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Original Article

GC-MS PROFILE OF *IN VIVO, IN VITRO* AND FUNGAL ELICITED *IN VITRO* LEAVES OF *HYBANTHUS ENNEASPERMUS* (L.) F. MUELL

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ABSTRACT

Objectives: Investigation of the bioactive compounds from the methanol leaf extracts of *in vivo, in vitro* and fungal elicited *in vitro* plants of *Hybanthus enneaspermus* through GC-MS analysis.

Methods: The leaf explants were cultured on MS (Murashige and Skoog) medium supplemented with different concentrations of NAA (1-naphthalene acetic acid) for callus induction. The calli was treated with four different fungal elicitors, namely, *Mucor prayagensis, Trichoderma viride, Fusarium moniliformis* and *Aspergillus niger* on suspension culture containing the same growth regulators for two weeks. Regeneration of shoots was achieved from the fungal treated and untreated calli on MS medium fortified with different concentrations of cytokinins. Rooting was achieved from the isolated shoots on half strength MS medium containing different concentrations of auxins. The phytochemical composition was analyzed from the methanol extracts of *in vivo, in vitro* and fungal treated leaves of *in vitro* plants using gas chromatography and mass spectroscopy (GC-MS).

Results: Of the different concentrations and combinations of NAA, well developed green compact reproducible calli were obtained on MS medium supplemented with 10 µmol NAA+4 µmol BAP (6-benzylaminopurine). Shoots were regenerated from the fungal treated and untreated calli on MS medium containing 10 µmol BAP+6 µmol KIN (kinetin-6-furfurylaminopurine). Rooting was achieved on half strength MS medium supplemented with 9 µmol NAA. The GC-MS analysis revealed that the leaves of *in vivo* and *in vitro* plants contained 16 different phytochemicals, whereas, the fungal treated *in vitro* plants showed more number of phytochemicals, i.e., 22 (*Mucor prayagensis*), 26 (*Trichoderma viride*), 19 (*Fusarium moniliformis*) and 21 (*Aspergillus niger*) compounds.

Conclusion: Synthesis of more number of phytochemicals in the fungal treated leaves, in the present study, shows that the fungal species can be used for the process of elicitation and to enhance the secondary metabolites in medicinal plants.

Keywords: *Hybanthus enneaspermus*, Suspension culture, Tissue culture technique, Fungal elicitors, GC-MS.

INTRODUCTION

Medicinal plants are the most important source for the discovery of new drugs and drug development. Most of the plant drugs are the secondary metabolites synthesized by various metabolic pathways. The secondary metabolites are economically important not only as drugs, but also used as flavour and fragrances, dye and pigments, pesticides, food additives, defense substances, etc. The utilization of plant cells for the production of natural or recombinant compounds of commercial interest has gained increasing attention over past decades [1].

Plant cell and tissue cultures hold great promise for controlled production of a great number of useful secondary metabolites on demand. Discovery of cell cultures, capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants, has accelerated in the last few years [2]. Large-scale plant tissue culture is found to be an attractive and alternative approach to traditional methods of plantation as it offers controlled supply of biochemicals, independent of plant availability [3]. Plant tissue culture has proved as an important technique for improving the utility sources and naturally occurring active metabolites in medicinally important plant system. This is a highly appreciable and acceptable biotechnological concept that contributes largely in exploring and conserving the natural sources of herbal medicines to achieve high product recovery. *In vitro* production of secondary metabolites in plant cell suspension cultures has been reported from various medicinal plants [4-7]. Different strategies for metabolic engineering are worked out by the scientists for qualitative and quantitative improvement of biologically active compounds. These strategies include use of suitable conditioned bioreactor, chemical and biological elicitors, increasing the cell permeability and alterations in media composition [8-10]. Elicitors are compounds that stimulate plant cell secondary metabolism and are usually derived from components of fungal or plant cell walls. Feeding of elicitors has been proven to be an effective way to enhance secondary metabolites in plant cell cultures. In recent years, the enhancement of secondary metabolites or phyto chemicals has been achieved in several plants by using biotic and abiotic elicitors [11-16]. The biotic elicitors are generally derived from pathogenic or non-pathogenic microorganisms, plant cell wall components, as well as chemicals released by plants at point of pathogen or herbivore attack [17-19]. Fungal elicitation has been an effective technique for enhancement of secondary metabolites [20, 21] and as a tool for studying metabolism [22]. The fact that the strongly and rapidly accumulating secondary metabolites, stimulated by the fungal elicitors, have recently attracted considerable attention [9, 23].

