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Efficient *In vitro* Micro Propagation of *Andrographis paniculata* and Evaluation of Antibacterial Activity from Its Crude Protein Extract

M. A. Al-Mamun[1], Rafica Akhter[2], A. Rahman[2] and Z. Ferdousi[3*]

[1]Protein Science Lab, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.
[2]Ali. Mohammad Eunus Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi 6205, Bangladesh.
[3]Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author MAA and RA contribute equally. Authors MAA and ZF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RA and AR managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The current study was designed to establish a cost effective protocol for rapid *in vitro* regeneration of *Andrographis paniculata* (Kalmegh) and also screening the antibacterial activity of its crude protein extracts against five human pathogenic bacteria.
Study Design: The whole investigation of in vitro micro propagation was carried out using three replications. Screening of antibacterial assay was carried out using disc diffusion method and measuring inhibition zone in millimeter.
Place and Duration of Study: The entire study was conducted in Prof. Ali. Mohammad Eunus Laboratory of the Department of Genetic Engineering and Biotechnology, University of Rajshahi,

**Corresponding author: E-mail: mamungeb26@gmail.com; ferdousi04@yahoo.com;*

Bangladesh between October 2012 to March 2014.
Methodology: The present research work was undertaken for *in vitro* shoot formation, shoot multiplication, root induction and establishment of whole plantlets from shoot tips and nodal segment of Kalmegh using MS media supplemented with BAP, Kn NAA and IBA, either alone or in combination. The extracted crude protein from the leaf of *Andrographis paniculata* was used for antibacterial screening against three gram negative and two gram positive pathogenic bacteria and measuring the antibacterial activity as zone inhibition in millimeter. Gentamicin was used as standard drug.
Results: BAP alone showed maximum (100%) shoot regeneration from nodal segment at a concentration of 0.5 mg/l. In combination, medium having 0.5 mg/l BAP + 0.1 mg/l NAA was found to be best for auxillary shoot proliferation (90%). Maximum rooting 100% with 12.4 roots per explants were recorded on the medium containing 0.2 mg/l of IBA. The crude extract showed dose dependent strong antimicrobial activity against the entire test organism by showing zone inhibition ranging between 7.91 to 17.5 mm.
Conclusion: The protocol for *in vitro* micro propagation has been described here, is very simple and cost effective, which can be easily utilized for mass regeneration of Kalmegh for the purpose of drug development due to the presence of potential antibacterial polypeptide in its leaf extracts.

Keywords: Andrographis paniculata; disk diffusion; growth regulator; microorganism; micropropagation; zoon inhibition; protein.

ABBREVIATIONS

***BAP,** 6-Benzylaminopurine; **Kn,** kinetin; **NAA,** 1-naphthaleneacetic acid; **IBA,** indole-3-butyric acid.*

1. INTRODUCTION

The growing demands of conventional drugs for maintaining public health from a past century and recent development of microbial resistance to the commercial therapeutic agent have leaded the scientist to explore the antimicrobial activity of herbal products. A range of people in developing countries relies on natural medicine for combating against the invention of micro organism because of their potent medicinal activity, lesser side effect, sufficiency and economic feasibility. A number of medicinal plants have already been evaluated for screening of antimicrobial activity and showing a significant inhibition of microbial growth compared with the conventional drug [1,2]. The medicinal values of plant products are unique to specific plant species or groups because of the disparity in the constituents of their primary and secondary metabolites [3]. Along with these secondary metabolites, recently a considerable attention of professionals is attracted to a specific group of plant polypeptides responsible for the antagonistic action against microbes. Therefore, exploring newer species of medicinal plants and fiund out its cost effective rapid regeneration protocol may play a vital role in the development of potential therapeutic agents for curing infectious disease.

