Journal of Applied Pharmaceutical Science Vol. 7 (04), pp. 083-088, April, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70411
ISSN 2231-3354

Comparative Phytochemical Analysis of Wild and Micropropagated *Cleome Viscosa* L.

M. Deventhiran, W. John Wyson, M. Sheik Noor Mohamed, K. Jaikumar, P. Saravanan, D. Anand*

Post Graduate & Research Department of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Mylapore, Chennai - 600 004. India.

ARTICLE INFO

Article history:
Received on: 12/11/2016
Accepted on: 18/01/2017
Available online: 30/04/2017

Key words:
In vitro propagation, MS medium, plant growth regulators, GC-MS analysis.

ABSTRACT

In the recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions. The *Cleome viscosa* are used in traditional systems of medicine for the treatment of many diseases in human. The present study aims to investigate the role of assorted plant growth regulators (PGRs) on *in vitro* propagation and comparison of similar and dissimilar compounds of wild plant *C. viscosa*. Nodal explants of 1.5-2.0 cm were used to induce multiple shoots in Murashige and Skoog (MS) medium supplemented with various concentration of different plant growth regulators (PGRs) such as 6-Benzylaminopurine (BAP), Kinetin (KIN), Naphthalene-3-acetic acid (NAA) and the bioactive constituent of wild and *in vitro* propagated *C. viscosa* plant was compared by analyzing polar and non polar extract of both the plants using Gas Chromatography - Mass Spectrometry (GC-MS) analysis. Multiple shoots were initiated within 28 days of inoculums and the various concentration of PGR had a significant role in the number of shoot formed and the *in vitro* regeneration of explants. The regenerated plantlets showed no morphological differences from the wild plant but the GC-MS analysis of ethanol extract showed the presence of eight compounds in wild plant and six in micropropagated while chloroform extract showed ten compounds in both plants.

INTRODUCTION

Cleome viscosa L., commonly known as wild or dog mustard, belongs to the Cleomaceae family. It is an annual, straight, considerably branched, 2-16 diameter tall, densely glandular, sticky herb found as a common weed all over the plains of India, Pakistan and throughout the tropics of the world (Mali, 2010, Chatargee and Pakrashi, 1991). The leaves, seeds and roots of the plant are widely used in traditional and folkloric systems of medicine as an anthelmintic, antiscorbutic, antiseptic, cardiac stimulant, carminative, and anticonvulsant (Shah *et al*., 1983) and it can be used in treatment of malarial fevers, skin diseases, leprosy, fever due to indigestion, blood disorders and uterine complications. Earlier pharmacological reports of *C. viscosa* indicated that it acts as hepatoprotective, analgesic, anti-inflammatory, antioxidant, immunomodulatory and antimalarial agent (Devi *et al*., 2003; Parimaladevi *et al*., 2003). The plant contains lignans, flavonoids, saponins, ascorbic acid, and polyunsaturated fatty acid. Some other chemical constituents isolated from *C. viscosa* are glucosinolates, cleomeolide, Stigmasta-5, kaempferide-3-glucuronide, and naringenin glycoside (Sudhakar *et al*., 2006). The tissue culture of important plants has shown promise in obtaining regenerates and clonal multiplication for domestication of wild populations, afforestation and economically important trees that have been cultivated for generations. Considering the importance of these medicinal plants for medicinal uses, pharmaceuticals and industries, it can be exploited at the commercial level. The medicinal value indicates that the use of various parts (leaves, roots, bark, etc.) of this plant extracts helpful in overcoming the disorders/disease predominant in the many rural areas of the country (Bonga and Durzan 1982).

* *Corresponding Author*
Dr. D. Anand, Assistant professor, Department of Botany Ramakrishna Mission Vivekananda College (Autonomous) Mylapore Chennai - 600 004, India. Phone:+91- 9841798900, E-mail: anandesingh@yahoo.co.in

Micropropagation is an efficient method to mass propagate good quality materials that may substantially improve production. It involves the use of defined growth media supplemented with appropriate growth regulators that enable morphogenesis to occur from naturally growing plant parts (Debergh and Read, 1991). Cytokinins are usually used on the micropropagation media to stimulate axillary shoot proliferation (Chawla, 2009; El-Agamy, 2009).