Hybanthus enneaspermus (L.) F. Muell. (*Ionidium suffruticosum* Ging.), a member of Violaceae, is a small suffrutescent perennial herb distributed in the tropical and subtropical regions of the world. It is popularly known as Rathanapurush, Pursharathna (Sanskrit, Hindi) and Orithazhthamarai (Tamil). This herb is considered to be extremely beneficial to men. In folklore, the plant is used in the case of pregnant and parturient women, and in case of gonorrhoea and urinary infections. The plant is reported in ancient Ayurvedic literature to cure conditions of "kapha" and "pitta", urinary calculi, strangury, painful dysentery, vomiting, burning sensation, wandering of the mind, urethral discharges, blood troubles, asthma, epilepsy, cough, and to give tone to the breasts. Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhea, leucorrhoea, dysuria and sterility [24, 25].

Several studies revealed that the plant has free radical scavenging activity [26-28], anti plasmodial [29], antiarthritic [30], antidiabetic [28], antimicrobial [31], antiinflammatory [32], anti-infertility [33]

and nephro protective activities [34]. The root is diuretic and is used in urinary infections and bowel complaints in children.

Based on this lime light and our early study on regeneration of *Hybanthus enneaspermus* from stem explants [35], the present investigation is aimed to regenerate the plantlets from leaf explants through organogenesis and to analyze the photochemical components of the leaves of *in vivo*, *in vitro* and fungal treated *in vitro* plants using Gas Chromatography-Mass Spectrophotometer (GC-MS).

MATERIALS AND METHODS

Plant material

In the present investigation, *Hybanthus enneaspermus* was selected for *in vitro* regeneration through callus culture from the leaf explants. The plant materials were collected from in and around the college campus. The leaf explants were washed thoroughly under running tap water for 20 min. Then they were rinsed with surfactant (Teepol) for 2 min. After that they were rinsed with distilled water for 4-5 times. The explants were disinfected with 70% alcohol (v/v) for 45 seconds followed by 0.1% mercuric chloride (w/v) for 3 min in the Laminar Air Flow Chamber. Finally, the explants were washed with sterile distilled water for 3-5 times to remove the traces of mercuric chloride.

Preparation of culture media

For the entire study MS medium [36] was used as the basal medium with 3% sucrose and respective growth regulators for callus culture, suspension culture and regeneration. The medium was solidified with 0.8% agar and the pH of the medium was adjusted to 5.8 using 0.1 N NaOH and 0.1 N HCl before autoclaving. The medium was autoclaved at 1.06 kg/cm2 at 121°C under 15 lbs/sq. ft. pressure for about 20 min.

Callus culture

The leaf explants were inoculated on MS medium fortified with different concentrations of NAA with low concentration of BAP for callus induction. The suitable concentration of growth regulator was optimized and further used for suspension culture.

Preparation of fungal elicitors

Fungal elicitors were prepared from cultures of *Mucor prayagensis* (Acc. No.3371), *Trichoderma viride* (Acc. No.793), *Fusarium moniliformis* (Acc. No.156) *and Aspergillus niger* (Acc. No.281) which were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTEC), Chantigarh, India. The fungal filaments were grown in 250 ml conical flasks containing 100 ml of SD broth (Sabouraud Dextrose Broth) at room temperature for 21 d under static condition. Fully grown mycelia with spores were homogenized and centrifuged at 4000 rpm after autoclaving for 20 min at 121ºC. The supernatants were collected and stored at 4 °C. These extracts were used as fungal elicitors [37, 38].

Suspension culture

The well developed green compact calli were transferred to 250 ml conical flasks containing 100 ml of MS liquid medium fortified with optimized concentration of NAA and different fungal elicitors (15 ml/l at 1.2 OD600). The cultures were maintained in an orbital shaker at 120 rpm/min for two weeks.

Regeneration of plantlets

After two weeks of incubation, the calli were transferred to MS solid medium fortified with optimized concentration of BAP with and without fungal elicitors for shoot regeneration. The regenerated shoots were transferred to half strength MS medium with different concentrations of NAA for rooting. The rooted plants were transferred to the field through hardening and acclimatization.

Extraction of leaf samples for GC-MS analysis

The leaf samples of *in vivo*, *in vitro* and fungal elicitated *in vitro* plants were collected, shade dried and ground to fine powder. The powders were extracted with methanol using soxhlet apparatus. The extracted materials were subjected to GC-MS analysis.

