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RESEARCH ARTICLE

Complete plant regeneration of *Valeriana wallichii* DC. on auxin enriched medium and phytochemical analysis

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ABBREVIATIONS
2-isopentyladenine (2iP), Murashige and Skoog (MS), 2,4-Dichlorophenoxyacetic acid (2,4-D), Naphthalene acetic acid (NAA), Indole-3-acetic acid (IAA), Thidiazuron (TDZ)

ABSTRACT

Valeriana wallichii DC. (Valerianaceae) is a well-known medicinal herb distributed in Northwest Himalayas. The herb is utilized in the treatment of numerous ailments and diseases such as diarrhoea, diabeties, gastrospans, ulcer and wound healing, etc. Overexploitation, especially collection of rhizomes on a large scale for the medicinal purpose has significantly declined the availability of the herb in natural stands. Hence, there is a requirement of development of cultivation practices and protocol for mass propagation to achieve sustained utilization along with conservation of the species. In the present study, *in-vitro* culture of nodal segments on MS+2iP+IAA+2,4-D and MS+2iP+IAA+NAA resulted in multiple shoot induction along with regeneration of *in-vitro* roots on the same medium making the protocol cost-effective, efficient and comparatively less time-consuming. Moreover, the regeneration of adventitious roots from regenerated shoots enhanced the total number of plants obtained per explant as shoots with adventitious roots were individually excised and were transferred to natural conditions through the process of acclimatization. GC-MS analysis of a methanolic extract of leaves of the mother plant and micropropagated plant revealed the presence of 37 and 36 phytocompounds respectively. Phytocompounds including eucalyptol, neophytidiene, hexadecenoic acid, dimethyl palmitamine were identified to be present in the leaves of both mother and micropropagated plants whereas other compounds such as eicosyne, lilial, behenic alcohol were confined to be present in extract prepared from leaves of mother plant. Similary some phytocompounds (phytol, vinicizer, retinol) were detected in methanolic extract prepared from leaves of micropropagated plants.

Introduction

Valeriana wallichii DC. commonly called as Tagara is a rhizome herb, belongs to the family Valerianaceae (1) and is known for several medicinal properties. *V. wallichii* has been reported to inhabit regions of Northwest Himalayas of India, Nepal, China and Afghanistan (2). It is generally distributed in the mountainous region at an altitude of 1500–3000 m (3). Roots of the plant is reported to posess several biologically active compounds including valerenic acid, valerenol, valerenone, valtrate, Isovaltrate, alkaloids, flavonoids (4). The plant is used in the treatment of wounds, arthritis, cough, skin diseases, fever, insomnia, epilepsy, antidiuretic, anti-constipation etc (5-6). The plant has also been reported to possess anticancerous and antioxidant activity (7). The roots derived phytocompounds are used as mild sedatives in the pharmaceutical industry. This activity is largely attributed to the presence of valepotriates. In most of the Valerian species, the active constituents are located in roots and rhizome. Beside root and rhizome, leaves of *V. wallichii* have also been reported to possess antimicrobial and anti-inflammatory activity (8). This plant contains 0.3–1% essential oil (3). Oil obtained from the herb has been reported to contain several bioactive phytocompounds, including valeric acid, terpinol, sesquiterpene, camphene etc. (9). Phytocompound hesperidin has been reported to

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be responsible for sedative and sleep-inducing enhancing effect of *V. wallichii* (10). The plant propagates mainly through rhizomes. Unrestricted harvesting of the herb from natural grounds for several medicinal purposes and lack of adequate commercial cultivation has resulted in rapid depletion of the plant (6). Owing to ever-increasing demand from the pharmaceutical sector there is a requirement to optimize *in-vitro* and *in-vivo* cultivation to conserve, and mass propagates the plant to meet the demand from traditional as well as industrial sector. The present study was conducted to develop easily reproducible and efficient mass propagation protocol for the conservation of *V. wallichii.* Culture medium utilized in the present study was fortified with auxin, considering auxin to be crucial plant growth regulator essential for organogenesis in plants (11).

Moreover auxin has been reported to possess potential to induce adventitious root formation (12). Along with auxin, culture medium was supplemented with 2iP considering that, 2iP along with auxin has been reported to induce proliferation of shoots in *Valeriana* species (13).

Materials and Methods

Explant

In the present study, the nodal segment obtained from the plant of *Valeriana wallichii* maintained in the Department of Biotechnology, Uttaranchal University, Dehradun were utilized as explants.

Sterilization of explants

Nodes excised from the mother plant were thoroughly washed with running water for about 10 minutes to remove dust impurities followed by which the explants were treated with tween 20. 500 ml beaker containing explants was half filled with 1% tween 20 solution and beaker was covered with a muslin cloth and kept under running water for 20 minutes. After removing all traces of tween 20, explants were transferred to Laminar Air Flow for further sterilization. Explants were rinsed with 70% ethanol for 50 seconds and washed with distilled water. Explants were surface sterilized with 0.1% $HgCl_2$ for 3 minutes. Sterilized explants were washed with sterile distilled water for 3–4 times and were dried using a sterile tissue paper. Explants were excised to appropriate size before inoculation onto culture medium (14).