Mass spectrophotometry coupled with chromatographic separations such as Gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants (Sermakkani and Thangapandian, 2012).

Hence, the aim of the present study was to develop simple and efficient method for *in vitro* micropropagation through high frequency multiple shoots regeneration of *C. viscosa* utilizing the least number and various concentrations of PGRs under aseptic laboratory condition and to compare the phytochemical analysis between polar and non polar extract of micropropagated and wild plants to ascertain the rationale for its use in traditional medicine.

MATERIALS AND METHODS

Collection of Plant Material

The healthy plants of *Cleome viscosa* L. were collected during the month of March from the natural habitats of Kanchipuram district, Tamil Nadu, India. The plant specimen was identified and authenticated by Botanical Survey of India (BSI) Coimbatore, Tamil Nadu, India.

Sterilization of Explant

The nodal segment of the plant was chosen as explants for the present investigation. Actively growing shoots were selected as the source for explants. The explants were pre-sterilized by washing with running tap water to remove the dust particles from the surface.

The explants were then wrapped in 25% (v/v) Clorox containing three drops of tween 20 solution for 10 min and again rinsed several times with sterile distilled water until all traces of Clorox were eliminated. Surface sterilization of explants were carried out by rinsing it with 0.01% mercuric chloride ($HgCl_2$) for 3 minutes and then washed 3 times with sterile distilled water (Muthusamy Govarthanan *et al*., 2015).

Inoculation in culture medium

The nodal segments were cut into 5 mm in size and carefully transferred to the sterile MS basal medium (pH 5.8) supplemented with 3% (w/v) sucrose, 0.8% agar and different concentration (Table 1) of PGRs such as 6-Benzylaminopurine (BAP), Kinetin (KIN), Naphthalene-3-acetic acid (NAA).

The inoculated cultures were maintained in growth chamber with regulated temperature (26±2ºC), relative humidity (55±5%), light and dark conditions of 16/8 hours photoperiod and 3000 lux intensity of constant light was provided in culture shelves by cool-white fluorescent tubes. Data was recorded after 4 weeks (Archana Sharma *et al*., 2013).

Preparation of Solvent extraction

Wild and Micropropagated plant

The whole wild and micropropagated plants were washed thoroughly in sterile distilled water. The plants were shade dried and ground to fine powder using mortar and pestle. One gram (dry weight) of powdered extract was soaked in 10 ml of ethanol for 3 hours and sonicated in an Ultrasonic Sonicator at 20 pulses for 20 min. The extract was centrifuged at 10,000 rpm for 10 min and the supernatant was freeze-dried and stored at 4^{o}C until further use (Singh and Tiwari, 2012).

Gas Chromatography- Mass Spectrometry Analysis

GC-MS analysis of the Ethanol extract of *C. viscosa* was performed in a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2μl employed (a split ratio of 10:1). The injector temperature maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay 0 to 2 min, and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2 (Bojaxa *et al*., 2012).

Identification of compounds

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Bojaxa *et al*., 2012).

RESULTS

C. viscosa was efficiently regenerated from nodal explants from field grown young plants on MS medium on supplemented with different concentration of cytokinins and auxins BAP, Kinetin and NAA (0.5 - 0.3mg/L, and 0.5 – 2.5) were tabulated in the table 1. The callus was observed in 15 days old

cultures on media tested and it was found the number of shoots developed on nodal explants exposed to 2.5, 3.0, 2.5 mg/l and 0.3, 0.5, 0.3 mg/l with an average no. of shoots per explants ranging 5.33 ± 1.15 and 4.16± 0.76, the shoot length 4.60 ± 0.52 and 3.70 ± 0.36 were recorded after 4 weeks of culture (Fig. 1a to 1c), but the growth regulator type and concentration did not significantly affect shoot length. GC-MS chromatogram of the ethanolic extracts of whole plant of wild and callus are reveled the presents of eight and six compounds respectively (Table 2 and 3, Fig 2 and 3). The chloroform extracts are exhibited the presence of ten compounds in both the whole plants extract of wild and callus (Table 4 and 5, Fig 4 and 5). The active principles with their molecular formula, molecular weight, retention times and peak area (%) are presented in Table 2, 3, 4 and 5.

