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.

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., 2003) 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

© 2017 M. Deventhiran et al. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlikeUnported License (http://creativecommons.org/licenses/by-nc-sa/3.0/).
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 micropropogation 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 micropropogation 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 micropropogated 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 (HgCl₂) 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±2°C), 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 Micropropogated plant
The whole wild and micropropogated plants were washed thoroughly in sterile distilled water. The plants were shade dried and ground to fine powder using mortal 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°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 revealed the presence 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.

Table 1: Effect of different concentrations of BAP, KIN and NAA in MS medium on multiple shoot induction from nodal explants of C. viscosa.

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

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

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).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Retention Time</th>
<th>Name of the compound</th>
<th>Molecular Formula</th>
<th>Peak (%)</th>
<th>MW g/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.03</td>
<td>Tetradecanoic acid</td>
<td>C_{14}H_{28}O_{2}</td>
<td>4.22</td>
<td>228.37</td>
</tr>
<tr>
<td>2</td>
<td>17.08</td>
<td>4',5,7-Trihydroxy isoflavone</td>
<td>C_{10}H_{15}O_{5}</td>
<td>4.48</td>
<td>270.23</td>
</tr>
<tr>
<td>3</td>
<td>17.77</td>
<td>Octadecanoic acid,2-(2-hydroxyethoxy) ethyl ester</td>
<td>C_{19}H_{38}O_{5}</td>
<td>25.69</td>
<td>372.58</td>
</tr>
<tr>
<td>4</td>
<td>18.82</td>
<td>4H-1-Benzopyran-4-one,2-(3,4-dimethoxyphenyl)-7-hydroxy-</td>
<td>C_{22}H_{26}O_{5}</td>
<td>6.83</td>
<td>298.29</td>
</tr>
<tr>
<td>5</td>
<td>19.45</td>
<td>E-9-Octadecenoic acid ethyl ester</td>
<td>C_{20}H_{38}O_{2}</td>
<td>25.85</td>
<td>310.51</td>
</tr>
<tr>
<td>6</td>
<td>19.67</td>
<td>Heptadecanoic acid, 15-methyl-, ethyl ester</td>
<td>C_{20}H_{36}O_{2}</td>
<td>15.96</td>
<td>312.53</td>
</tr>
<tr>
<td>7</td>
<td>21.48</td>
<td>Pregn-4-ene-3,20-dione,16-methyl-6methylene-,(16a')-</td>
<td>C_{23}H_{38}O_{2}</td>
<td>1.67</td>
<td>388.50</td>
</tr>
<tr>
<td>8</td>
<td>23.13</td>
<td>Estra-1,3,5(10),6-tetraene-3,17-diol, diacetate,(17α')-</td>
<td>C_{22}H_{30}O_{6}</td>
<td>15.26</td>
<td>354.43</td>
</tr>
</tbody>
</table>
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 shoots, 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, ZZ-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 (Serrmakkani and Thangapandian, 2012). The isouromadendrene 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.


Devi BP, Booninathan 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.


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

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


How to cite this article: