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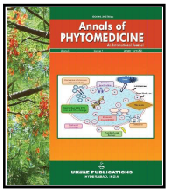

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Innovative technique for rapid *in vitro* multiplication of rootless shoots in *Andrographis paniculata* (Burm. f) Nees: A plant with immense pharmaceutical value

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Abstract

Andrographis paniculata (Burm. f) Nees is a treasured medicinal plant having higher amounts of its principal bioactive compound andrographolide in leaves. In the present study, a novel and rapid *in vitro* propagation technique has been developed for rootless shoot cultures in *A. paniculata* by evaluating different culture techniques. Rootless shoot cultures were optimally cultivated on Murashige and Skoog (MS) liquid/solid medium, supplemented with 2 mg l^{-1} benzylaminopurine. Various techniques such as only liquid media, liquid media over blotting paper, liquid media on cotton block, and solid media were evaluated for fast multiplication of *A. paniculata* rootless shoots. Amongst the culture techniques evaluated, a maximum of 131.66 ± 11.98 shoots were obtained in liquid medium over blotting paper method after 21 days of culture. The liquid culture technique facilitated shoot multiplication frequency up to 71.66 ± 8.1. The liquid medium over blotting paper technique for multiplication of shoots was found the best as compared to other methods evaluated. HPLC analysis in these four techniques revealed that, the shoots grown in liquid media promoted highest andrographolide accumulation of (4.0 % DW) compared to culture of shoots in solid media normally practiced. The evaluation of different culture techniques revealed the efficacy of culture methods for rapid propagation of *A. paniculata* as a constant source of this elite plant material.

1. Introduction

Andrographis paniculata (Burm. f) Nees commonly known as "Kalmegh", belongs to family Acanthaceae, is an important medicinal plant and used in traditional medicine in China, South Asian countries. This is also called as "King of bitters" due to the presence of andrographolide (Sajeeb *et al.,* 2015). It has various pharmacological applications (Dai *et al.,* 2019) like, antimicrobial (Xu *et al.,* 2016), antihyper glycemic, anti-inflammatory (Gan *et al.,* 2019), anti-cancerous (Sarkar *et al.,* 2019) and immune-stimulatory effects (Subramanian *et al.,* 2012; Kumar *et al.,* 2014) anti HIV properties (Yang *et al.,* 2010). It also showed antimalarial (Widyawaruyanti *et al.,* 2014) hepato protective (Negi *et al.,* 2008) properties. It is used to treat upper respiratory tract infection (Poolsup *et al.,* 2004). The analogues of andrographolide have been reported to possess immune-modulator and antiatherosclerotic effects (Chao *et al.,* 2010). In addition to this, *A. paniculata* has been generally used for common cold and flu as a conventional medication. It was mentioned in the past that this plant also used as an wonder drug during 1919 Spanish flu pandemic alike current COVID-19 crisis worldwide (Hancke *et al.*, 1995). In a recent report, andrographolide nanocrystals have demonstrated hepatoprotective activity to drug induced liver injury (Basu *et al.,* 2020) and antivenom (Ghosh *et al.,* 2020). Andrographolide extracted from various parts of the plant such as leaves, stems, roots of this plant, the highest content was found in leaves (Sharma *et al.,* 2013). Keeping in view the importance of diterpene lactone andrographolide, the conventional methods for andrographolide production were not sufficient (Padmanabhan *et al.,* 2017). Thus, to overcome the limitations in the continuous supply of andrographolide and related compounds, biotechnological intervention by *in vitro* propagation (micropropagation) of these plants can be an alternative (Avila, Treviño *et al.,* 2017).

Andrographolide is produced in two important pathways, *i.e.,* Methyl erythritol phosphate (MEP) pathway (plastidal) and Mevlonate (MVA) pathway (cytosolic), with simultaneous contribution of both the pathways (Srivastava and Akhila 2010). However, the andrographolide production may be influenced by different climatic and other conditions (Pandey *et al.,* 2019). Our laboratory is engaged in research initiatives on distribution of *A. paniculata* and related species, pharmacological study and yield enhancement of bioactive compounds, using biotechnological, genomic, proteomic and bioinformatics approaches (Neeraja *et al.,* 2015; Zaheer and Giri, 2015; Parlapally *et al.,* 2016; Giri and Zaheer, 2016; Zaheer and Giri, 2017 (a, b); Bindu *et al.,* 2017; Srinath *et al.,* 2017; Shailaja *et al.,* 2018). Besides the enhanced production of secondary metabolites with particular reference to andrographolide, the elicitation of plant cell, tissue and organ cultures, understanding of molecular basis of elicitation process and secondary metabolism in general at functional level is our envisaged ongoing research goal (Srinath *et al.,* 2020; Bindu *et al.,* 2020). There is a continuous need of *in vitro* grown plants/plantlets for their exploitation in answering questions on secondary metabolism manipulation, regulation in general and with

