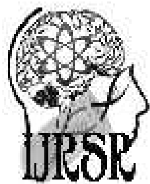
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Research Article

STUDY ON THE PHYTOCHEMICAL ANALYSIS OF WILD AND MICROPROPAGATED *CLEOME RUTIDOSPERMA* DC

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ABSTRACT

Cleome rutidosperma is traditionally used in Indian ayurvedic medicine for the treatment of wide number of health disorders. Plant tissue culture techniques are considered as easy and consistent methods for rapid propagation of plants, especially rare and endangered plant species. *In vitro* propagation of *C.rutidosperma* was investigated to develop reliable protocols for direct shoot regeneration induced by culturing nodal explants and comparison the components between wild and micropropagated. The nodal explants were cultured on MS medium supplemented with different concentration of plant growth regulators like 6-Benzylaminopurine and NAA. The bioactive compound of chloroform extracts was identified by Gas chromatography–mass spectrometry method. Multiple shoot generation was achieved after 28 days of incubation with average five shoots per explants. The wild and micropropagated plant showed the presence of eleven and nine compounds respectively. The study concluded that callus formation yield mass multiplication and chloroform extracts showed more compounds than that of micropropagated plant.

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INTRODUCTION

The plants have traditional value such as ethno-medicine and pharmacology, which is now increasingly recognized in the source of many modern human and veterinary medicine (Maydell Hans, 1990). It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant substances (Pezzuto, 1996). *Cleome rutidosperma* belongs to the family: Cleomaceae which is a low-growing herb, up to 70 cm tall, found in waste grounds and grassy places with trifoliate leaves small, violet-blue flowers, which turn pink as they age, elongated capsules display the asymmetrical and dull black seeds (Widespread, 1972).The plant is native to West Africa, although it has become naturalized in various parts of tropical America as well as Southeast Asia (Waterhouse and Mitchell, 1998). *C.rutidosperma* is traditionally used in the treatment of paralysis, epilepsy, analgesic, anti-inflammatory, antimicrobial, antioxidant, antiplasmodial pain and skin disease (Bidla *et al.*, 2004; Bose *et al.*, 2010; Burkill, 1985).

In vitro propagation refers to true type propagation of selected genotypes under laboratory conditions. Different explants such as single cells, protoplasts, pieces of leaves or roots can be used to generate a new plant on culture media with required nutrients (Mohamed, 1991). *In vitro* culture is being used in an increasing number of botanic gardens for the propagation and conservation of medicinal and endangered plant species (Fay, 1992). *In vitro* propagation or tissue culture of plants holds tremendous potential for the production of high-quality plant-based medicines and different strategies, using an *in vitro* system, have been extensively studied to improve the production of plant chemicals are responsible for medicinal activity. These are non-nutritive chemicals that have protected human from various diseases (Savithramma *et al.*, 2011).

Gas Chromatography Mass Spectroscopy (GC-MS) a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants (Ronald Hites, 1997). Hence, the aim of the present study was to develop high frequency multiple shoots regeneration of *C. rutidosperma* utilizing the least number and various concentrations of PGRs under aseptic laboratory condition and to compare the phytochemical constituents between chloroform 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 rutidosperma* DC., were collected during the month of January from the natural habitats

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of Kancheepuram district, Tamil Nadu, India. The plant specimen was identified and authenticated by Botanical Survey of India (BSI) Coimbatore, Tamil Nadu, India. Plant voucher No.BSI/SRC/Tech/358.

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 three times with sterile distilled water (Muthusamy Govarthanan, 2015).

Inoculation in culture medium

The nodal segments were cut into 5 mm in size and carefully transferred to the sterile MS (Murashige and Skoog) basal medium (pH 5.8) supplemented with 3% (w/v) sucrose, 0.8% agar and different concentration of PGRs such as 1-Naphthaleneacetic acid (NAA), 6-Benzylaminopurine (BAP) (Table.1). The inoculated cultures were maintained in growth chamber with regulated temperature (26±2ºC), relative humidity (55±5%), light conditions 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, 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 chloroform 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 stored at 4°C until further use (Singh and Tiwari, 2012).