Gas chromatograph-Mass spectrometer (GC-MS) analysis

GC-MS analysis was carried out on a Thermo GC-Trace Ultra Ver.5.0 Thermo MS DSQ II System. The chromatography was performed by using the DB5-MS Capillary Standard Non-Polar Column. Helium gas was used as a carrier as well as eluent at a constant flow of 1 ml/min. About 1 µl methanol extract was injected using a micro syringe. Injection temperature was set at 260 °C. The oven temperature was programmed from 70 °C with an increase of 6 °C/min raised to 260 °C, 1 min isocratic and cooled to 70 °C, followed by the additional 5 min delay. The ion trace integration was done using the mass lab finds target method for the characteristic fragment of assigned peaks. Total GC running time was 34 min.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the data base of the National Institute Standard and Technology (NIST) and Wiley Spectra Libraries having more than 62,000 patterns. Spectra of the unknown components were compared with the spectra of known components stored in the NIST Library. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from NIST and Wiley Spectra Libraries were recorded. Prediction of biological activities of each compound was based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA [39].

RESULTS

In vitro plant regeneration and fungal elicitation

The leaf explants of *Hybanthus enneaspermus* was cultured on MS basal medium supplemented with different concentrations of auxins with low concentration of cytokinins for callus induction. Of the different concentrations of growth regulators, the best results were obtained from 10 µmol NAA+4 µmol BAP. Shoots were regenerated from the untreated and fungal elicitor treated calli. More number of shoots was regenerated on MS medium supplemented with 10 µmol BAP in combination with 6 µmol KIN. Rooting was achieved on half strength MS medium supplemented with 9 µmol NAA. (fig. 1).

Phytochemical analysis by GC-MS

The GC-MS analysis revealed that various bioactive compounds were identified in the *in vivo, in vitro* and four different fungal elicited *in vitro* leaves of *Hybanthus enneaspermus* (table 1; fig. 2A-F).

The mass spectrum of the leaves of *in vivo* and *in vitro* plants revealed the presence of 16 different phyto chemicals. Of these 16 compounds, 5 compounds were similar in both *in vivo* and *in vitro* plants. Other 11 compounds were different from each other (table 1; fig. 2a, b). Octadecanoic acid, phytol, vitamin E, Bis (2-ethylhexyl) phthalate, n-Hexadecanoic acid, Ethanone, Dodecanoic acid, oxirane, etc. were the major compounds present in the leaves of *in vivo* plants. The *in vitro* leaves showed 2, 3-dimethyl-benzofuran, 4-butoxy-1-butanol, Ethyl alpha-d-glucopyranoside, 3-methyl-1H-Indole, lactose, n-Hexadecnoic acid, Octadecatrienoic acid, etc. as major compounds.

The fungal treated leaves, however, contained the greater number of compounds than that of *in vivo* and *in vitro* untreated plants. The number of compounds varied from 19 to 26. Interestingly nearly 12 similar compounds were identified in the leaves of all the fungal treated plants (table 1; fig. 2C-F).

The leaves of *in vitro* plants treated with the extract of *Mucor prayagensis* showed 22 different compounds, of which 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one, 2,3-dihydro-benzofuran, 4-butoxy-1-butanol, Ethyl alpha-d-glucopyranoside, 3-methyl-1H-indole, n-Hexadecanoic acid, Octadecatrienoic acid, vitamin E, etc. were the major compounds (table 1; fig. 2C).

The leaves of *Trichoderma viride* treated plants showed the maximum number of 26 different compounds. 2,4-dimethylfuran, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one, 2,3-dihydro-

benzofuran, N,N'-dibutyl-N,N'-dimethyl-urea, 1-chlorododecane, Cyclotetradecane, 2,5-Difluorobenzoic acid, 4-dodecyl ester, Ethyl alpha-d-glucopyranoside, 1-Dodecanethiol, beta.-D-Glucopyranose, n-Hexadecanoic acid, 1-(1-Hydroxybutyl)-2,5-dimethoxybenzene, phytol, Octadecatrienoic acid, vitamin E, 7-hexadecanoic acid, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)-ethanone, etc. were the major compounds (table 1; fig. 2dD).

The *in vitro* plants treated with *Fusarium moniliformis* showed 19 different compounds, of which the major compounds were 2,4-dimethylfuran, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one, 2,3-dihydro-benzofuran, 2-methylphenol, N,N'-dibutyl-N,N'-dimethyl-urea, cyclododecane, 2,4-Difluorobenzoic acid, 4-dodecyl ester, Ethyl alpha-d-glucopyranoside, 3-methyl-1H-indole, 2,6,6-trimethyl-,(1. alpha.,2. beta.,5. alpha.)-bicyclo[3.1.1]heptane, n-Hexadecanoic acid, 1-(1-Hydroxybutyl)-2,5-dimethoxybenzene, Phytol, Octadecatrienoic acid, Vitamin E, 7-hexadecanoic acid, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)-ethanone, etc. (table 1; fig. 2E).