Table1: Effect of different concentrations of BAP, KIN and NAA in MS medium on multiple shoot induction from nodal explants of *C. viscosa*.

Hormone Con. (mg/L) BAP + KIN + NAA	Shoot length (Mean ± S.D)	No. of shoots/explants (Mean ± S.D)
0.3 + 0.5 + 0.3	4.16 ± 0.76	4.33 ± 1.52
0.5 + 1.0 + 0.5	4.00 ± 1.00	3.0 ± 1.0
1.0 + 1.5 + 1.0	4.76 ± 0.25	5.33 ± 1.15
1.5 + 2.0 + 1.5	3.70 ± 0.36	3.66 ± 0.57
2.0 + 2.5 + 2.0	4.01 ± 0.40	4.33 ± 0.57
2.5 + 3.0 + 2.5	4.60 ± 0.52	4.33 ± 0.57

Medium: MS+ additives; mean± SD, n= 6 replicates

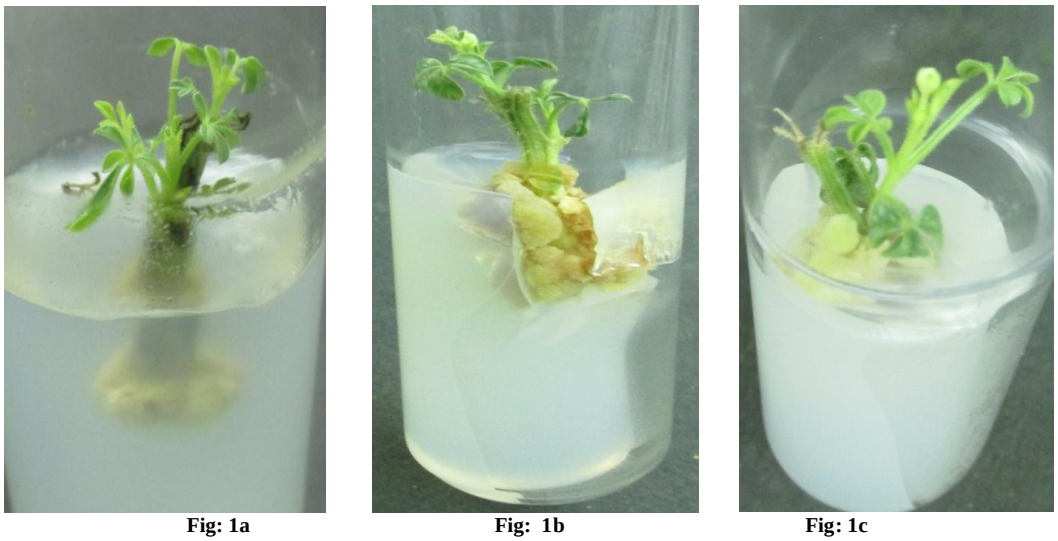

Fig: 1a **Fig: 1b** **Fig: 1c**

Fig. 1a, b, c: *In vitro* shoot proliferation of *C. viscosa* on MS Medium with different concentration of cytokinins and auxins.