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particular reference to *A. paniculata*. The methods for shoot proliferation *in vitro* have been used for rapid propagation of plants. In the past, attempts have been made for biomass magnification through multiple shoot cultures using different culture techniques such as solid, liquid and temporary immersion in liquid (Pérez-Alonso *et al.,* 2012; Zuma *et al.,* 2017; Aziz *et al.,* 2018; Arano-Avalos *et al.,* 2019). To substantiate this objective in the present communication, rapid multiplication of shoots for enhanced biomass production in *A. paniculata* is of utmost importance and significance. On this above background, evaluation of different culture techniques was undertaken for the rapid multiplication of shoots in *A. paniculata*.

2. Materials and Methods

2.1 Establishment of *A. paniculata* plants in net house conditions

The dried seeds of *A. paniculata* were procured from Regional Centre of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad, India. Seeds were sown in net house plot (cement structure filled with soil) at Centre for Plant Molecular Biology (CPMB), Hyderabad. The plants were established by regular supplement of Hoagland solution twice daily (Hoagland *et al.,* 1938).

2.2 Germination of seeds from pods of *A. paniculata*

The immature green and matured brown pods from 5 months old *A. paniculata* plants, were collected and washed with Teepol for 10 min and then surface sterilized with 0.1% (w/v) $HgCl_2$ for 5 min followed by 5 times washing with sterile distilled water, 5 min each. The pods were dissected and seeds were inoculated into MS (Murashige and Skoog 1962) medium and incubated at 25 ± 2°C temperature for germination in dark.

2.3 Multiple shoot induction from seedlings of *A. paniculata in vitro*

MS + BAP (2 mg l^{-1}) medium was prepared and pH was adjusted to 5.8 ±2 before adding 0.9% (w/v) agar and was autoclaved at 121°C, 15 lb pressure for 15 min. The cotyledonary nodes from seedlings of *A. paniculata* were transferred aseptically onto MS nutrient medium for initiation of multiple shoots. The cultures were incubated under cool white fluorescent tube lights at 25 ± 2°C temperature. The sub-culturing of shoots was done once at four week of regular intervals. The shoots were maintained in same solid medium up to two sub culture passages.

2.4 Technique for rapid propagation of *A. paniculata* multiple rootless shoot cultures

Different techniques carried out for multiplication of rootless shoot cultures and are as follows:

a. Semi solid media
b. Liquid media
c. Liquid media over blotting paper
d. Liquid media over cotton block

Semi solid medium: MS + BAP (2 mg l^{-1}) medium was prepared with 9 % (w/v) agar and the pH was set to 5.8 ± 0.2. About 25 ml melted agar medium was added to each 150 ml flask and closed with cotton plugs.

Liuid medium: Liquid medium with MS + BAP (2 mg l^{-1}) was prepared without agar and 25 ml medium was added to each flask.

Liquid media over blotting paper: Blotting paper sheets were cut into small (*i.e.*, one and half inch length and breadth), four slices of paper were taken and placed at the bottom of each flask. Twenty five ml of same liquid medium MS + BAP (2 mg l^{-1}) was added to the flasks.

Liquid media over cotton block: The cotton was cut into blocks of one and half inch length and breadth and single cotton block was placed at the bottom of the flask containing 25 ml MS + BAP medium.

2.5 Inoculation of multiple shoots onto medium using different culture techniques

Single shoot bunch consisting of a minimum of 15 shoots weighing 1.5 gm were taken as explants, inoculated aseptically onto 150 ml Erlenmeyer flasks from 8-week old cultures initiated and established on both semi solid and liquid MS + BAP (2 mg l^{-1}) medium. The cultures were incubated under 3000 lux light intensity provided by cool white fluorescent tube lights at 25 ± 2°C temperature. Observation was taken after three weeks to evaluate the efficacy of different culture techniques.