The leaves of *in vitro* plants elicitated by *Aspergillus niger* showed 21 different compounds. 2,4-dimethylfuran, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one, 2,3-dihydro-benzofuran, 6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide, cyclododecane, 2,4-Difluoro-benzoic acid, 4-dodecyl ester, Ethyl alpha-d-glucopyranoside, n-Hexadecanoic acid, phytol, Octadecatrienoic acid, vitamin E, 7-hexadecanoic acid, 2-(2-benzothiazolylthio)-1-(3,5-dimethyl-pyrazolyl)-ethanone, etc. were the major compounds (table 1; fig. 2F).

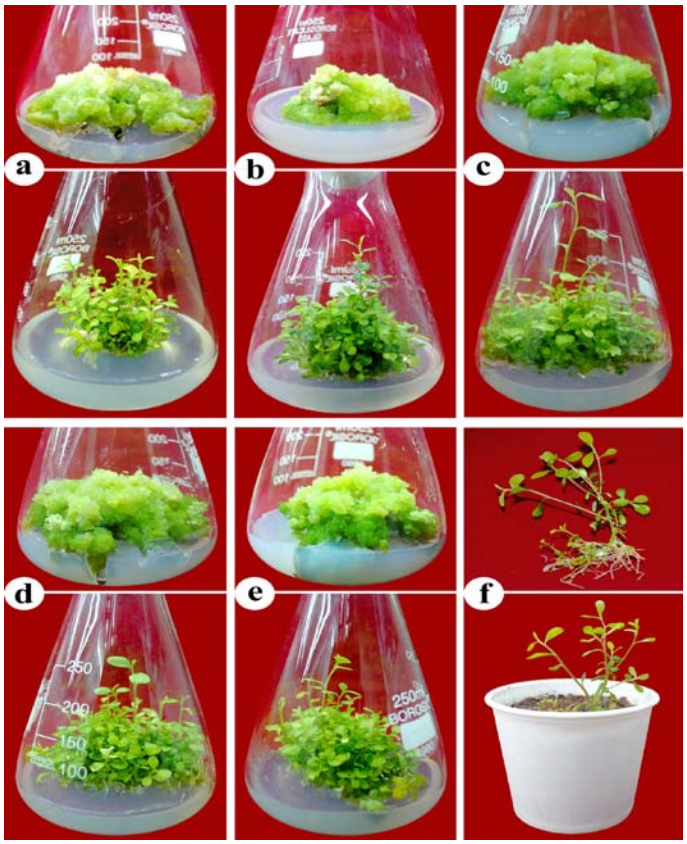

Fig. 1: Callus culture and regeneration of plantlets from the untreated and fungal treated callus of *Hybanthus enneaspermus*. A. control; B. treated with *Mucor prayagensis*; C. treated with *Trichoderma viride*; D. treated with *Fusarium moniliformis*; E. treated with *Aspergillus niger*; F. rooting and hardening of plantlets

Table 1: Comparative GC-MS analysis of *Hybanthus enneaspermus*-Compounds identified in the leaf extracts of *in vivo, in vitro* and *in vitro* plants treated with the extracts of different fungal species

S. No.	RT	Name of the compounds	Percentage of Peak Area					
			In vivo Plants	*In vitro* Plants	*In vitro* plants treated with			
					Mucor prayagensis	*Trichoderma viride*	*Fusarium moniliformis*	*Aspergillus niger*
1	2.19	3-Amino-2-oxazolidinone	--	--	0.37	--	--	--
2	2.46	Cyclopentene, 3-ethyl-	--	--	--	0.60	0.69	0.92
3	2.48	1-Ethylcyclopentene	--	--	0.86	--	--	--
4	2.81	1,3-Oxathiolane, 2,2-dimethyl-	--	0.69	--	--	--	--
5	3.41	2,4-Dimethylfuran	--	--	1.94	1.11	1.27	2.17
6	7.87	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	--	--	3.05	1.33	2.66	2.64
7	9.19	Benzofuran, 2,3-dihydro-	--	5.38	3.55	3.68	4.37	5.46
8	11.99	Bicyclo[2.1.0]pentane, 1,4-dimethyl-	--	0.88	--	--	--	--
9	11.99	Phenol, 2-methyl-	--	--	--	--	1.28	2.20
10	12.00	Pyrazole, 4-aminomethyl-3,5-dimethyl-	--	--	0.87	--	--	--
11	12.01	1,2,5-Trimethylpyrrole	--	--	--	0.87	--	--