Establishment of cultures and Data analysis

Surface sterilized nodal segments (2-3 cm) were inoculated on freshly prepared culture medium. Murashige and Skoog (15) medium was utilized as a basal culture medium. All media combinations utilized were uniformly supplemented with 4 µM 2iP and 4 µM IAA. In addition to 2iP and IAA, in one experimental setup, media (pH 5.8) was supplemented with different concentrations of 2,4-D (2–10 µM), and in another set of experiment different concentrations of NAA (2–10 µM) was utilized. For each concentration of 2,4-D and NAA independent experiment was set with a minimum of 15 cultures, and each experiment was repeated thrice. Tabulated data represents average of number of shoots followed by standard error. Incubation conditions comprised of 25±2 °C temperature and a photoperiod of light (15 uE/m2/s irradiance).

Acclimatization of *in-vitro* regenerated plants

In-vitro regenerated plants with well-developed roots were removed from culture tubes/flasks under aseptic conditions. Roots of plants were gently washed with sterile water to remove media attached to roots. Plants were transferred to beakers (100–150 ml) with 10–15 ml solution of MS salts covered with aluminium foil and incubated under similar conditions utilized for *in-vitro* regeneration of plants. After 7–8 days, when plants exhibited normal growth, autoclaved soil and sand (1:1) was added to beakers, and a small incision was made on aluminium foil. Plants showing normal growth along with the emergence of new leaves were transferred from the incubation chamber to normal laboratory conditions. After an interval of 7–10 days, plants were removed from the beaker and transferred to garden soil under natural conditions.

GC-MS analysis

50 gm fresh leaves were thoroughly grinded and added to round bottom flas containing 200 ml methanol. Soxhlet apparatus was operatefor 48 hrs to prepare methanolic extract (16, 17). The extract was concentrated to final volume of 10 ml using a water bath. Methanolic extract prepared from leaves of mother and *in-vitro* raised plants was subjected to GC-MS analysis. Compounds were identified based on retention time, date of the mass spectrum, molecular mass being compared with that of standard compounds in NIST data bank and Wiley library. The relative percentage of each phytocompound was determined by comparing individual peal area of a compound to a total area. GC Clarius Pakin Elmer Analyzer was used for analysis. Carrier gas utilized was He (with 1 ml/ min flow rate). Mass spectrum of phytocompounds was obtained through electron ionization at 70 Ev with detector operated in scan mode (40–45 amu) with 0.5 seconds of scan interval.

Results and Discussion

In-vitro culture of the nodal segment of *V. wallichii* on MS medium containing 4 µM 2iP + 4 µM IAA along with the varying concentration of either 2, 4-D (2–10 µM) or NAA (2–10 µM) was found to be an extremely efficient method for *in-vitro* mass propagation of *V. wallichii* as multiple shoot regeneration was achieved on all media combinations (Table 1). On medium MS + 2iP + 4 µM IAA + 2 µM 2, 4-D about 88.4% explants developed multiple shoots with a maximum and the average number of shoots to be 7 and 4.4 ± 0.4 respectively. When the concentration of 2, 4-D was enhanced (4–10 µM), multiple shoot induction and proliferation was achieved in all the explants (100%). However, the medium containing 4 µM and 6 µM 2, 4-D (Fig. 1. f & g) was found to be most effective as the average number of shoots (per culture) obtained was 8.4 ± 1.2 and 10 ± 0.8, respectively. A maximum of 13