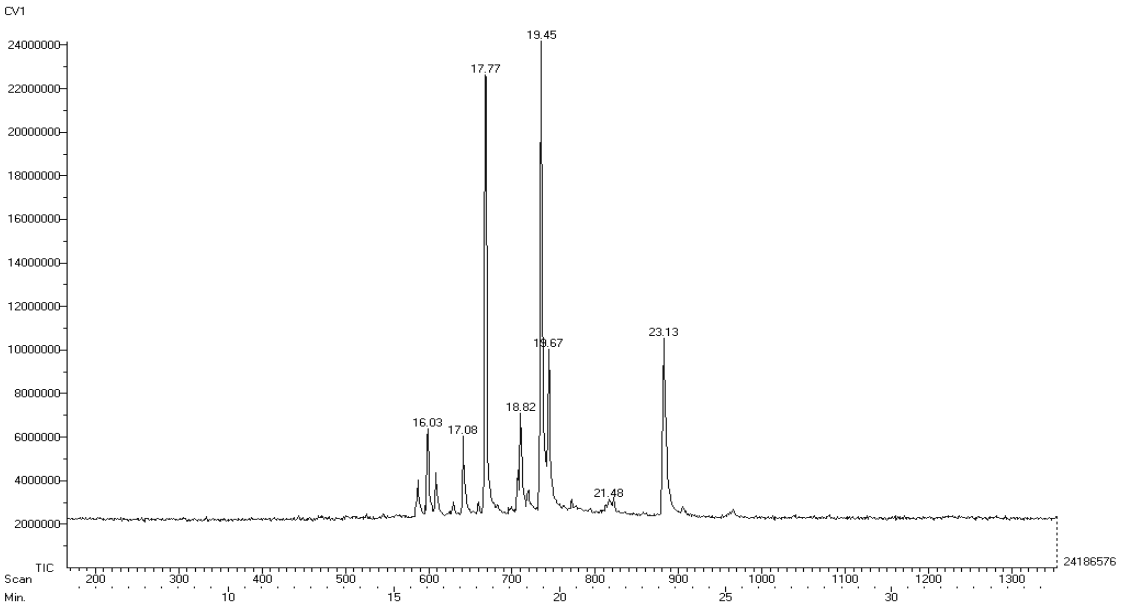

Fig. 2: GC-MS Chromatogram of ethanolic extract of C. viscosa (***Wild***).

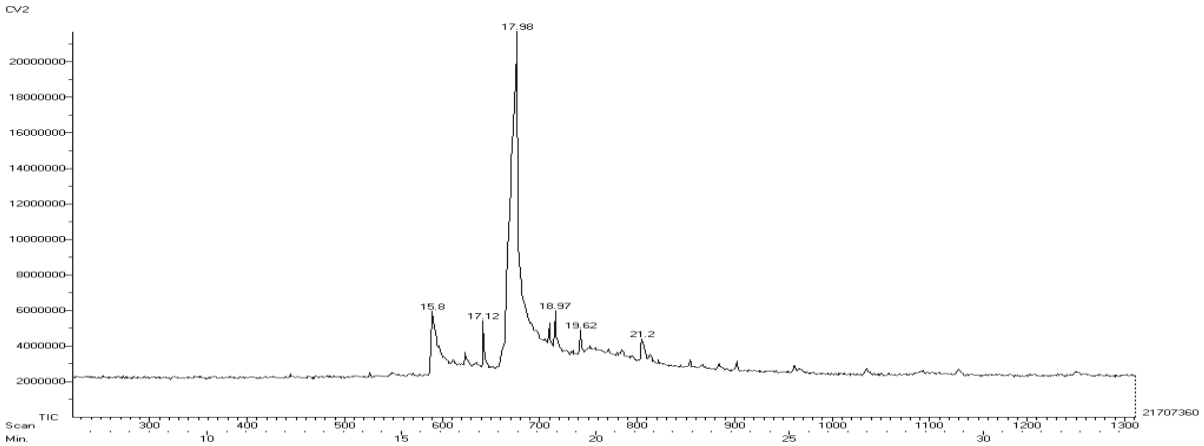

Fig. 3: GC-MS Chromatogram of ethanolic extract of *C. viscosa* (callus).