12	12.36	6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide	--	--	--	--	--	6.88
13	12.37	Urea, N,N'-dibutyl-N,N'-dimethyl-	--	--	--	5.68	10.66	--
14	12.41	1-Butanol, 4-butoxy-	--	19.06	6.91	--	--	--
15	12.52	Metformin	--	--	--	--	--	1.20
16	12.53	Dodecane, 1-chloro-	1.31	--	--	2.13	--	--
17	12.59	Chloroacetic acid, 2-tridecyl ester	--	--	1.75	--	--	--
18	12.59	Cyclododecane	--	--	--	--	2.18	2.36
19	12.60	Cyclotetradecane	--	--	--	2.84	--	--
20	12.80	2,5-Difluorobenzoic acid, 4-dodecyl ester	--	--	--	1.23	--	--
21	12.81	2,6-Difluorobenzoic acid, 4-tridecyl ester	--	--	1.28	--	--	--
22	12.81	2,4-Difluorobenzoic acid, 6-dodecyl ester	--	--	--	--	2.05	1.81
23	13.02	Arginine	--	3.23	--	--	--	--
24	13.66	Dodecanoic acid	5.3	--	--	--	--	--
25	14.62	Ethyl. alpha.-d-glucopyranoside	--	21.68	30.58	35.50	6.80	23.89
26	14.77	1H-Indole, 3-methyl-	--	10.84	25.24	--	14.70	--
27	15.00	Oxirane, [(dodecyloxy)methyl]-	5.09	--	--	--	--	--
28	15.01	1-Dodecanethiol	--	--	--	5.27	--	--
29	15.71	Cyclododecane	1.79	--	--	--	--	--
30	15.93	Lactose	--	4.12	--	1.80	--	--
31	15.94	Tetradecanoic acid	2.04	--	0.97	--	--	--
32	16.04	. beta.-D-Glucopyranose	--	--	--	3.37	--	--
33	16.04	D-Glucose, 6-O-. alpha.-D-galactopyranosyl-	--	--	1.54	--	--	--
34	16.71	1,2-Dihexylcyclopropene	2.15	--	--	--	--	--
35	16.71	(3-Fluorophenyl)(furan-2-yl)methanol	--	--	--	0.32	--	--
36	16.71	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1. alpha.,2. beta.,5. alpha.)-	--	--	--	--	1.57	--
37	16.71	6-Octen-1-ol, 3,7-dimethyl-, propanoate	--	--	--	--	--	0.49
38	18.02	n-Hexadecanoic acid	20.02	8.52	5.49	5.96	9.08	9.01
39	18.43	1-(1-Hydroxybutyl)-2,5-dimethoxybenzene	--	--	--	1.39	--	--
40	18.51	Oxirane, tetradecyl-	2.98	--	--	--	--	--
41	19.31	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	--	1.54	--	--	--	--
42	19.33	Methyl 8,11,14-heptadecatrienoate	--	--	--	--	--	0.85
43	19.44	Phytol	7.62	--	0.56	1.01	2.20	1.42
44	19.70	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	24.36	14.76	6.68	9.74	14.82	12.68
45	19.91	2-Methyl-Z,Z-3,13-octadecadienol	--	--	--	--	--	1.34
46	19.92	Octadecanoic acid	2.7	--	0.89	0.86	--	--
47	19.92	9,17-Octadecadienal, (Z)-	--	--	--	--	1.33	--
48	22.45	2-Nonadecanone	--	--	--	0.25	--	--
49	22.54	L-Glutamic acid 5-ethyl ester	--	--	--	0.59	--	--
50	22.55	Dodecahydropyrido[1,2-b]isoquinolin-6-one	1.88	--	--	--	--	--
51	22.55	Piperidine, 1-[(2,3,4,5-tetramethylphenyl)sulfonyl]-	--	1.14	--	--	--	--
52	23.01	Bis(2-ethylhexyl) phthalate	5.81	--	--	--	--	--
53	23.01	Diisooctyl phthalate	--	--	0.61	0.49	--	--
54	23.02	Adamantane, 1-isothiocyanato-3-methyl-	--	--	--	--	--	1.07
55	27.46	Vitamin E	6.81	2.96	3.75	7.02	1.47	7.58
56	27.47	dl-alpha. Tocopherol	--	--	--	--	17.33	--
57	29.23	7-Hexadecenoic acid, methyl ester, (Z)-	--	2.25	0.96	1.69	1.94	2.37
58	29.40	Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)-	10.12	1.76	1.37	2.79	3.21	4.60
59	29.90	9-Octadecenoic acid (Z)-, methyl ester	0.04	1.19	0.80	2.39	3.32	3.54
		Total number of compounds	16	16	22	26	20	21

DISCUSSION

Plant tissue culture technique is frequently used as an efficient renewable resource for producing a variety of phyto chemicals that are difficult to synthesize artificially or produced in extremely low concentration in field-grown plants. Many workers periodically reviewed the progress in this subject [40-43].