Andrographis paniculata Nees usually known as "King of Bitters" belongs to the family Acanthaceae, an erect annual herb normally 30 to 110 cm in height and widely distributed in southern India and tropical Asia. Traditionally the leaves and aerial parts of this plant have long been used for fighting off colds and infection [4]. Enormous pharmacological implications of kalmegh have already been reported against a range of diseases including hepatitis, bronchitis, colitis, cough, fever, mouth ulcers, tuberculosis, bacillary dysentery, urinary tract infections and acute diarrhoea [5]. The bioactive components of kalmegh showed significant antimicrobial [6], anti-cancer [7], anti-diabetes [8,9], anti-inflammatory [10], antioxidant [11] Anti HIV [12], hepatoprotective [13], cardio-protective [14], anti-protozoan [15] insecticidal [16] and immunostimulatory activity [17]. Antibacterial activity of methanolic, ethanooic, aqueous, acetone and chloroform extracts of *Andrographis paniculata* has been documented by many worker and showing potential inhibition of bacterial growth [18-20]. Among them methanloic extract showed highest antagonist action to pathogenic organism [20]. Hence, the screening of antibacterial activity of crud protein extract of kalmegh may emerge a new prospect to surmount the growing problem of multiple drug resistance and toxicity of commercially available antibiotic. Active crude extracts of *Andrographis*

paniculata have already been standardized toward drug development in Nigeria [21]. After finding out the potential natural compound, cost effective mass production of plant material is the subsequent step toward drug development. Clonal regeneration of medicinal plants requires the establishment of successful *in vitro* plant tissue culture methods. A number of potential medicinal plants have already been successfully *in vitro* propagated with greater performance [22,23]. The limitation of plant regeneration through conventional techniques are low germination rate, pathogenic attract, higher life span and insufficiency of germination material [24,25]. Therefore, there is an utmost need to practical understanding of the mass multiplication, conservation, cost effective and sustainable usage of *Androgra phispaniculata* clones within a reasonable time frame [26]. Therefore, the current study was conducted to evaluate comparative response of auxin-cytokinin either alone or in combination on shoot multiplication and root induction from shoot tip and nodal explants of kalmegh and also screening of antibacterial activity of crude protein extract against human pathogenic bacteria.

2. MATERIALS AND METHODS

2.1 *In vitro* Micro Propagation

2.1.1 Collection of plant materials

Entire kalmegh plants were collected from the Botanical garden of Rajshahi University by author. The identity of the plants was verified by a taxonomist professor Mahbubur Rahman at the Department of Botany of Rajshahi University, Bangladesh.

2. 1.2 Maintaining aceptic condition

Sterilization of leaves sample was carried out by washing under running tap water for 30 minutes then taken in a reagent bottle containing distilled water with 1% savlon (v/v) and 4 drops of Tween-80 (wetting agent) with constant shaking for 8 minutes to remove gummy substance. This was followed by second washing with autoclaved distilled water. The explants were kept into 3 sterile conical flasks containing different concentrations of $HgCl_2$ for 1-8 minutes to ensure surface sterilization followed by washing 5-7 times with sterile double distilled water promptly to remove all traces of $HgCl_2$.

2.1.3 Media and culture condition

The culture media containing Murashige and Skoog (MS) salt supplement with different macro and micro element sucrose 3% (w/v) as carbon source and agar 0.8% (w/v) as gelling agent previously described by Murashige and Skoog, [27]. The growth regulators (Auxin and cytokinine) of the culture media were prepared separately as stock solutions for ready use during the preparation of culture media. Sterilization of the media was carried out by adjusting 15-1bs./sq. inch pressure and temperature at 121ºC for 20 minutes. The cultures were maintained in the culture room at 25±2ºC under a 16h photoperiod (cool-white fluorescent tube supplying). Light intensity varied from 2000-3000 lux.

2.1.4 Shoot initiation and multiplication

For shoot proliferation and multiplication, the explants were cultured on MS medium supplemented with various concentrations of plant growth regulators (cytokines rich) viz.6-Benzylaminopurine (BAP), kinetin (Kn) either alone or in combination with 1-naphthaleneacetic acid (NAA) andindole-3-butyric acid (IBA). Shoots were rescued aseptically from the culture vessels and were cut into several small pieces having auxiliary buds and again cultured to freshly prepared medium containing different combinations of hormonal supplements for multiplication of shoots at regular intervals of every 3 per weeks. Among the regenerated explants which showed adventitious shoots proliferations were recorded after 8 weeks of culture.