2.6 Study of different culture techniques for biomass, andrographolide production in multiple shoot cultures of *A. paniculata* and quantitative analysis of andrographolide

Three weeks old multiple shoots were taken out from medium and soaked using tissue paper to remove surface moisture and the FW of the shoots were taken. Shoots were further dried in oven dryer for 1 week at 68ºC. Dry weight of shoots was taken, powdered and stored in vials. The samples in methanol were sonicated for half an hour and the resultant extract was filtered using Whatman filter paper No. 41. Further, the final filtration was done by passing extract through 0.45 µM membrane (Millex HV, Millipore, Ireland), 500 µl of filtrate was taken and transferred to HPLC vials. The different plant samples and shoots established in different techniques were air dried and finely powdered. The fine powder of plant sample (50 mg) was placed in a vial containing 5 ml of HPLC (high performance liquid chromatography) grade methanol and incubated for 24 h at room temperature. The vials were subjected to ultrasonication for 30 min in ultrasonic cleaning bath. The resultant extract was filtered using Whatman filter paper No. 41. Further, the final filtration was done by passing extract through 0.45 µm membrane filter (Millex HV, Millipore, Ireland). About 500 µl of filtrate solution was taken and transferred into HPLC vials.

2.7 HPLC protocol

The samples were analyzed for andrographolide content using HPLC (Waters, USA). The column employed was Spherisorb C 18 column (250 × 4.6 mm, 5 µM). Compounds separation was carried out by isocratic elution with HPLC-grade methanol as mobile phase at 1 ml min^{-1}. Flow rate of the injected sample volume was 0.02 ml. The column was maintained at 25°C and elution was monitored at 230 nm. The software used was Empower pro and detector was photodiode array detector (PDA) with a retention time of 2.6 ± 0.3 min. Authentic standard andrographolide of 98% purity procured from Sigma Aldrich, USA was used for analysis. Andrographolide content was expressed as % dry weight (DW) of control and elicited multiple shoot culture samples.

2.8 Statistical analysis

The experiments on *in vitro* multiplication of shoots were performed three times with a minimum of 9 replicates each. Each replicate is having 15 shoots as a bunch. Latin square experimental design was followed for the experiments and subsequent statistical analysis. The mean value in tables shows the average response of 9 replicates. Analysis such as, Mean, SE, two-way ANOVA, one-way ANOVA and multiple comparisons was done using Matlab Version 5.3, and SPSS version 10.0 Math Works Inc. (USA) statistical packages.

3. Results

3.1 Germination of seedlings and multiple shoot induction on solid medium

The immature pod seeds were germinated with a frequency of 73.3 ± 10.32 % in MSO medium with an average of 7 ± 2 days to germination (Figure 1).

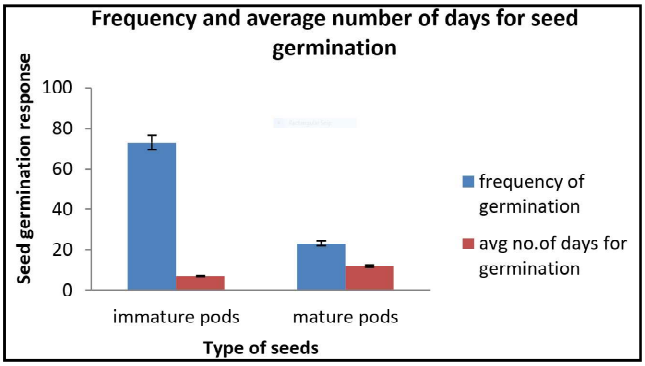

Figure 1: Frequency and average number of days for seed germination from immature and mature pods of *A. paniculata*.

Whereas, the mature pod seeds germinated with a frequency of 23.3 ± 5.16 % with an average of 12 ± 3 days for obtaining germination (Figure 2a). Each seedling gave an average of 3.16 ± 0.40 shoots on 2.0 mg l^{-1} BAP supplemented medium after 4 weeks of culture. Further, sub-culturing of multiple shoots for duration of 8 weeks on same 2.0 mg l^{-1} BAP supplemented medium generated 15.5 ± 0.42 shoots (Figures 2b, 2c).

3.2 Influence of different techniques on multiplication of *A. paniculata* rootless shoots

Growth of shoots using various techniques gave variable response on multiple shoot induction. When the initial inoculums with a small number of shoots (*i.e.*, less than ten shoots) was inoculated into liquid medium, any further shoots were not obtained from these inoculated shoots. But, when a minimum of 15 shoots bunch was inoculated, the growth of shoots was found better.

The shoot number was increased to 61.44 ± 8.32 in only liquid, 109.88 ± 11.3 in liquid media over blotting paper, 34.55 ± 3.79 in liquid media over cotton, 32.22 ± 3.41 in solid media after 14 days of culture (Tables 1, 2, 3; Figure 2 and 4). Further, observations revealed 71.66 ± 8.1, 131.66 ± 11.9, 39 ± 2.5 and 36.88 ± 4.1 shoots in sole liquid, liquid media over blotting paper, liquid media over cotton block and solid media, respectively; after 21 days. The highest number of multiple shoots, *i.e.*, 131.66 ± 11.98 was seen in liquid media over blotting paper compared to all other techniques (Tables 3, 4, 5; Figure 3 and 4). Thus a mass scale multiplication of shoots was achieved in liquid media compared to blotting paper.