Though callus and cell suspension cultures are preferred for producing increased quantity and quality of phyto chemicals, in most cases the biosynthesis of these compounds has been found to be linked with higher level of cellular or tissue differentiation in the form of organized shoots or roots or both [43, 44].

This situation is more demanding when desired metabolites are synthesized or accumulated in specialized cells or tissues of the plants [45, 46]. In the present study, biotic elicitors derived from fungi, namely, *Mucor prayagensis, Trichoderma viride, Fusarium moniliformis* and *Aspergillus niger,* were tested to enhance phytochemical compounds in the *in vitro* plants of *Hybanthus enneaspermus*. The results showed that, all the fungal treated *in vitro* plants synthesized more number of compounds when compared to that of *in vivo* field grown plants and also *in vitro* plants without fungal elicitor treatment (table 1). Results of the present study confirmed the findings of the early studies that the elicitors not only enhanced the phytochemicals but also synthesized new medicinally important compounds that were not synthesized in the non treated plants (table 2).

Of the four fungal elicitors treated, maximum number of 26 chemical compounds was identified on the *in vitro* leaves treated with *Trichoderma viride* followed by *Mucor prayagensis*. The earlier findings show that the members of the genus *Trichoderma* are well known for their effect on plant growth promotion and are frequently used as bio-protectant [17, 47-50]. Studies dealing with the mechanism of interaction between *Trichoderma* and plants have indicated that culture filtrate of this fungus contains

macromolecules and low molecular weight compounds that induce strong changes in cytosolic calcium ion level in plant cells and activate defense responses including the accumulation of plant secondary metabolites [51].