2.1.5 Rooting of micro shoot

When *in vitro* raised shoots grew about 4-5 cm in length, they were separated aseptically from the culture vessels and transferred to freshly prepare rooting media containing different combinations of NAA and IBA (0.1, 0.2, 0.5, 1.0, 2.0 µM). Data were also documented on percentage of rooting, number and length of roots after 4 weeks of transferring to rooting media.

2.1.6 Transplantation and acclimatization of plantlets

Plantlets with sufficient rooting system were then transferred carefully from the culture vessels and washed under running tap water followed by transferring to polar pots containing different

planting substrates viz. garden soil, sand and compost (2:1:1) under diffuse light (16/8 h photoperiod). All polar pots were covered with a moist polythene bag immediately to prevent desiccation. After 13-14 days the polythene bags were gradually exposed to acclimatization to natural condition. However the plants were successfully adapted to the natural environment and were transferred to the field condition. In this way, the regenerated plants showed 80% success in field condition.

2.2 Antibacterial Assay

2.2.1 Extraction of total protein content

The simplified protocol for extraction of total plant protein described previously Wang et al. [28]. The leaves sample were blended with Tris-hydrochloric acid (Tris-HCl) buffer until making paste with mortar and spatula. Then the paste materials were mixed with Tris-HCl buffer (1 ml/10 mg leaves) as well as β-mercapto ethanol (1 µl) then vortexes for homogenization. The homogenized mixtures were centrifuged at 10000 rpm for 20 minutes. Out of three layers found in the epindroff tube, the middle layer was taken carefully with the help of micropipette and stored in the eppendorf tube at 4ºC.

2.2.2 Test microorganism

Antimicrobial activity of *Androgra phispaniculata* protein extracts was investigated against three gram-negative human pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus* and two gram-positive bacteria namely *Bacillus subtilis* and *Mycobacterium smegmatis* collected from the Microbiology laboratory, Department of Genetic engineering and biotechnology, University Rajshahi, Bangladesh.

2.2.3 Agar diffusion assays

The agar diffusion assay was used for screening of antibacterial activities of *A. paniculata*crud protein extract according to the method described by Bagamboula et al. [29]. 6 µm paper disks were punched into the agar media with sterile cork borer containing 0.5, 1, 2 and 4 mg/disccrude protein extracts with micropipette and kept them at laminar air flow hood for dryness (5-10 minutes). The test organisms (100 µL) were inoculated on the surface of the solid agar medium with a sterile spreader. The agar plates inoculated with test organism were incubated for one hour before placing the protein sample impregnated paper disks on the plates for diffusion. The bacterial plates impregnated with a paper disk containing different concentrations of crude protein were incubated at 37±0.1ºC for 24 hours. Gentamicine (30 µg/ disc) was used as positive control for comparing the antibacterial assay. Antimicrobial activity was determined by measuring the zone of inhibition in millimeter around each wall (excluding the diameter of the wall).

3. RESULTS AND DISCUSSION

3.1 *In vitro* Micro Propagation of Kalmegh

Different morphogenetic responses with different concentrations and combinations of plant growth regulators were documented from nodal segment and shoot tip of kalmegh.

3.1.1 Standardization of $hgcl_2$ treatment for field grown explants

We measured the exact time duration of 0.1% $HgCl_2$ treatment with getting the highest number of viable cultures during surface sterilization. Among the different time of treatments with 0.1% $HgCl_2$, the treatment for 5 min produced 100% contamination free cultures and subsequently yielded 95% aseptic plantlets. (Table 1). When treatment duration was more than 5 minutes, the survivability of the culture was also decreased. Neglecting some drawbacks of inhibitory effect, $HgCl_2$ has emerged as a potent surface sterilizing agent in modern plant tissue culture [30].