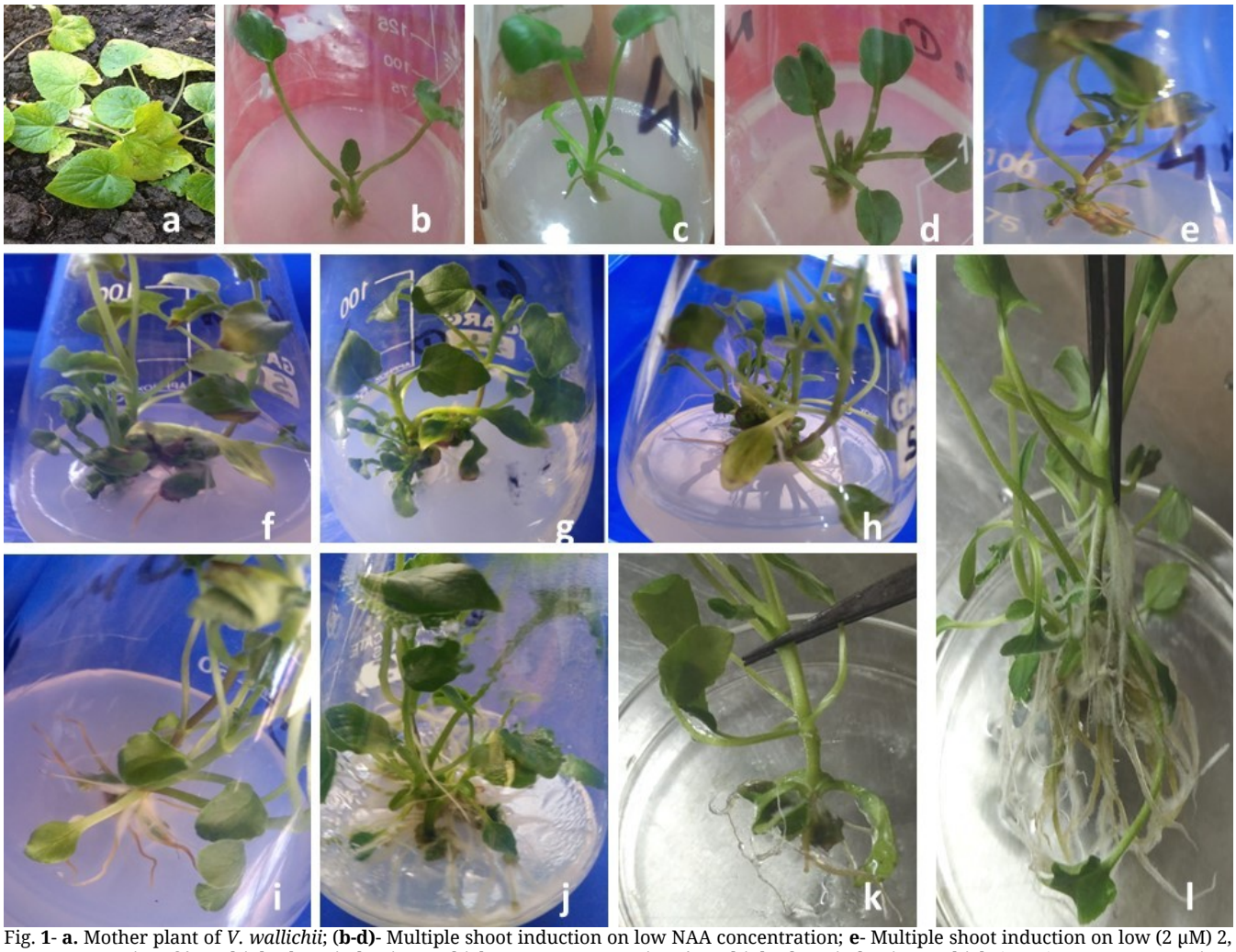

Fig. **1- a.** Mother plant of *V. wallichii*; **(b-d)**- Multiple shoot induction on low NAA concentration; **e**- Multiple shoot induction on low (2 µM) 2, 4-D concentration; **f,i**- Multiple shoot induction on high 2, 4-D concentration; **j**- Multiple shoot induction on high (8 µM) NAA concentration; **(h-j)** Growth of roots over the surface of media (instead of penetrating into media); **m**- Callus (extremely low proliferation) development on media with high (8-10 µM) 2, 4-D concentration; **l, m**- Extensive development of roots; **n-** Adventitious root development

Table 1. *In-vitro* culture of nodal segment of *V. wallichii* on MS + 2ip (2 µM) + IBA (4 µM) + 2,4-D (2-10 µM) and MS + 2ip (2 µM) + IBA (4 µM) + NAA (2-10 µM)

Media MS + 2ip (2 µM) +IBA (4 µM) +	Conc. (µM)	% Response	Avg. No of shoots	Max. No. of shoots	% explants developing callus	extent of callusing
2, 4-D	2	88.4	4.4±0.5	7	NR	NR
	4	100	8.4±0.5	12	NR	NR
	6	100	10±1.2	13	NR	NR
	8	100	7.8±0.4	09	56.4	+
	10	100	8.0±0.4	11	62.8	+
NAA	2	100	4.0±0.5	6	NR	NR
	4	100	7.0±1.2	10	NR	NR
	6	100	8.4±0.5	12	36.2	NR
	8	100	8.6±0.8	10	42.6	NR
	10	100	9.0±0.5	12	52.4	+

(NR= no response; '+'= extremely low growth); Average number of shoots represent average shoots per explant on particular hormone concentration followed by standard error of data

(from single explant) shoots was attained on medium MS + 2iP + IAA + 6 µM 2, 4-D. In another experimental setup, where 2, 4-D was replaced by NAA; MS + 4 µM 2iP + 4 µM IAA + NAA (2–10 µM) regeneration of multiple shoots was achieved from cultured nodal segments on all concentrations of NAA. However, the number of shoots regenerated varied with variation in the concentration of NAA. On culture medium containing a low concentration of NAA MS + 2iP + IAA + 2 µM NAA; the average number of shoots regenerated was 4 ± 0.8 with a maximum of 6 shoots obtained on this medium composition (Fig.1. b & c). On enhancing the concentration of NAA to 8 µM (MS + 2iP + IAA + 8 µM NAA), the average number of shoots per explant enhanced to 7 ± 0.2 with a maximum of 10 shoots (Fig.1. h). On medium, MS + 2iP + IAA + 10 µM NAA maximum and the average number of shoots was 12 and 9.0 ± 0.4 respectively. In earlier studies conducted pertaining to *in-vitro* propagation of *V. wallichii* regeneration of shoots from nodal / shoot apex segments onto MS medium fortified with BAP, Kn or TDZ have been reported (18,

19). An earlier study (20) have reported regeneration of adventitious shoots from *in-vitro* culture of leaf segments on MS medium containing plant growth hormone BAP, NAA and 2ip. Similarly, regeneration of shoots from *in-vitro* cultured shoot tips onto MS medium containing TDZ or Kn is also reported (21). In the present study on media enriched with a higher concentration of 2, 4-D (8–10 µM) or NAA (6–10 µM), development of callus was also achieved along with multiple shoot regeneration. However, the extent of callus regenerated was low irrespective of the hormone 2, 4-D (8–10 µM) or NAA (6–10 µM) present in culture medium and exhibited almost negligible proliferation (Fig. 1k) and was easily excised during acclimatization. *In-vitro* multiple shoot regeneration was the prominent morphogenic response obtained in the present study achieved.