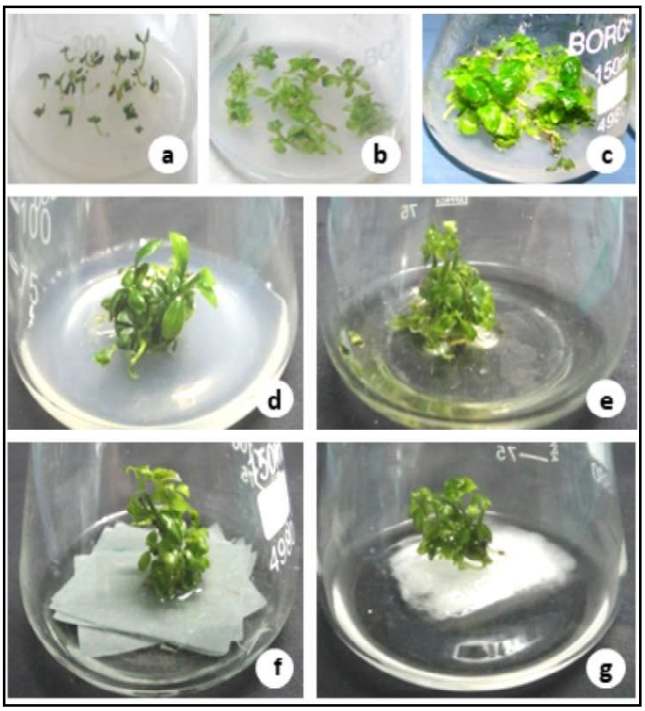

Figure 2: Germination, growth, establishment and evaluation of different culture techniques using multiple shoot cultures of *A. paniculata*. (a) Germinating seedlings from immature pods in MSO medium. (b) Elongation and growth of seedlings (c) Further growth and establishment of multiple shoot cultures. 1st day of multiple shoot inoculation in MS + BAP (2 mg l^{-1}) (d) Semi solid medium (e) Liquid media (f) Liquid media over blotting paper (g) Liquid media over cotton.

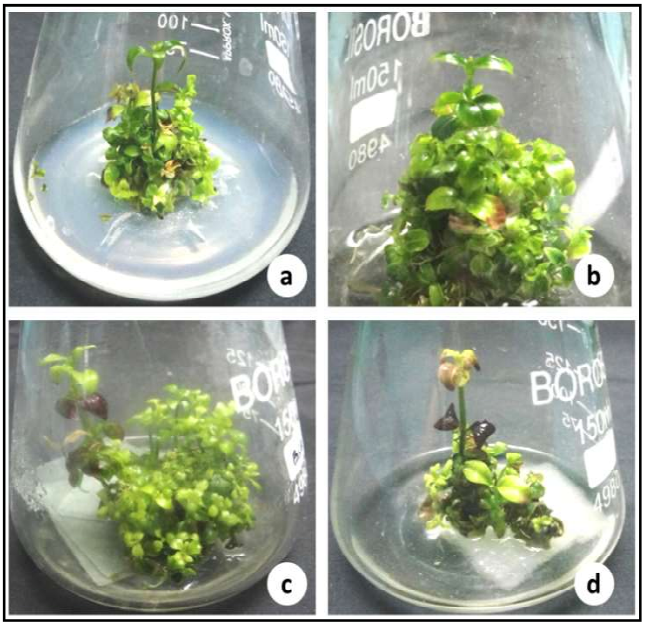

Figure 3: Evaluation of different culture techniques using multiple shoot cultures of *A. paniculata*. 21 days of multiple shoot inoculation in MS + BAP (2 mg l^{-1}) (a) Semi solid medium (b) Liquid media (c) Liquid media over blotting paper (d) Liquid media over cotton.

