidenedifuran
8	12.345	2225274	1.98	Benzoic acid, 4-ethoxy-, ethyl ester
9	13.124	1098304	0.98	Benzenepropanoic acid, 4-hydroxy-, methyl ester
10	13.960	2480795	2.21	Comarin, 3,4,4a,5,6,8a-hexahydro-6,8a-epidioxy-4a,6-dimethyl
11	14.527	31554248	28.12	Mome inositol
12	14.627	3161268	2.82	Mome inositol
13	15.054	488368	0.44	1H-Indole-6-carboxylic acid, 7-(acetyloxy)-2,5-dimethyl-, methyl ester
14	15.453	1117043	1.00	3-heptadecanol
15	15.652	1117980	1.00	Isopropyl myristate
16	15.815	1049728	0.94	2,6,10-trimethyl,14-ethylene-14-pentadecne
17	16.269	375142	0.33	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[R*,R*-(E)]]-
18	16.706	5492512	4.89	Hexadecanoic acid, methyl ester
19	17.111	5863024	5.22	Pentadecanoic acid
20	18.384	3769260	3.36	9,12-octadecadienoic acid (z,z)-, methyl ester
21	18.432	8015058	7.14	9-octadecenoic acid (z)-, methyl ester
22	18.563	327215	0.29	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[R*,R*-(E)]]- (t-phytol)
23	18.649	1811797	1.61	Methyl stearate
24	18.783	1705852	1.52	9,12-octadecadienoic acid (z,z)
25	18.825	4274753	3.81	Octadec-9-enoic acid
26	19.014	1278112	1.14	Octadecanoic acid
27	19.224	721265	0.64	9,12-octadecadienoic acid (z,z)-, methyl ester
28	20.111	717396	0.64	N-Nonadecanol-1

Table 5 (*continued*)

Peak	R. Time	Area	Area %	Name
29	20.430	130880	0.12	Methyl 18-methylnonadecanoate
30	20.638	261715	0.23	Hexadecane, 2,6,10,14-tetramethyl
31	21.008	378836	0.34	Eicosane
32	21.304	134951	0.12	Oxalic acid, pentadecyl propyl ester
33	21.692	290277	0.26	1,3,5-trisilacyclohexane
34	21.811	1232417	1.10	Pentatriacontane
35	22.070	1082302	0.96	Docosanoic acid, methyl ester
36	22.309	668582	0.60	1,2-benzenedicarboxylic acid
37	22.624	728646	0.65	Tetracontane
38	23.535	764425	0.68	Pentacosane
39	24.588	504919	0.45	Tetracontane
40	25.044	705433	0.63	Squalene
41	25.840	536420	0.48	Tetracontane
42	28.544	1334768	1.19	Phenol, 2-[3,4-dihydro-8-methyl-8-(4-methyl-3-pentenyl)-2H,8H-benzo [1,2-b:3,4-b′]dipyran-3-yl]-5-methoxy-
43	30.392	2001767	1.78	Unknown
44	33.857	2401960	2.14	Stigmasta-5,22-dien-3-ol, (3.beta.,22E)-
45	35.581	1394177	1.24	Gamma.-Sitosterol
46	40.396	2939852	2.62	17-Androstanone, 3-(3,4-dimethylphenyl)-3-methyl
47	42.226	1424452	1.27	Methyl commate A

Table 6

The activity of major bioactive compounds depending upon % area screened in the roots of two year old *G. glabra*.

Retention time	Compound	Chemical formula	% Area	Medicinal importance
7.898	Ethyl pipecolinate	$C_8H_{15}NO_2$	3.91	No activity reported
10.448	5-Oxo-Pyrrolidine-2-carboxylic acid Methyl ester	$C_6H_9NO_3$	2.95	Anti-inflammatory, Anti-arthritis
11.349	Cyclopentanol	$C_5H_{10}O$	2.99	Pharmaceutical and perfumery solvent
14.527	Mome Inositol	$C_7H_{14}O_6$	28.12	Anti-proliferative, Anti-alopecic, Anti-cirrhotic, Anti-neuropathic, Cholesterolytic, Lipotropic, Sweetener
16.706	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	4.89	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
17.111	Pentadecanoic Acid, ethyl ester	$C_{15}H_{30}O_2$	5.22	Antioxidant, lubricant, hypercholesterolemic, cancer preventive, cosmetic
18.384	9,12-Octadecadienoic Acid (Z,Z)-	$C_{19}H_{34}O_2$	3.36	Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antieczemic, Antiacne
18.432	9-Octadecenoic Acid (Z)-methyl ester	$C_{19}H_{36}O_2$	7.14	Antimicrobial
18.825	Octadec-9-enoic Acid	$C_{18}H_{34}O_2$	3.81	Antimicrobial
33.857	Stigmasta-5,22-dien-3-ol, (3.beta.,22e)-	$C_{29}H_{48}O$	2.14	Antihepatotoxic, antiviral, antioxidant, cancer preventive, hypocholesterolemic
40.396	17-Androstanone, 3-(3,4-dimethylphenyl)-3-methyl-	$C_{28}H_{40}O$	2.62	No activity reported

Note: Refer to references [37–41].