Most of the tissue culture studies conducted for conservation, propagation of several plant species such as *Withania somnifera* (22); *Withania coagulans* (23), *Ephedra* (24), *Rauvolfia serpentina* (25) have reported either direct or indirect regeneration from various explant sources. In either case shoot (either regenerated from callus or directly from explant) need to be aseptically excised and subcultured on rooting medium making the whole process comparatively complex, time-consuming, moreover rooting may or may not be achieved on all media utilized and percentage of culture developing roots also varies depending upon nature and concentration of hormones, culture conditions etc. Contrary to this in the present study along with multiple shoot regeneration from nodal segments, the formation of roots was also achieved. Roots regenerated were white, exhibited extensive growth after 7–8 days of induction (Fig. 1 j & k). Regenerated roots exhibited growth over the surface of media (Fig. 1 h-j) rather than penetrating into media which made the removal of plant from media convenient and efficient during acclimatization. Besides the regeneration of roots from the basal part of the shoot (which was in direct contact of media), aerial (adventitious) roots developed from the node of regenerated shoots (Fig. 1 l). Formation of adventitious roots enhanced the overall number of complete plant regenerated per explant as shoots with adventitious roots was aseptically excised and transferred for acclimatization. A maximum of 60.4% *in-vitro* regenerated plants were successfully acclimatized to natural conditions. Acclimatized plant exhibited normal growth and development. In a previous study also, micropropagated plants of *Valeriana* species when acclimatized to natural conditions were reported to exhibit normal and identical growth (26).

GC-MS of methanolic extract of the leaves of micropropagated and mother plant

GC-MS analyze of methanolic extract prepared from the leaves of mother plant of *V. wallichii* revealed the presence of 37 peaks, each of which corresponds to a specific phytocompound. Fig. 2 represents Gas chromatogram and Table 2 enlists phytocompounds identified. The major compounds, in terms of amount, found to be present include dimethyl palmitamine, gamma sitosterol, hexadecanoicacid, trans-sesquisabinene hydrate, octadecanoic acid methylester, neophytadiene, bis (2-ethyl hexyl) phthalate, phosphoric acid, dioctadecyl ester, 1-azabicyclo (2,2,2) octan-2-ol, 4-phenyl, 1-heptacosanol. Fig. 4 depicts mass spectroscopy peaks of major compounds found to be preset in methanolic extract of leaves of the mother plant.

Methanolic extract of leaves of *in-vitro* regenerated plant when subjected to GC-MS analysis exhibited presence of 36 phytocompounds. Fig. 3 represents Gas chromatogram and Table 3 enlists phytocompounds identified. Major compounds include eucalyptol, retinol, didrovaltrate, phytol, hexadecanoic acid, dimethylpalmitamine, neophytadiene, stigmast-5-en-ol (3BETA), (2-ethylhexyl) phthalate. The concentration of common compounds exhibited variation. Fig. 5 depicts mass spectroscopy peaks of major compounds found to be preset in methanolic extract of leaves of mother plant. Eucalyptol was present in higher amount in the extract of micropropagated plant (29.9%) as compared to extract of mother plant 1.57%). On the contrary dimethyl palmitate was present in higher concentration in mother plant (41.19%) as compared to micropropagated plant (24.5%). Compounds including octadecanoic acid methyl ester, hexadecanoic acid and derivatives, phthalic acid (derivatives) were also found to be present in the wild as well as micropropagated plants. However, some phytocompounds were found to be specifically present in extract prepared from leaves of mother plant and absent in extract prepared from *in-vitro* regenerated leaves and vice versa. The difference in phytocompounds found to be present among *in-vitro* regenerated plants as compared to mother plant is expected to have occurred due to probable impact of culture media, plant growth regulator or *in-vitro* cultural conditions. Terpenoline, 1-decanol, phytol, retinol, didrovaltrate were found to be present only in the extract of leaves of tissue culture raised plants. Whereas compounds such as isobornyl acetate, hexadecanol, lilial, 3-eicosyne, trans-5 esquisabinene, hydrate, glyeidyl palmitate, Behenic alcohol were present only in extract from leaves of wild plants. Most of the phytocompounds identified to be present in the extract of leaves have been reported to possess biological activities which are responsible for the overall medicinal potential of plant. Terpinolene has been reported to anticancer and antioxidant in function (20). Compound eucalyptol is utilized in the treatment of bronchitis, sinusitis, asthma and chronic rhinitis (27). Gamma Terpinene also acts as an antioxidant and is also protective against the effects of high fructose. Phytol is a well-known anti-inflammatory agent (28). Neophytadiene has been reported to have an inhibitory effect against several microbes (29). Di-methyl palmitamine is also antioxidant along with antifungal properties (30). Phytocompound didrovaltrate is reported to be hypotensive, inotropic and antiaerhythmic (31). Retinol plays an important role in vision. Lilial is also found to have insect-repellent activity (32). Iso-bornyl acetate is a compound present in the extract which is useful as a chemotaxonomic marker (33). Trans sesquisabinene is also found to be anticancerous in function. 3-eicosyne has been reported to possess antimicrobial activity (34). Octadecanoic acid

methylester is a compound which is present in the plant found to have anti-viral activity (35). Hexadecanol has also been reported to possess anti-

Table 2. Phytocompounds present in methanolic extract of the leaves of wild plants of *V. wallichii*