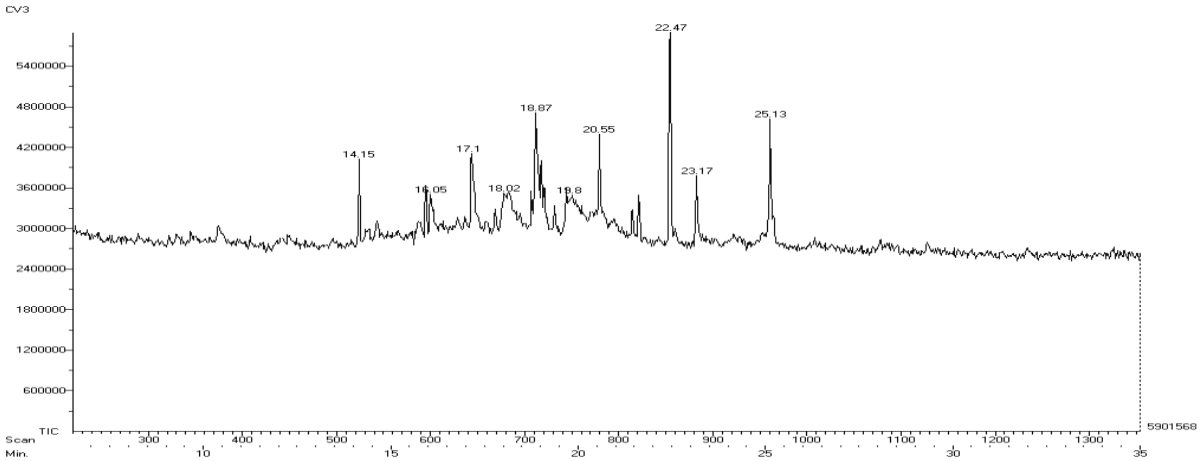

Fig. 4: GC-MS Chromatogram of chloroform extract of *C. viscosa* (Wild).

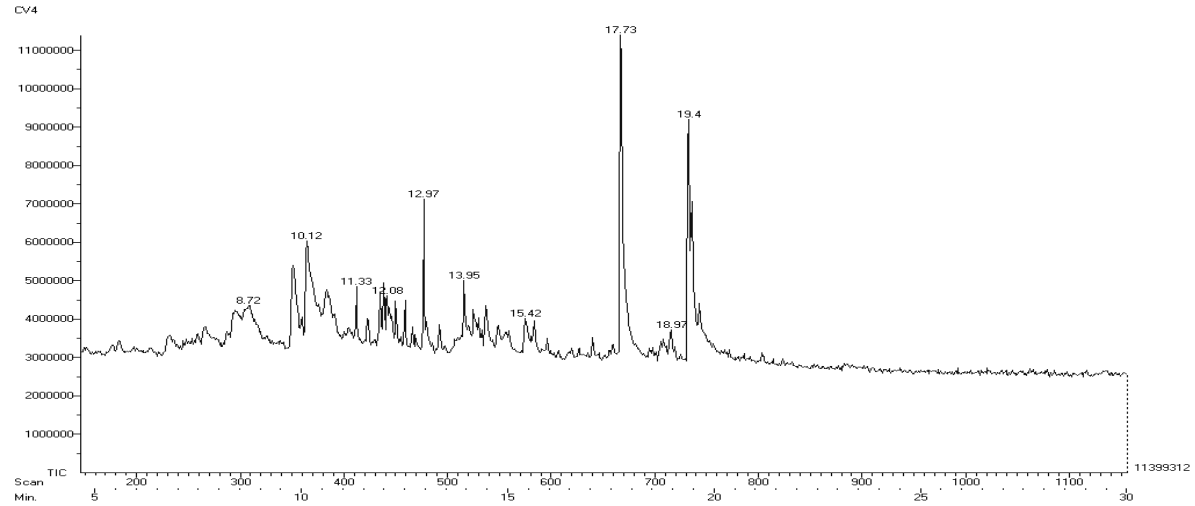

Fig. 5: GC-MS Chromatogram of chloroform extract of *C. viscosa* (callus).

Table 2: Phytocomponents identified in the ethanolic extract of *C. viscosa* (wild).

S. No	Retention Time	Name of the compound	Molecular Formula	Peak (%)	MW g/mol
1	16.03	Tetradecanoic acid	$C_{14}H_{28}O_2$	4.22	228.37
2	17.08	4',5,7-Trihydroxy isoflavone	$C_{15}H_{10}O_5$	4.48	270.23
2	17.77	Octadecanoic acid,2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	25.69	372.58
4	18.82	4H-1-Benzopyran-4-one,2-(3,4-dimethoxyphenyl)-7-hydroxy-	$C_{17}H_{14}O_5$	6.83	298.29
5	19.45	E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	25.85	310.51
6	19.67	Heptadecanoic acid, 15-methyl-, ethyl ester	$C_{20}H_{40}O_2$	15.96	312.53
7	21.48	Pregn-4-ene-3,20-dione,16-methyl-6methylene-,(16a')-	$C_{23}H_{32}O_5$	1.67	388.50
8	23.13	Estra-1,3,5(10),6-tetraene-3,17-diol, diacetate,(17a')-	$C_{22}H_{26}O_4$	15.26	354.43

Table 3: Phytocomponents identified in the ethanolic extract of *C. viscosa* (callus).