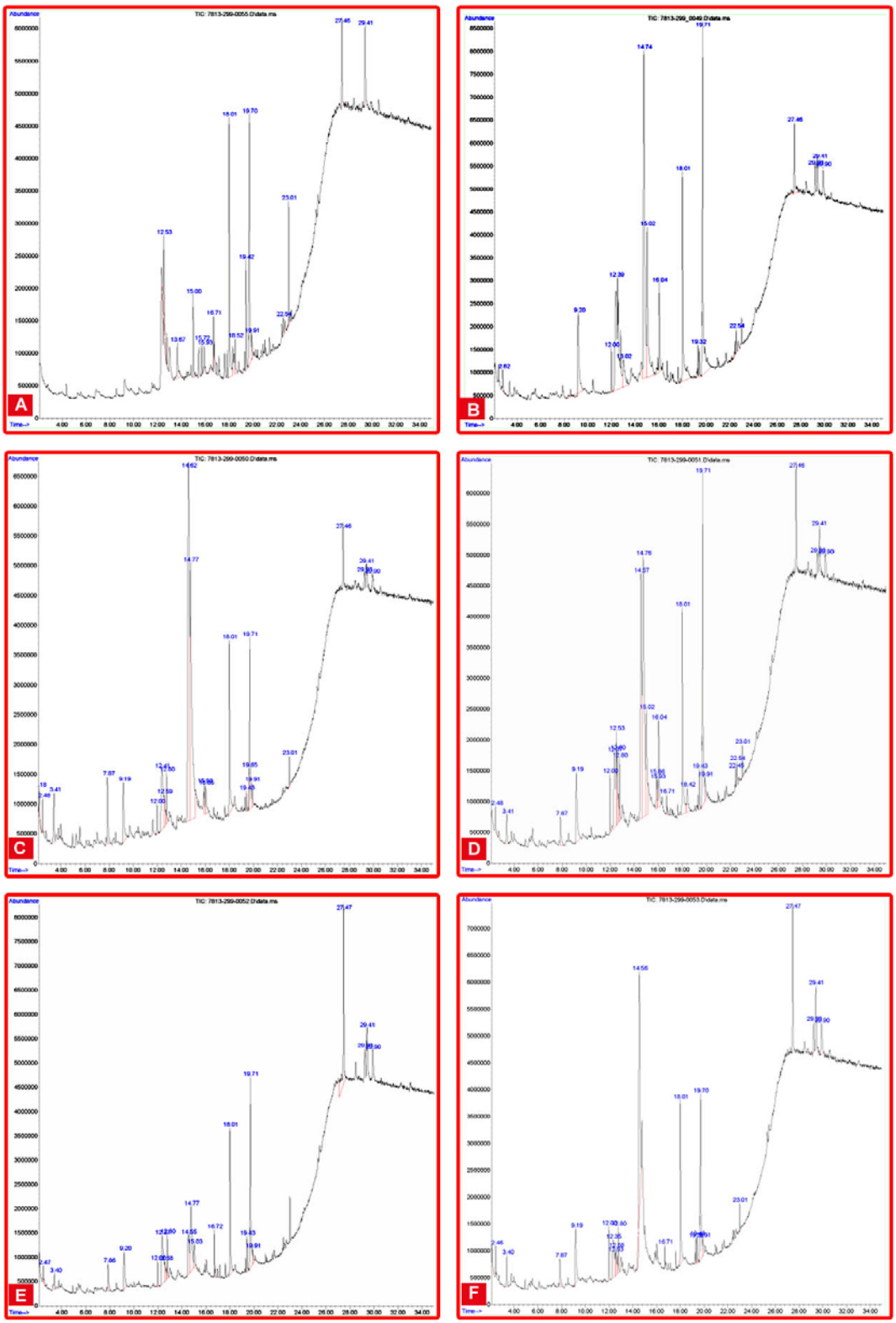

Fig. 2: GC-MS Spectra of *in vivo*, *in vitro* and fungal treated *in vitro* leaves of *Hybanthus enneaspermus*. A. spectrum of *in vivo* leaf; B. spectrum of *in vitro* leaf; C. spectrum of *in vitro* leaf treated with *Mucor prayagensis*; D. spectrum of *in vitro* leaf treated with *Trichoderma viride*; E. spectrum of *in vitro* leaf treated with *Fusarium moniliformis*; F. spectum of *in vitro* leaf treated with *Aspergillus niger*

Majority of the chemical compounds derived from all the sources, namely, *in vivo, in vitro* and fungal treated *in vitro* leaves were found to be bioactive and medicinally important. Various biological activities of these compounds were listed in table 2.

Table 2: Biological activities of the compounds derived from the leaf of *in vivo, in vitro* and fungal treated *in vitro* plants of *Hybanthus enneaspermus*

S. No.	RT	Name of compound	Nature of compound	Biological activities
1	2.19	3-Amino-2-oxazolidinone	Metabolite of Furazolidone and Nitrofuran	Not intended for diagnostic or therapeutic use
2	2.46	Cyclopentene, 3-ethyl-	Cycloalkene	Antiallergic, anti-inflammatory, anti-tumour
3	2.48	1-Ethylcyclopentene	Cycloalkene	Antiallergic, anti-inflammatory, anti-tumour
4	2.81	1,3-Oxathiolane, 2,2-dimethyl-	---	Anticancer
5	3.41	2,4-Dimethylfuran	Furan derivative	Biofuel
6	7.87	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	---	Antimicrobial, anti-inflammatory, Antiproliferative.
7	9.19	Benzofuran, 2,3-dihydro-	Coumaran	Antilipidemic, Anti-inflammatory, anti-helminthics, antidiarrheal
8	11.99	Bicyclo[2.1.0]pentane, 1,4-dimethyl-	---	No activity reported
9	11.99	Phenol, 2-methyl-	Cresol (phenol derivative)	Anaesthetic, Analgesics Antiseptic, antipruritic (itch reliever), Antidotic, Antioxidant
10	12.00	Pyrazole, 4-aminomethyl-3,5-dimethyl-		Analgesic, Antiinflammatory, Antipyretic
11	12.01	1,2,5-Trimethylpyrrole	Pyrrolizidine Alkaloid	Hepatoprotective, Antitumour
12	12.36	6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide	---	No activity reported
13	12.37	Urea, N,N'-dibutyl-N,N'-dimethyl-	Urea derivative	Not intended for diagnostic or therapeutic use
14	12.41	1-Butanol, 4-butoxy-	Alcohol	Volatile Biomarker for gastric cancer
15	12.52	Metformin	---	Antidiabetic
16	12.53	Dodecane, 1-chloro-	Chloroalkane	No activity reported
17	12.59	Chloroacetic acid, 2-tridecyl ester	Ester	Herbicide
18	12.59	Cyclododecane	Cyclic alkane	Non-toxic, No activity reported
19	12.60	Cyclotetradecane	Cyclic alkane	No activity reported
20	12.80	2,5-Difluorobenzoic acid, 4-dodecyl ester	Ester	Not intended for diagnostic or therapeutic use
21	12.81	2,6-Difluorobenzoic acid, 4-tridecyl ester	Ester	Not intended for diagnostic or therapeutic use
22	12.81	2,4-Difluorobenzoic acid, 6-dodecyl ester	Ester	Not intended for diagnostic or therapeutic use
23	13.02	Arginine	Amino acid	Antihepatatic, Antiimpotence, Antiinfertility, Aphrodisiac, Diuretic, Spermigenic, Vasodilator
24	13.66	Dodecanoic acid	Fatty acid	Antibacterial, Antioxidant, Antiviral, Candidcide
25	14.62	Ethyl. alpha.-d-glucopyranoside	Glucoside	Antituberculous Activity, Antioxidant,alpha amylase inhibitory activity, Hypolipemic activity, Anticonvulsant
26	14.77	1H-Indole, 3-methyl-	Skatole	Antituberculosis
27	15.00	Oxirane, [(dodecyloxy)methyl]-	Cyclic ether	No activity reported
28	15.01	1-Dodecanethiol		Not intended for therapeutic use
29	15.71	Cyclododecane	Cyclic alkane	Antidote, emergency treatment
30	15.93	Lactose	Sugar	Anticephalopathic, Antihepatotic, Sweetener
31	15.94	Tetradecanoic acid	Fatty acid	Antioxidant, Cancer preventive, Cosmetic, Hypercholesterolemic, Nematicide
32	16.04	. beta.-D-Glucopyranose	Sugar (cyclic glucose)	Biofuel
33	16.04	D-Glucose, 6-O-. alpha.-D-galactopyranosyl-	Sugar moiety	No activity reported
34	16.71	1,2-Dihexylcyclopropene	Unsaturated hydrocarbon	No activity reported
35	16.71	(3-Fluorophenyl)(furan-2-yl)methanol	Alcohol	Antiepileptic
36	16.71	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1. alpha.,2. beta.,5. alpha.)-	---	Antimicrobial
37	16.71	6-Octen-1-ol, 3,7-dimethyl-, propanoate	Ester	Fragrance, Flavour
38	18.02	n-Hexadecanoic acid	Fatty acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic
39	18.43	1-(1-Hydroxybutyl)-2,5-dimethoxybenzene	Benzene derivative	No activity reported
40	18.51	Oxirane, tetradecyl-	Cyclic ether	No activity reported
41	19.31	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Ester	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, Antiandrogenic
42	19.33	Methyl 8,11,14-heptadecatrienoate	Ester	Antibiotic
43	19.44	Phytol	Diterpene	Antimicrobial Anti-inflammatory Anti cancer Diuretic
44	19.70	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Fatty acid	Antiinflammatory, Insectifuge, Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, Antiandrogenic, Antiarthritic, Anticoronary,
45	19.91	2-Methyl-Z,Z-3,13-octadecadienol	Terpenoid	Pesticide, Herbicide, Insecticide, Pheromone