Peak	R. time	Area%	Name of the compound	Chemical formula
1	8.907	0.91	Cyclohexene, 1-methyl-4-(1-methylethenyl)	$C_{10}H_{16}$
2	9.001	1.57	Eucalyptol	$C_{10}H_{18}O$
3	10.381	0.81	Undecane, 3-methyl-	$C_{12}H_{26}$
4	14.018	1.19	Isobornyl acetate	$C_{12}H_{20}O_2$
5	14.329	0.38	1-hexadecanol	$C_{16}H_{34}O$
6	16.346	0.89	1,5,9,11-Tridecatetraene, 12-methyl-, (E,E)-	$C_{14}H_{22}$
7	17.713	0.95	Phenol, 3,5-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$
8	18.005	1.30	Lilial	$C_{14}H_{20}O$
9	18.443	0.23	(S)-1-phenyl-4-(triisopropylsilyloxy)-1-buta	$C_{19}H_{34}0_2Si$
10	18.744	2.22	(2,6,6-trimethyl-1-cyclohexen-1-yl)acetalde	$C_{11}H_{18}O$
11	21.387	1.27	[Dodecanoyl(methyl)amino]acetic acid	$C_{15}H_{29}NO_3$
12	22.286	2.97	Neophytadiene	$C_{20}H_{38}$
13	22.361	0.74	(2E)-3,7,11,15-tetramethyl-2-hexadecene	$C_{20}H_{40}$
14	22.612	1.06	Phthalic acid, trans-dec-3-enyl undecyl ester	$C_{29}H_{46}O_4$
15	22.847	0.64	3-Eicosyne	$C_{20}H_{38}$
16	23.181	41.19	Dimethyl palmitamine	$C_{18}H_{39}N$
17	23.421	2.04	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$
18	23.934	4.11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
19	24.199	1.37	Cis-caryophyllene	$C_{15}H_{24}$
20	25.443	0.65	Yinylcyclooctane	$C_{10}H_{18}$
21	25.524	2.12	Hexadecadienoic acid, methyl ester	$C_{17}H_{34}O_2$
22	25.648	3.07	Ethyl linial	$C_{12}H_{22}O$
23	26.032	2.63	trans-Sesquisabinene hydrate	$C_{15}H_{26}O$
24	26.313	2.57	4 isopropyl benzaldehyde	$C_{10}H_{12}O$
25	26.524	1.12	1-Cyclohexylethanol, trifluoroacetate	$C_{10}H_{15}F_3O_2$
26	27.704	0.90	Glycidyl palmitate	$C_{19}H_{36}O_3$
27	28.020	0.55	Octylmethoxycinnamate	$C_{18}H_{26}0_3$
28	28.530	0.32	Squalene	$C_{30}H_{50}$
29	30.082	2.10	Phosphonic acid, dioctadecyl ester	$C_{36}H_{75}O_3P$
30	30.570	2.17	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$
31	32.056	2.22	1-Azabicyclo[2.2.2]octan-2-ol, 4-phenyl-	$C_{13}H_{17}NO$
32	32.281	1.90	1-Heptacosanol	$C_{27}H_{56}O$
33	33.390	1.18	Cyclohexanone, 4,4'-(1,2-diethyl-1,2-ethaned	$C_{18}H_{30}O_2$
34	34.451	1.26	Behenic alcohol	$C_{22}H_{46}O$
35	37.278	2.53	STIGMAST-5-EN-3-OL, (3.BETA.)-	$C_{29}H_{50}O$
36	39.438	1.89	2,4a,8,8Tetramethyldecahydrocyclopropa[d]naphthalene	$C_{15}H_{26}$
37	42.005	4.97	gamma.-Sitosterol	$C_{29}H_{50}O$
		100.00		