S. No	Retention Time	Name of the compound	Molecular Formula	Peak (%)	MW g/mol
1	15.80	Tetradecanoic acid	$C_{14}H_{28}O_2$	10.90	228.37
2	17.12	4`,5,7-Trihydroxy isoflavone	$C_{15}H_{10}O_5$	3.92	270.23
3	17.98	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	76.61	256.42
4	18.97	Phytol	$C_{20}H_{40}O$	4.51	296.53
5	19.62	Z,Z-3,13-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	0.70	308.49
6	21.20	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	3.32	324.54

Table 4: Phytocomponents identified in the chloroform extract of *C. viscosa* (wild).

S. No	Retention Time	Name of the compound	Molecular Formula	Peak (%)	MW g/mol
1	14.15	Flavone	$C_{15}H_{10}O2$	3.53	222.23
2	16.05	Dodecanoic acid, 10-methyl-, methyl ester	$C_{14}H_{28}O_2$	7.89	228.37
3	17.10	4',5,7-Trihydroxy isoflavone	$C_{15}H_{10}O_5$	10.41	270.23
4	18.02	6,7-Dimethyl-4-thiophen-3-yl-3,4-dihydro-1H-quinolin-2-one	$C_{15}H_{15}NOS$	9.80	257.35
5	18.87	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	20.22	296.48
6	19.80	Ethanol, 2-(9-octadecenyloxy)-,(Z)-	$C_{20}H_{40}O_2$	0.37	312.53
7	20.55	Elaidic acid, isopropyl ester	$C_{21}H_{40}O_2$	2.66	324.54
8	22.47	1,4-Diazabicyclo(4.3.0) nonane,2,3-bis(4-methoxyphenyl)-4-methyl-,(2R,3R,6S)-	$C_{22}H_{28}N_2O_2$	15.12	352.47
9	23.47	Benzo(d,E)isoquinoline-1,3(2H)-dione,2-(2-(1-benzotriazolyl)-2-oxoethyl)-	$C_{20}H_{12}N_4O_3$	7.03	356.33
10	25.13	3-Piperidinopropyl 2-ethoxy-2,2-diphenylacetate	$C_{24}H_{31}NO_3$	12.77	381.50

Table 5: Phytocomponents identified in the chloroform extract of *C. viscosa* (callus).

S. No	Retention Time	Name of the compound	Molecular Formula	Peak (%)	MW g/mol
1	8.72	2-Propyl-tetrahydropyran-3-ol	$C_8H_{16}O_2$	4.51	144.21
2	10.12	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	10.63	150.17
3	11.33	ç-Elemene	$C_{15}H_{24}$	3.31	204.00
4	12.08	Azulene,1,2,3,4,5,6,7,8-octahydro -1,4-dimethyl-7-(1-methylethyl idene)-, (1S-cis)-	$C_{15}H_{24}$	9.30	204.35
5	12.97	ç-Elemene	$C_{15}H_{24}$	8.72	204.00
6	13.95	Isoaromadendrene epoxide	$C_{15}H_{24}O$	5.39	220.35
7	15.42	Tetradecanoic acid	$C_{14}H_{28}O_2$	4.10	228.37
8	17.73	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	27.62	284.47
9	18.97	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	4.09	268.39
10	19.40	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	22.29	308.49

DISCUSSION

The MS medium was the most effective for callusing of explants from *C. viscosa*. The morphogenetic response of the explants is mainly based on the type and concentration of the hormone used. The explants cultured on MS medium supplemented with different concentration of Cytokinins and auxins of BAP, KIN and NAA showed varied response for callusing. In this present research that the callus cultured on MS medium, hormones such as BAP, KIN and NAA produced micro shoots. High frequency (95%) multiple shoot induction with 5.33 ± 1.15 number of shoots and an average length of 4.76 ± 0.25 shoots in MS medium supplemented with 1.0 + 1.5 + 1.0 mg/ml BAP, KIN and NAA, was observed. Similarly, two cytokinins used BAP induced significantly higher percentage of shoot initiation and mean number of shoot, whereas higher mean shoot length was obtained in the shoots obtained on media supplement with KIN (Gokhale and Bansal, 2009). All three types of PGRs were found to be most effective at different concentrations tested for shoot production. The findings are in agreement with those observed with optimum level of TDZ along with various levels of auxins, IAA/NAA/IBA for frequency of shoot multiplication (Vijayakumar *et al*., 2014). In that case, the addition of TDZ (3.0 mg/L) and IAA (0.7mg/L) to the medium also induced 100% shooting response with maximum of 24.9±1.0 numbers of micro shoots with small amount of light green mucilaginous type of basal calli observed from cotyledonary leaf explants culture.