4. Discussion

The compositional constituents of encapsulation matrix significantly contribute to synseed conversion into plantlets [13]. The second important factor for synseed re-growth (formation of shoots) or conversion (simultaneous formation of shoots and roots), is the composition of planting medium. Hegde *et al.* [16] found that encapsulation matrix containing MS salts was more effective in inducing synseed conversion as compared to the matrix devoid of MS salts, however, with no significant difference. While, full - strength MS liquid medium without carbohydrate used for encapsulation matrix preparation encouraged non - aseptic synseed regrowth [15]. In the present investigation, matrix prepared in DDW without MS salts or carbohydrates supplementation effectively resulted in synseed regrowth and conversion when placed on suitable planting medium either composed of single cytokinin or in combination with auxins. However, cytokinin in combination with auxins was more effective in % conversion response. Synergistic effects of cytokinins and auxins have also been found to be beneficial in enhancing re-growth frequency [14,27]. The only report on encapsulation of *G. glabra* [28] shows a preliminary study and the trend of re-growth or conversion frequency pattern on different media composition is lacking.

The storage at lower temperature (4 °C) was encouraged as reported in previous studies [13,29], this reduces pathogenic activities and dehydration of beads as compared to the storage at room temperature (25 °C) as described [28]. In due course of storage at low temperature, the regenerative ability of the encapsulated propagules was depicted to fall [13,19,20]. For efficient conversion, the findings suggest that encapsulates can be stored for a considerable period of time which in the present case is standardised for 6 weeks.

The plantlets developed through *in vitro* culture under controlled conditions risk survival when directly transferred to the greenhouse [30], thus plantlets require slow adjustment to controlled conditions after transfer to the planting substrate. Soilrite is an effective substrate for this purpose. Plantlets recovered from synthetic seeds grew healthy after 4 weeks of transfer to the soilrite and were finally planted in the garden soil + manure (1:1) to adapt to the natural condition.

Among the chemical constituents, Mome inositol (28.12%) - a polysaccharide, was identified as the major compound which was followed by the presence of many fatty acids or their esters like 9-Octadecenoic Acid (Z)- methyl ester (7.14%), pentadecanoic acid, ethyl ester (5.22%), hexadecanoic acid, methyl ester (4.89%) *etc*.

The analysis further displayed the presence of 2.21% Comarin ($C_{11}H_{14}O_4$) which showed isomerism with Dimalone ($C_{11}H_{14}O_4$) – an insect repellent [31]. The root extract also contained 1.61% of methyl stearate, an important biodiesel compound (renewable resource) and easy substitute for petroleum diesel [32]. Besides, some compounds were detected in minor quantities and possess significant bioactive properties. Among them are isopropyl myristate (1.0%), benzoic acid (1.98%) and anabasine (0.56%). isopropyl myristate is a potential water based micro-emulsion used for drug delivery system [33] and has been efficiently employed for topical delivery of fluconazole, used against activity of *Candida albicans* [34]. Besides, it is also used in food products and cosmetics as lubricant and thickening agent [35]. Benzoic acid and its derivatives show antifungal [36,37], antibacterial [38] and antiparasitic activity [39]. Anabasine is known to possess fungicidal, aphicidal [40] and pesticidal [41] properties.

The primal bioactive compound – glycyrrhizin, was not detected in the present study. This could be attributed to the fact that the presence or absence of glycyrrhizin depends on the time of harvesting, climatic condition, age of the plant (mostly 3–5 year old) *etc* [6]. However, 0.63% area of Squalene ($C_{30}H_{50}$) – a precursor of glycyrrhizin, was detected, and its biosynthesis involves mevalonic acid pathway. Squalene following a series of oxidative reactions forms β-amyrin as the possible intermediate and finally produces glycyrrhizin [1]. Squalene, apart from its activity as a precursor for glycyrrhizin, also possesses chemo-preventive properties, which is used in cosmetics and as an immunologic adjuvant in vaccines [22].

It is best suggested that the encapsulation of *G. glabra* propagules in DDW matrix and lower concentration of PGRs singly or in combination as the planting medium could be employed for high frequency synseed conversion. The encapsulates can be best stored for 6 weeks at 4 °C to maintain viability and morphogenic ability of the germplasm, avoiding pathogenic attacks which are prone to occur at room temperature (25 °C). Moreover, a range of important phyto-compounds can be harvested from the methanolic extract of roots for commercial and pharmacological applications.

Conflict of interest statement

All authors declare no conflict of interest.

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