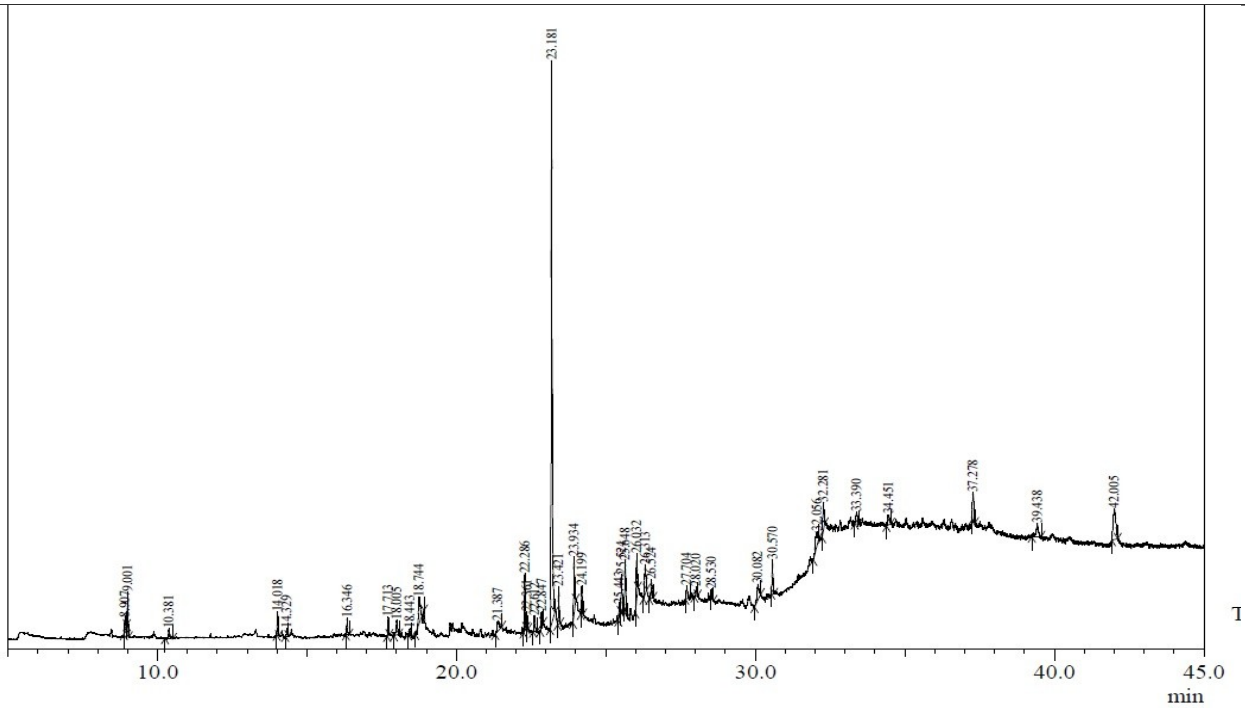

Fig. 2. GC-MS chromatogram of methanolic extract of leaves of wild plants of *V. wallichii.*

Table 3. Phytocompounds present in methanolic extract of leaves of micropropagated plants of *V. Wallichii.*

Peak	R. time	Area%	Name of the compound	Chemical formula
1.	6.763	0.53	2-Thujene	$C_{10}H_{16}$
2.	7.655	0.51	DELTA-3-CARENE	$C_{10}H_{16}$
3.	7.77	0.13	B-geraniolene	$C_{10}H_{16}$
4.	8.015	0.42	Mycrene	$C_{10}H_{16}$
5.	8.639	0.25	Terpinoline	$C_{10}H_{16}$
6.	8.813	1.92	O-Cymene	$C_{10}H_{14}$
7.	8.947	6.51	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{20}O_2$
8.	9.003	29.9	Eucalyptol	$C_{10}H_{18}O$
9.	9.538	0.91	gamma.-Terpinene	$C_{10}H_{16}$
10.	9.876	0.26	1,2,3,6-Tetrahydrobenzylalcohol, acetate	$C_9H_{14}O_2$
11.	10.381	0.28	Decane, 3-methyl-	$C_{11}H_{24}$
12.	11.949	0.94	Bingpian	$C_{10}H_{18}O$
13.	12.099	0.93	cis-Sabinenhydrate	$C_{10}H_{18}O$
14.	14.182	0.37	Hexyl octyl ether	$C_{14}H_{30}O$
15.	14.334	0.6	Decanedioic acid, didecyl ester	$C_{30}H_{58}O_4$
16.	14.477	0.41	Hexyl octyl ether	$C_{14}H_{30}O$
17.	17.718	1.46	2,4-Di-t-butylphenol	$C_{14}H_{22}O$
18.	18.788	1.41	Beta.-apo-8-carotenal	$C_{11}H_{18}O$
19.	22.288	2.3	Neophytadiene	$C_{20}H_{38}$
20.	22.363	0.51	1-Dodecanol	$C_{12}H_{26}O$
21.	22.625	1.28	Phthalic acid, butyl undecyl ester	$C_{25}H_{36}O_4$
22.	22.847	0.67	Butyric Acid	$C_{14}H_{26}O_2$
23.	23.185	24.53	Dimethyl palmitamine	$C_{18}H_{39}N$
24.	23.426	1.58	Hexadecanoic acid, methyl ester	:$C_{17}H_{34}O_2$
25.	23.936	2.52	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
26.	24.604	0.34	Isopropyl palmitate	$C_{19}H_{38}O_2$
27.	25.453	0.69	5-Eicosyne	$C_{20}H_{38}$
28.	25.525	3.8	2-monolinolenin	$C_{21}H_{36}O_4$
29.	25.663	1.89	Phytol	$C_{20}H_{40}O$
30.	26.311	1.81	6-Allyl-2-cresol	$C_{10}H_{12}O$
31.	30.573	1.44	Vinicizer	$C_{24}H_{38}O_4$
32.	31.852	1.27	Didrovaltrate	$C_{22}H_{32}O_8$
33.	32.514	0.48	S-Propyl hexanethioate	:$C_9H_{18}OS$
34.	32.844	1.19	Hexanethioic acid, s-ethyl ester	$C_8H_{16}OS$
35.	37.287	1.68	Stigmast-5-en-3-ol, (3.beta.)	$C_{29}H_{50}O$
36.	42.034	4.25	Retinol	$C_{20}H_{30}O$