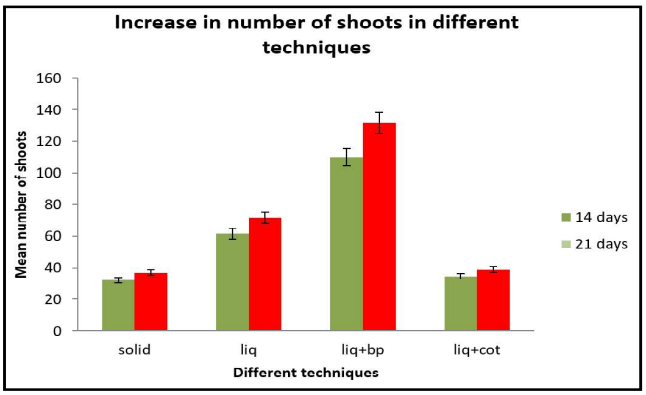

Figure 4: Frequency of multiple shoot induction using different culture techniques in *A. paniculata*. liq: liquid medium; liq+bp: liquid medium over blotting paper; liq+cot: liquid medium over cotton.

Table 1: One-way analysis of multiple shoots growth using different techniques in *A.paniculata* after 14 days

Culture techniques	Mean	Std. error
Semi solid	32.2222	3.41475
Liquid	61.4444	8.32184
Liquid media over blotting paper	109.8889	11.35347
Liquid media over cotton	34.5556	3.79368

Observations were recorded after 14 days of inoculation. The experiment was repeated thrice by maintaining minimum of 9 replicates each time. Each replicate consists of 15 ± 3 healthy shoots. Mean is the average response of 9 replicates and the mean % value in the table shows the average frequency of 3 repeated experiments.

Table 2: Univariate ANOVA analysis illustrating the effect of different techniques on growth of multiple shoots after 14 days

Source	Sum of squares	df	Mean square	F	Sig.
Between groups	35182.083	3	11727.361	23.247	.000
Within groups	16142.889	32	504.465		
Total	51324.972	35			

Table 3: Multiple comparison test for growth of multiple shoots of *A. paniculata* using different culture techniques after 14 days

Target (I)	Target (J)	Mean difference (I-J)	Std. Error	Sig
Solid	[a]liq	-29.22222*	10.58789	.009
	[b]Liq+bp	-77.66667*	10.58789	.000
	[d]Liq+ cot	-2.33333	10.58789	.827
Liquid	[b]liq+bp	-48.44444*	10.58789	.000
	[c]liq+cotton	26.88889*	10.58789	.016
	[d]solid	29.22222*	10.58789	.009
Liquid Blotting paper	[a]liq	48.44444*	10.58789	.000
	[c]Liq+cotton	75.33333*	10.58789	.000
	[d]solid	77.66667*	10.58789	.000
Liquid Cotton	[a]liq	-26.88889*	10.58789	.016
	[b]liq+bp	-75.33333*	10.58789	.000
	[d]solid	2.33333	10.58789	.827

Observations were recorded after 14 days of inoculation. [a]Liq: Liquid medium; [b]Liq+bp: Liquid medium over blotting paper; [c]Liq+cot: Liquid mediumover cotton;[d]solid: semi solid medium with 8% agar. Based on observed means the mean difference is significant at the 0.05 level.

The analysis was carried out through two-way analysis of variance (ANOVA) for on all data under the different techniques for rapid production of multiple shoots. The individual test for the experiment's significance was tested in Post-Hoc analysis through multiple comparison tests of different techniques (Table 3 and 6). The data was evaluated through two-way ANOVA for the overall data of different techniques for shoot proliferation. The experiment with different techniques was found significant at LSD (Least significant difference) ≤ 0.05 in terms of number of multiple shoots (Table 4 and 7).

Table 4: One-way analysis of multiple shoots growth using different techniques in *A. paniculata* after 21 days

Culture techniques	Mean	Std error
Semi solid	36.8889	4.16815
Liquid	71.6667	8.11891
Liquid media over blotting paper	131.6667	11.98958
Liquid media over cotton	39.0000	2.59272
Total	69.8056	7.43200

Observations were recorded after 21 days of inoculation. The experiment was repeated thrice by maintaining minimum of 9 replicates each time. Each replicate consists of 15 ± 3 healthy shoots. Mean is the average response of 9 replicates and the mean % value in the table shows the average frequency of 3 repeated experiments.