Previous authors claimed that chloroform leaf extract of *Cleome burmanni* exposed sixteen compounds where as in the current study *Cleome viscosa* showed ten divergent compounds (Lakshmi *et al*., 2013). The GC MS analysis of the whole ethanolic extracts of wild and callus of *C. viscosa* was reveled the two similar compounds namely Tetradecanoic acid and 4',5,7-Trihydroxy isoflavone. The dissimilar compounds namely Octadecanoic acid,2-(2-hydroxyethoxy) ethyl ester, 4H-1-Benzopyran-4-one,2-(3,4-dimethoxyphenyl)-7-hydroxy-, E -9-Octadecenoic acid ethyl ester, Heptadecanoic acid, 15-methyl-, ethyl ester, Pregn-4-ene-3,20-dione,16-methyl-6methylene-,(16a'), Estra-1,3,5(10),6-tetraene-3,17-diol, diacetate,(17a')- and n-Hexadecanoic acid, Phytol, Z,Z-3,13-Octadecadien-1-ol acetate, 4,8,12,16-Tetramethylheptadecan-4-olide. The chloroform extracts didn't exhibit any similar compounds in both extracts. Earlier authors reported that aqueous extract of callus of *Pisonia alba*

revealed the presence of thirteen compounds whereas in the present study ethanol extract of callus of *C. viscosa* showed six dissimilar compounds (Saritha and Karpagam, 2015). The identified phytochemical compounds have biological properties. For instance, n-Hexadecanoic acid and Phytol reported to contain antioxidant, hypocholesterolemic nematicide, pesticide, lubricant, antiandrogenic and antimicrobial, anticancer, cancer preventive, diuretic antiinflammatory (Sermakkani and Thangapandian, 2012). The isoaromadendrene epoxide was high anti microbial activity reported on Mohammad Majdi et al. (2016). The *Cleome viscosa* are natural source of bioactive compounds to treat many diseases.

CONCLUSION

In conclusion, the present study was observed on the combination of the different hormones and their concentration showed a varying effect on the germination of the explants. The explants gave a positive response under various combinations of plant growth regulators and the complete germination of whole plantlet of *C. viscosa* was observed. Using this technique, it is possible to produce healthy and disease free clones which could be released to their natural habitat in large scale. Due to the *in vitro* cultivation the synthesized of compounds are varied from the wild variety. In spite of the compound variation, the structural organization of the plants was not modified. Divergent compounds from the callus will be helpful in advanced pharmacological activities.

ACKNOWLEDGEMENT

The authors are thankful to the Secretary and the Principal, Ramakrishna Mission Vivekananda College (Autonomous), Mylapore, Chennai, India for providing all facilities and we specially thank Sophisticated Analytical Instrument Facility (SAIF) Laboratory, Indian Institute of Technology, Chennai, India for carrying out GC-MS studies and validation of the results.

Financial support and sponsorship: NIL.

Conflict of Interests: There are no conflicts of interest.

REFERENCES

Mali, RG. *Cleome viscosa* (wild mustard): A review on ethnobotany, phytochemistry, and pharmacology. Pharm. Biol. 2010; 48(1); 105-112.

Chatargee A, Pakrashi SC. The treatise on Indian Medicinal Plants, Vol I PID, CSIR, New Delhi. 1991; 155.

Shah, GL, Yadav SS, Badri N. Medicinal plants from Dahanu Forest division in Maharashtra State. J. Econ.Tax. Bot. 1983; 4; 141-151.