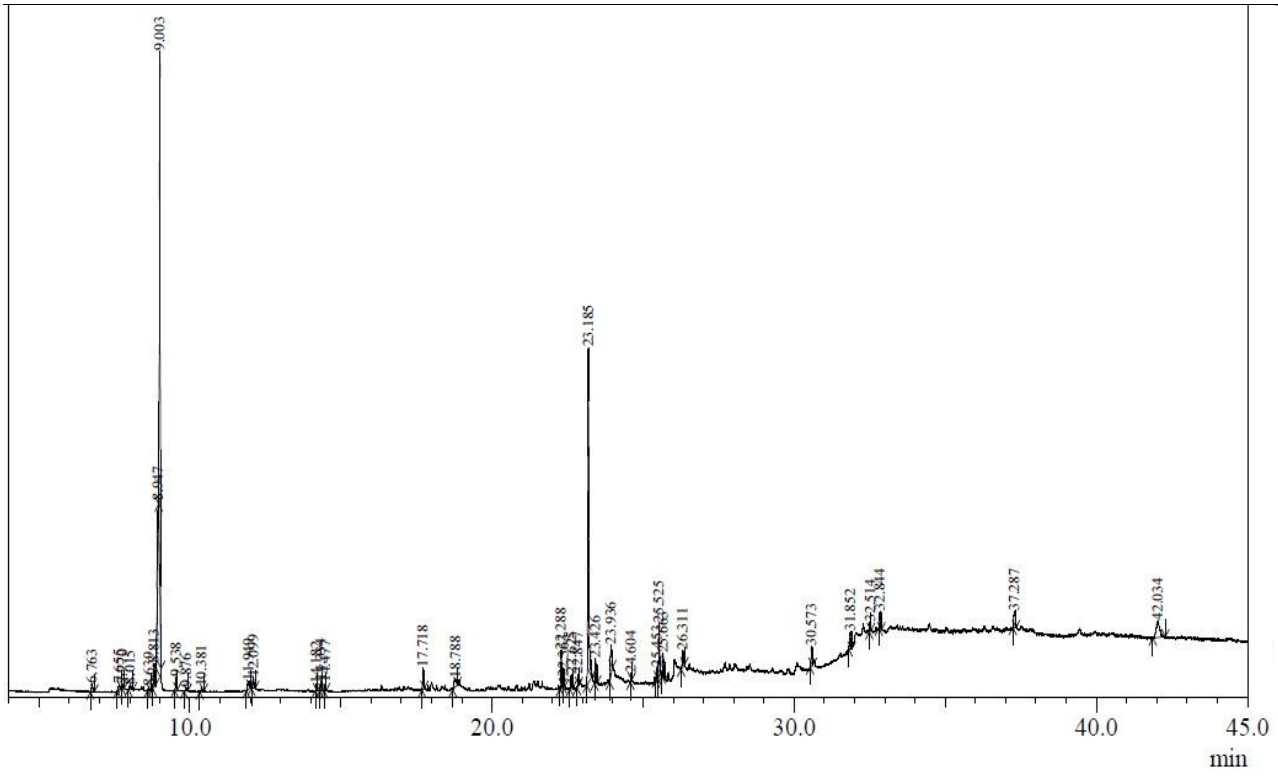

Fig. 3. GC-MS chromatogram of methanolic extract of leaves of micropropagated plants of *V. wallichii.*

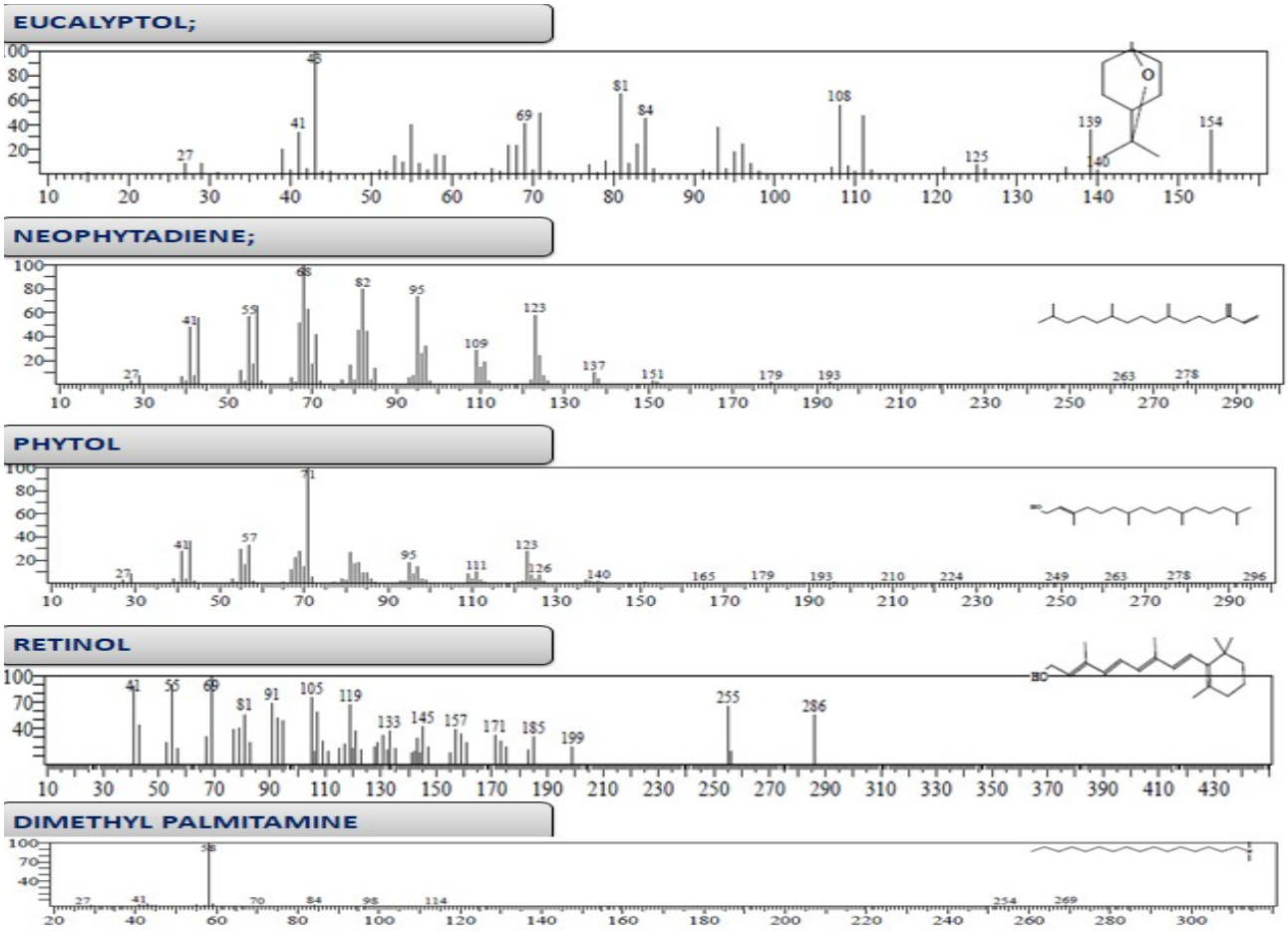

Fig. 4. MS peaks of major compounds present in methanolic extract of leaves of mother plant.