46	19.92	Octadecanoic acid	Fatty acid	Hypocholesterolemic, Cosmetic, Flavour, Lubricant
47	19.92	9,17-Octadecadienal, (Z)-	---	No activity reported
48	22.45	2-Nonadecanone	Ketone	Not intended for therapeutic use
49	22.54	L-Glutamic acid 5-ethyl ester	Ester	Antiepileptic; Antiprostatitic; Antiretardation; Anxiolytic; Neurotoxic;
50	22.55	Dodecahydropyrido[1,2-b]isoquinolin-6-one	Ester	No activity reported
51	22.55	Piperidine, 1-[(2,3,4,5-tetramethylphenyl)sulfonyl]-	Heterocyclic amine derivative	Antienzymatic, Diaphoretic, Pesticide
52	23.01	Bis(2-ethylhexyl) phthalate	---	Plastisizer
53	23.01	Diisooctyl phthalate	Ester	Antiandrogenic
54	23.02	Adamantane, 1-isothiocyanato-3-methyl-	Cycloalkane derivative	Anticancer, Cocaine analogue
55	27.46	Vitamin E/dl-alpha. Tocopherol	Vitamin	Antiageing, Analgesic, Antidiabatic, Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary, Antiinfertility
56	29.23	7-Hexadecenoic acid, methyl ester, (Z)-	Ester	Antioxidant, Flavor, Hypocholesterolemic Pesticide, Anti-inflammatory, hypocholesterolemic, nematicide, antiandrogenic
57	29.40	Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)-	---	Antimicrobial
58	29.90	9-Octadecenoic acid (Z)-, methyl ester	Ester	Allergenic, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene-D4 (Anti-platelet activating factor), Dermatitigenic Insectifuge Perfumery, Cancer-Preventive, Choleretic, Flavor, Hypocholesterolemic, Irritant

CONCLUSION

Plant tissue culture can be used as an alternative technique not only for the mass production of plantlets in a short period but also for the enhancement of the phytochemical composition due the presence of ambience culture condition, growth regulators, elicitors, etc. Synthesis of more number of phytochemicals in the fungal treated leaves, in the present study, shows that the fungal species, especially *Trichoderma* spp., can be used for the process of elicitation and to enhance the secondary metabolites in medicinal plants.

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CONFLICT OF INTERESTS

The authors do not have any conflict of interest to declare

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