Devi BP, Boominathan R, Mandal SC. Evaluation of antipyretic potential of *Cleome viscosa* Linn. (Capparidaceae) extract in rats. J.Ethnopharmacol. 2003; 87(1); 11-13.

Parimaladevi B, Boominathan R, Mandal SC. Studies on analgesic activity of *Cleome viscosa* in mice. Fitoterapia. 2003; 74(3); 262-266.

Sudhakar M, Rao Ch V, Rao PM, Raju DB. Evaluation of antimicrobial activity of *Cleome viscosa* and *Gmelina asiatica*. Fitoterapi. 2006; 77(1); 47-49.

Bonga J. Vegetative propagation in relation to juvenility, maturity, and rejuvenation. In: Tissue culture in forestry. Bonga, JM, Durzan, DJ. (eds.). Martinus Nijhoff/ Dr. W. Junk Publishers. The Hague: 1982; 387-412.

Debergh PC, Read PE. Micropropagation. In: Debergh PC, Zimmerman RH. (eds.), Micropropagation, Technology and Application, Kluwer Academic Publishers, Netherlands. 1991; 1-13.

Chawla HS. Introduction to plant biotechnology. (Enfield NH: seience Publishers) Springer. 2009; 3rd ed.; 499 -500.

El-Agamy SZ. *In vitro* propagation of some grape root stocks. Acta Hort. 2009; 839; 125-132.

Sermakkani M, Thangapandian V. GC-MS Analysis of *Cassia italica* leaf methanol extract. Asian J. of Phar. and Cli. Rese. 2012; 5 (2); 90-94.

Muthusamy Govarthanan, Rathika Rajinikanth, Seralathan Kamala-Kannan, & Thangasamy Selvankumar. A comparative study on bioactive constituents between wild and *in vitro* propagated *Centella asiatica*. J. of Gene. Engi. and Biotech. 2015; 13(1); 25-29.

Archana sharma, Shikha bhansali, Ashwani kumar. Micropropagation of *Eclipta alba* hassk. An important medicinal plant of traditional medicine. International journal of life science and pharma research. 2013; 3 (2); 47-51.

Singh J, Tiwari KN. *In vitro* plant regeneration from decapitated embryonic axes of *Clitoria ternatea* L. An important medicinal plant. Industrial Crops and Products. 2012; 35; 224-229.

Bojaxa A. Rosy PJ, Rosakutty. GC-MS analysis of methanol wild plant and callus extracts from three Cissus species. J. Chem. Pharm. Res. 2012; 4(7); 3420-3426.

Gokhale M, Bansal YK. Direct *in vitro* regeneration of a medicinal tree *Oroxylum indicum* (L.) Vent. Through tissue culture. African Journal of Biotechnology. 2009; 8; 3777- 3781.

Vijayakumar J, Shobana Rathi G, Bhuvaneshwari SM, Ranjitha Kumari BD, Enrique Castaño. Thidiazuron-induced shoot organogenesis of *Cleome viscosa* (L) through cotyledonary explants culture. Afr. J. Biotech. 2014; Vol.13 (9); 1027-1036.

Lakshmi S, Pillai, Bindu R, Nair. GC-MS analysis of chloroform extract of *Cleome burmanni* w. and a. (Cleomaceae). IJPSR. 2013; Vol. 4(5); 1930-1933.

Saritha B, Karpagam S. Phytochemical Content of Leaf and *In Vitro* Established Callus Culture of Pisonia alba Span. IJSR. 2015; Vol 4(1); 2502 – 2505.

Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia italica* leaf methanol extract. Asian J Pharm Clin Res. 2012; Vol 5(2); 90-94.

Mohammad Majdi, Dara Dastan, Hossein Maroofi. Chemical Composition and Antimicrobial Activity of Essential Oils of *Ballota nigra* Subsp. *kurdica* From Iran. Jundishapur J Nat Pharm Prod. In Press (In Press): e36314. Published online 2016; September 3.

How to cite this article:

Deventhiran M, John wyson W, Sheik Noor Mohamed M, Jaikumar K, Saravanan P, Anand D., Comparative Phytochemical Analysis of Wild and Micropropagated *Cleome Viscosa* L. J App Pharm Sci, 2017; 7 (04): 083-088.