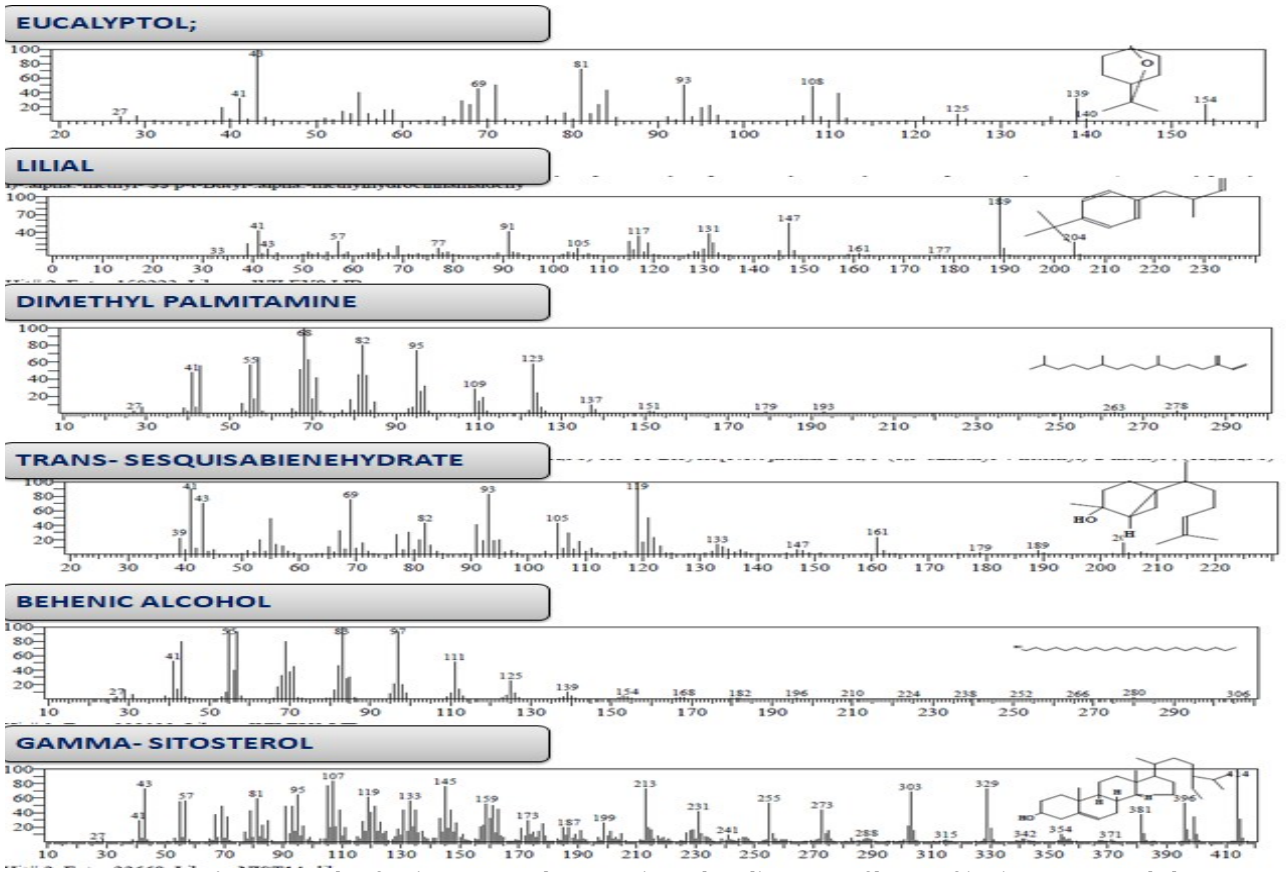

Fig. 5. MS peaks of major compounds present in methanolic extract of leaves of *in-vitro* regenerated plant.

bacterial activity (36). Presence of several phytocompounds with reported biological and pharmacological activities support utilization of leaves of the plant for medicinal purposes. Further studies need to be conducted to develop, optimize and validate protocols for authenticated utilization of leaves of *V.wallichii* in a similar manner as the roots/ rhizome of the plant are utilized for medication in traditional as well as modern medicine system.

Conclusion

Present study reports an efficient and rapid method of mass propagation of *V. wallichii.* Besides GC-MS analysis have revealed the presence of several phytocompounds possessing biological activities (reported in earlier studies) in the leaves of mother as well as micropropagated plants. Hence, there exists a promising scope for assessment of medicinal potential of leaves of *V. wallichii* and implementation of same for medicinal purpose in the similar way as the roots are utilized.

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Authors' contributions

NS conceptualized the study. NS, RR, FL carried out a literature survey and conducted experiments. AS & VB analyzed the GC results. NS and AS prepared the first draft of the manuscript. RR & VB has revised the manuscript. All authors read and approved the final manuscript.

Conflict of interests

There exists no conflict of interest.

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