Gas Chromatography- Mass Spectrometry Analysis

GC-MS analysis of the chloroform extract of *C. rutidosperma* 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%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2μl was employed (a split ratio of 10:1). The injector temperature was 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 was 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.

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.

Fig 1a

Fig 1b

Fig 1c

Figures 1a Callus induction, **1b:** Initiation of multiple shoots from callus and **1c:** shoot elongation

RESULTS

Nodal explants of *C.rutidosperma* were incubated on MS medium supplemented with different concentrations of BAP, NAA and the results were tabulated in Table 1. After fourteen days of incubation the callus was observed and shoots were developed at the nodal explants to 0.5 + 0.3 mg/l and 3.0 + 2.5 mg/l with an average no. of shoots per explants ranging 5.33 ± 1.15 and 4.16 ± 0.76, the shoot length 7.49 ± 0.36 cm and 4.12 ± 0.72 cm were recorded after 4 weeks of culture (Fig. 1a-1c). GC-MS chromatogram of the chloroform extracts of wild and micropropagated plants reveled of eleven and nine peaks respectively (Fig 2, 3). The active principles with their molecular formula, molecular weight, retention times and peak area (%) are presented in Table 2, 3. On comparison of the mass spectra of the constituents with the NIST library the eleven compounds from wild and nine compounds from micropropagated plants were identified and characterized.

Table 1 Effect of different concentrations of BAP, NAA and MS medium on multiple shoot induction from nodal explants of *C. rutidosperma.*

PGR Con.(mg/L) BAP + NAA	Shoot length (Mean ± S.D)	No. of shoots/explants (Mean ± S.D)
0.5 + 0.3	4.12 ± 0.72	4.33 ± 1.52
1.0 + 0.5	3.96 ± 0.96	3.0 ± 1.0
1.5 + 1.0	4.72 ± 0.21	5.33 ± 1.15
2.0 + 1.5	7.06 ± 0.32	3.66 ± 0.57
2.5 + 2.0	4.56 ± 0.48	4.33 ± 0.57
3.0 + 2.5	7.39 ± 0.36	4.33 ± 0.57

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

Table 2 Phytocomponents identified in the chloroform extract of *C. rutidosperma* (wild)

Fig 2 GC-MS Chromatogram of chloroform extract of *C. rutidosperma* (Wild)

Fig 3 GC-MS Chromatogram of chloroform extract of *C. rutidosperma* (Callus)

DISCUSSION

The various studies on micropropagation of medicinal plants reported that MS medium was the best culture medium for the shoot regeneration. The plant growth regulators dependent growth reported that the higher concentration of the PGRs generates higher number of shoots and the optimum level restricted the shoot regeneration (Vincent *et al*., 1992; Sen and Sharma, 1991). The basal medium alone were failed to regenerate shoots, where as supplemented with PGRs was induced shoot proliferation (Subhash Sirangi, 2015).

Table 2 Phytocomponents identified in the chloroform extract of *C. rutidosperma* (wild)

S.No	Retention Time	Name of the compound	Molecular Formula	Peak area (%)	Molecular Weight
1	12.53	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	5.93	206.32
2	15.02	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	2.74	242.39
3	15.95	Ethyl(2E,4E)-3,7,11-trimethyl-2,4-dodecadienoate	$C_{17}H_{30}O_2$	3.77	266.42
4	16.9	11-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	3.83	268.43
5	17.12	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	30.48	270.45
6	18.12	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	0.46	284.47
7	18.52	13-Hexyloxacyclotridec -10-en-2-one	$C_{18}H_{32}O_2$	3.94	280.44
8	18.85	10-Octadecenoic acid, methyl ester	$C_{19}H36O_2$	12.08	296.48
9	20.38	N-Isobutyl-11-(3,4methylenedioxyphenyl)-2E,4E,10E-undecatrienoic amide	$C_{22}H_{29}NO_3$	2.72	355.47
10	20.73	3a',12a'-Dihydroxy-bisnor-5,7-cholenic acid	$C_{24}H_{38}O_3$	7.16	374.56
11	23.22	[2-Cyclohex-1-enyl-ethyl]-[4,6-di-morpholin-4-yl-(1,3,5)triazin-2-yl]-amine	$C_{19}H_{30}N_6O_2$	3.59	374.48