Table 5: Univariate ANOVA analysis illustrating the effect of different techniques on growth of multiple shoots after 21 days

Source	Sum of squares	df	Mean square	F	Sig.
Between groups	52764.750	3	17588.250	33.440	.000
Within groups	16830.889	32	525.965		
Total	69595.639	35			

Table 7: Multiple comparison tests for growth of multiple shoots of *A. paniculata* in different techniques after 21 days

Target (I)	Target (J)	Mean difference (I-J)	Std. Error	Sig
Solid	[a]liq	-34.77778*	10.81116	.003
	[b]Liq_bp	-94.77778*	10.81116	.000
	[d]Liq_cotton	-2.11111	10.81116	.846
Liquid	[b]liq_bp	-60.00000*	10.81116	.000
	[c]liq_cotton	32.66667*	10.81116	.005
	[d]solid	34.77778*	10.81116	.003
Liquid Blotting paper	[a]liq	60.00000*	10.81116	.000
	[c]Liq_cotton	92.66667*	10.81116	.000
	[d]solid	94.77778*	10.81116	.000
Liquid Cotton	[a]liq	-32.66667*	10.81116	.005
	[b]liq_bp	-92.66667*	10.81116	.000
	[d]solid	2.11111	10.81116	.846

Observations were recorded after 21 days of inoculation. [a]Liq: Liquid medium; [b]Liq+bp: Liquid medium over blotting paper; [c]Liq+cot: Liquid medium over cotton; [d]solid: semi solid medium with 8% agar. Based on observed means the mean difference is significant at the 0.05 level.

The growth of shoots in liquid medium was encountered with thickening of leaves showing fructification tendency which were not suitable further differentiation and also could not be sub-cultured for multiplication. Apart from that, shoots growing in solid, only

liquid, over cotton on liquid medium became brown and showed affinity towards senescence within two weeks. The shoots became dark green and no further shoot formation with emergence of new shoot buds was observed. However, amongst the culture techniques evaluated shoots grown in liquid media over blotting paper was found best with higher multiplication rate of healthy green shoots (Figure 5). Although, in liquid media over blotting paper culture technique a negligible sign of leaf browning was observed at localized areas of shoot bunch, the nodal parts remained healthy and rejuvenated.

Figure 5: Mass scale multiplication of shoots of *A. paniculata* in liquid media over blotting paper (a) In 250 ml Erlenmeyer flask (b) 300 ml screw capped jam bottles.

3.3 Andrographolide analysis of rootless multiple shoots grown using different culture techniques

The amount of andrographolide in shoots by HPLC analysis revealed variation in content a of rootless multiple shoots amongst different culture techniques evaluated. Significantly, the shoots grown in liquid have shown highest andrographolide content, *i.e.*, 4.00 % DW compared to all other culture techniques (Figure 7 and 8). Whereas, in shoots grown in liquid media over blotting paper was 2.35 % DW, liquid media over cotton block was 2.44 % DW without much difference in the content increase. Although, the blotting paper method did not promote higher andrographolide production, but best growth of highly proliferating shoots with increased biomass could be achieved compared to other techniques where early senescence and browning of shoots was a common limitation (Figure 6). The shoots grown in solid media has shown least content of andrographolide with 1.59 % DW. A 2.51-fold higher andrographolide accumulation was observed in multiple shoots grown in liquid medium than solid.

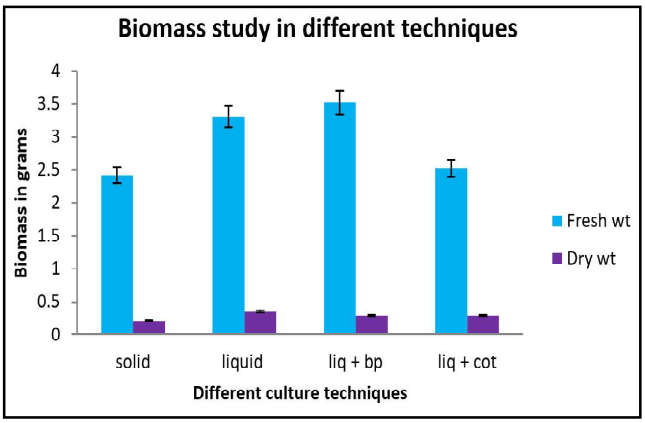

Figure 6: Effect of different culture techniques on biomass production of *A. paniculata* multiple shoots. liq: liquid medium; liq+bp: liquid medium over blotting paper; liq+cot: liquid medium over cotton.

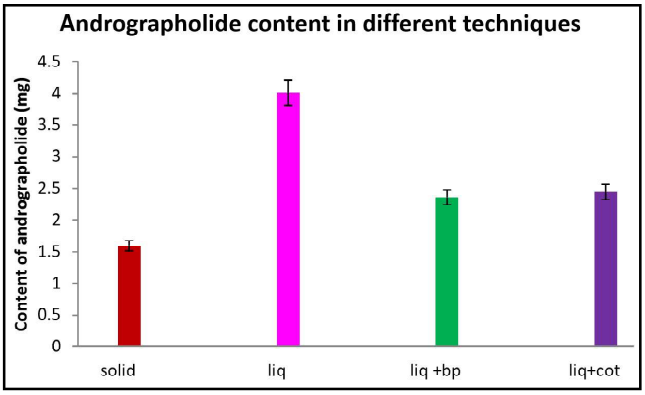

Figure 8: Influence of different culture techniques on andrographolide content in *A. paniculata.* multiple shoots. liq: liquid medium; liq+bp: liquid medium over blotting paper ; liq+cot: liquid medium over cotton.

4. Discussion

The germination of immature green seeds was faster in a few days compared to mature pod seeds. Similar results were obtained previously with immature pod seeds (Purkayastha *et al.,* 2008). In an earlier study of sub-culturing similar results were observed with *A. paniculata* and *Withania somnifera* seedlings on MS medium supplemented with BAP (2 mg l^{-1}) for shoot induction (Purkayastha *et al.,* 2008). In another report, BAP was supplemented with spermadine to improve the shoot multiplication in *Withania somnifera* (Sivanandhan *et al.,* 2013).

The liquid medium compared to solid medium was found advantageous for *in vitro* propagation of shoots as the media supplementation is easy. besides other advantages. It showed a faster and simple way to increase the biomass of shoots in liquid media compared to solid media from our previous reports (Roy *et al.,* 2009, Zaheer and Giri, 2015). In a previous report after 21 days of culture in temporary immersion systems, the biomass of shoots was also found to be 7 times higher and was best in terms of FW and DW in *Stevia rebaudiana* indicating large scale multiplication potential (Vives *et al.,* 2017).

In cultivars of *Eucalyptus, Bulbine latifolia, B. frutescens* and *Dianthus caryophyllus* the temporary immersion technique was considered better large scale propagation alternative for shoot production and elongation (Ahmadian *et al.,* 2017; Zhang *et al.,* 2018). In *Dianthus caryophyllus* and *Anthurium andreanum* shoot length, number of nodes and fresh weight was found to be increased 3 times in temporary immersion system with liquid medium compared to conventional solid medium (Park and Jeong *et al.*, 2019; Martínez-Estrada *et al.,* 2019). These reports of temporary immersion technique supports our finding on the superiority of shoot growth using liquid media over blotting paper for rapid propagation in *A. paniculata*. In the present study, More biomass can be generated in the form of multiple shoots using blotting paper technique compare to other methods. Because, there are several limitations such as, thickening of leaves, callusing at the base of the shoots, browning of leaves, during *in vitro* propagation of *A. paniculata* plants using semi solid, liquid in our study and adopting temporary immersion cultures in the past. Generally, in the solid cultures although aeration is possible but uniform nutrient uptake and availability is not appropriate but further the accumulation of CO_2 and ethylene, still remain a common problem.