Table 3 Phytocomponents identified in the chloroform extract of *C. rutidosperma* (callus)

S. No	Retention Time	Name of the compound	Molecular Formula	Peak area (%)	Molecular Weight
1	15.07	Tridecanoic acid, 12-methyl-,methyl ester	$C_{15}H_{3}0O_2$	2.55	242.39
2	16.95	11-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	5.48	268.43
3	17.13	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	27.84	270.45
4	18.13	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	1.01	284.47
5	18.52	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	6.34	280.44
6	18.87	10-Octadecenoic acid, methyl ester	$C_{19}H36O_2$	13.02	296.48
7	19.05	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	26.44	298.50
8	20.73	Octadecanedioic acid, dimethyl ester	$C_{20}H_{38}O_4$	7.90	342.51
9	23.23	Carbamic acid, N,N-dimethyl-,[6-methyl-2-(4-methylphenyl)-5propyl-4-pyrimidinyl]ester-	$C_{14}H_{20}N_2O_2$	4.71	248.32

The shoot regeneration of the present study was achieved by the different combination of BAP and NAA which is also confirmed by (Khan mubashir *et al*., 2014) reported that shoot regeneration of *Artemisia amygdalina* was achieved with supplemented BAP and NAA.

The GC MS analysis of the whole plant chloroform extracts of wild and callus of *C.rutidosperma* reveled six similar compounds namely Tridecanoic acid, 12-methyl-,methyl ester, 11-Hexadecenoic acid, methyl ester, Hexadecanoic acid, methyl ester, Hexadecanoic acid, 14-methyl-, methyl ester, 13-Hexyloxacyclotridec -10-en-2-one, 10-Octadecenoic acid, methyl ester. Five dissimilar compounds exhibited in the wild variety namely Phenol, 2,4-bis(1,1-dimethylethyl)-; Ethyl(2E,4E)-3,7,11-trimethyl-2,4-dodecadienoate; N-Isobutyl-11-(3,4methylenedioxyphenyl)-2E,4E,10E-undecatrienoic amide; 3a',12a'-Dihydroxy-bisnor-5,7-cholenic acid; [2-Cyclohex-1-enyl-ethyl]-[4,6-di-morpholin-4-yl-(1,3,5)triazin-2-yl]-amine; and three dissimilar compounds revealed in callus namely Heptadecanoic acid, 16-methyl-, methyl ester; Octadecanedioic acid, dimethyl ester; Carbamic acid, N,N-dimethyl-,[6-methyl-2-(4-methylphenyl)-5propyl-4-pyrimidinyl]ester-.

Earlier authors reported that methanolic wild and callus extracts of *Cissus xavierensis, Cissus quadrangularis* and *Cissus vitiginea* revealed the presence of sixteen, twenty three and twenty three in wild variety and forty five, forty six, and twenty seven compounds was present in callus. The number of compounds detected from *in vitro* biomass was higher than those obtained from wild extracts whereas, in the present study chloroform extracts of wild plant is comparatively higher than that of micropropagated plant (Bojaxa and Rosakutty, 2012).

The compounds identified from *C. rutidosperma* have pharmacological activity. Abdullah *et al*., 2011 reported that Phenol, 2, 4-bis (1, 1-Dimethyl ethyl) of Malaysian mango kernel have antibacterial activity. Hexadecanoic acid was exhibits inflammatory and cytotoxicity activities (Othman, 2015). Twenty compounds were identified from chloroform extracts of wild and callus which can be used for various Pharmacological applications.

CONCLUSION

In vitro regeneration systems favor rapid production of large numbers of plantlets, without seasonal dependency, for *ex situ* commercial cultivation purposes and reduces the risk of voluble medicinal plants being sampled from wild habitats thus safeguarding the existing natural populations. The combination of the different hormones and their concentration showed a varying effect on the germination of the explants. The micropropagation of *C. rutidosperma* and biological activity of wide range of bioactive compounds from the micropropagated and wild variety plants can serve as an alternative to conventional medicines and a basis for pharmacological evaluation.

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