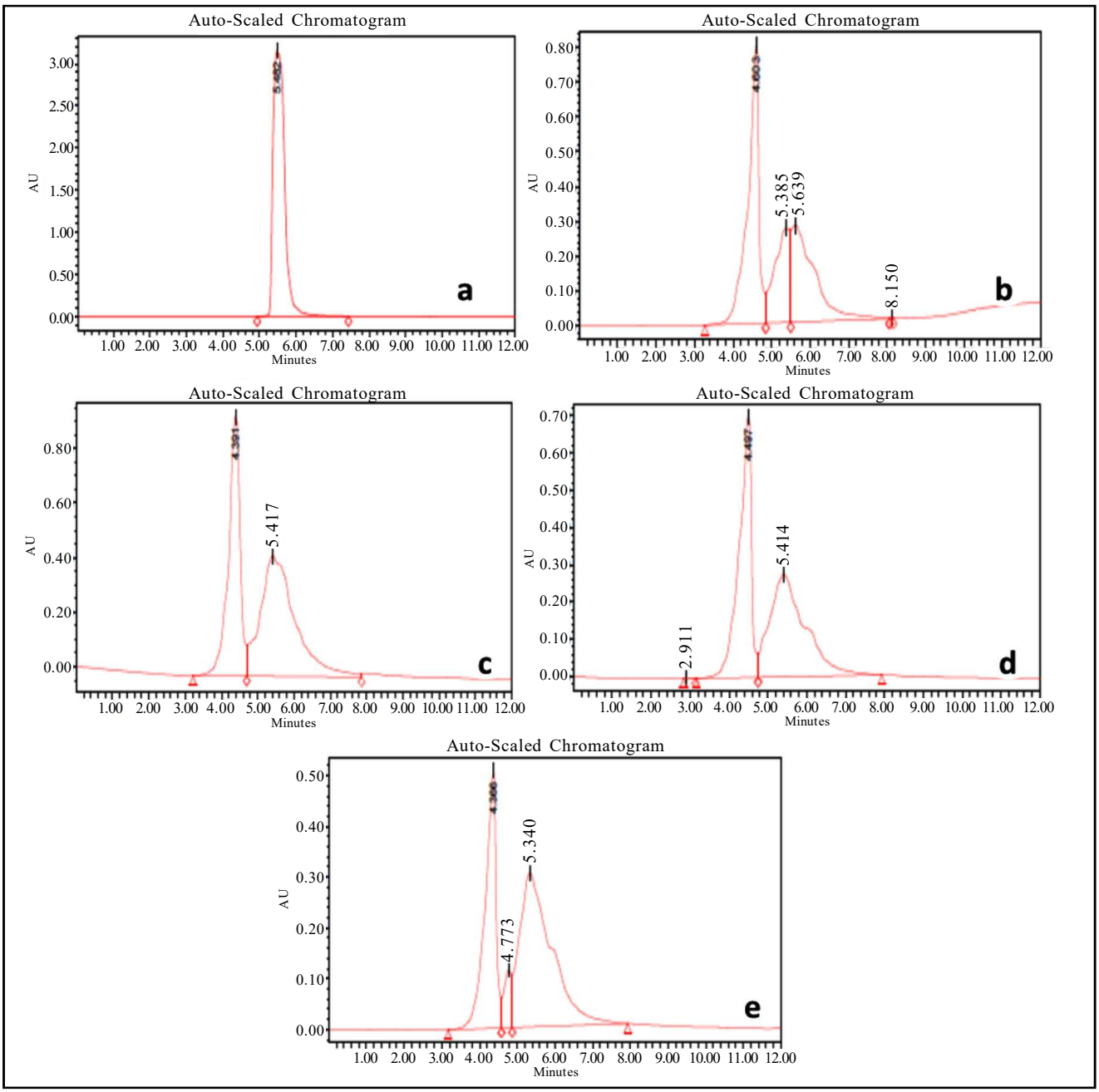

Figure 7: HPLC profile analysis for andrographolide in multiple shoot cultures of *A. paniculata*. (a) Standard (b) Shoots grown in semi solid media (c) Liquid media (d) Liquid medium over blotting paper (e) Liquid media over cotton.

On the contrary in general, comparatively nutrient uptake is uniform in liquid media grown cultures. However, the thickening of leaves with the symptom of hyperhydricity is a common limitation in liquid cultures which leads to oxidative stress, malformation of leaves and other physiological disorders even in temporary immersion methods (Berthouly and Etienne 2005; Chakrabarty *et al.,* 2006; Regueira *et al.,* 2017). To circumvent these problems, the temporary immersion technique could serve as a better alternative. Similar study on these three culture techniques, *i.e.*, solid, liquid, temporary immersion showed the increase in shoot multiplication and biomass in temporary immersion techniques. It has several advantages over other culture techniques such as prevention of asphyxia and hyperhydricity (Etienne and Berthouly, 2002). However, practically the temporary immersion technique is tedious and involves constant monitoring for the time of temporary immersion. On the other hand our present finding provides an extremely simple technique through the use of easily available low-cost blotting paper culture method to generate multiple shoots at increased rate in laboratory conditions for enhanced biomass production. In a previous report, steviol glycosides production was best in *Stivea rebaudiana* using temporary immersion technique (Vives *et al.,* 2017). In another report the temporary immersion technique had also revealed positive effect in *Digitalis* plants for cardiotonic digoxin and lanatoside C accumulation (Arano-Avalos *et al.,* 2019; Pérez-Alonso *et al.,* 2009).

5. Conclusion

An improved technique has been developed to establish and quicken the regeneration capacity of *A. paniculata* multiple shoots. This is the first report of *A. paniculata* multiple shoot cultures in liquid media with higher multiplication rate compared to the shoot multiplication in solid medium. It showed a faster and simple way to increase the biomass of shoots in liquid media compared to previous reports. The slow and continuous supplementation of liquid media over blotting paper as support system without the submergence of shoots might be one of the reasons for fast and consistent growth. The blotting paper platform may be facilitating sufficient aeration to the inoculated shoots for obtaining optimum growth. Thus, the improved culture technique in the present study has made a difference in terms of shoot multiplication frequency compared to shoots inoculated onto both liquid and solid medium in the long run. The rapid biomass production using improved technique, subsequent increased production of andrographolide in shorter duration of time may be exploited *in vitro* as a constant source of this pharmaceutically important bioactive diterpene lactone to meet the demand and for germplasm conservation in curtailing exploitation of *A. paniculata* from its natural habitat.

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Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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