3.1.2 Shoot multiplication from shoot tips and nodal segments

Nodal segments (1.0-1.5 cm) and shoot tips (1.0 cm) were cultured in MS medium supplemented with BAP and Kn singly or combinely with IBA and NAA for shoot proliferation and multiplication. Among the various cytokinins tested, BAP alone resulted in maximum number of explants initiating the shoots. The highest 100% explants induced multiple shoots from nodal segment in medium containing 0.5 mg/l BAP alone with mean number of shoots per explants was 8.4 and mean length of shoots was 4.7 against 80% from shoot tip for the same concentration of BAP (Table 2). On the other hand, the highest 83% explants induced multiple shoots from nodal segments in medium containing 0.5 mg/l Kn against 70% from shoot tip (Table 2).

Table 1. Effect of 0.1% $HgCl_2$ solution used at varying periods of minutes on surface sterilization of explants and their viability

Duration of treatment in minutes	No. of explants cultured	% of contamination free culture	% of survival cultured explants
3	20	30.03±1.2	15.02±1.0
4	20	60.12±1.5	46.13±0.6
4.3	20	75.10±1.6	60.42±0.8
5*	20	100.00±1.2	95.89±1.2
6	20	100.00±1.8	85.01±1.7
7	20	100.00±2.0	63.12±1.5
8	20	100.00±2.5	50.15±0.9

The airstrike mark in the table mentioned best result in the current study

Table 2. Effects of different concentration of BAP and Kn on shoot multiplication from both the explants. (Data were collected after 6 weeks of culture)

Treatment (mg/l)	Shoot tips			Nodal segments		
	% of culture responde	Mean No. of shoots per explant	Mean length of longest shoot in cm	% of culture responded	Mean No. of shoots per explants	Mean length of longest shoots in cm
BAP						
0.1	50.05±1.6	2.1±0.1	2.0±0.06	72.04±1.2	3.5±0.2	3.5±0.0
0.2	70.12±2.5	4.9±0.3	3.8±0.4	90.09±2.0	6.6±0.2	4.0±0.3
0.5*	80.09±1.9	6.8±0.2	4.0±0.3	100.0±2.3	8.4±0.3	4.7±0.6
1.0	62.02±1.8	4.0±.01	3.0±0.2	83.06±1.9	6.2±0.4	3.8±0.01
2.0	55.01±1.7	3.2±0.2	2.2±0.1	55.11±1.5	4.6±0.2	2.5±0.02
Kn						
0.1	40.12±1.3	1.1±0.3	1.0±0.0	53.15±0.9	2.1±0.2	2.2±0.2
0.2	50.16±1.9	2.0±0.2	2.0±0.1	73.16±1.9	3.1±0.3	3.1±0.1
0.5*	70.10±2.0	2.6±0.4	2.2±0.2	83.11±1.6	4.0±0.1	3.5±0.4
1.0	60.14±1.8	1.2±0.1	1.1±0.1	67.41±1.5	2.3±0.0	1.4±0.3
2.0	30.09±1.6	1.0±0.0	0.9±0.1	30.09±1.8	2.0±0.2	1.2±0.0

The airstrike mark in the table mentioned best result in the current study. Data were presented as Mean±Standard deviation, n=3

The results from the present study were in agreement with the findings reported by Fatima et al. [31] which showed that maximum shoot bud induction and shoot sprouting was obtained on MS medium supplemented with BA (2.5 µM) from both nodal segments and shoot tip explants. Similar results were also obtained during *in vitro* micro-propagation of cotyledon node explants of *Lallemantia iberica* cultured on MS medium containing 0.50 mg/L BAP [32]. The dominance of BAP over Kn as well as other cytokinins has been reported in a number of familier studies using a variety of explants [33]. The stimulatory effect of BAP on multiple shoot induction and proliferation has been reported previously for other medicinal plant [34]. The findings from our current study also indicated that shoot regeneration was partially inhibited by callus regeneration. Highest (100%) shoot multiplication was induced on the medium containing 0.5 mg/L BAP, where there was no callus induction. The possible explanation of more stability of BAP over other cytokinins was mainly attributed due to the presence of naturally occurring ribosides and nucleotides in BAP. Increasing concentration of BAP beyond the optimal level (0.5 mg/L) suppressed the overall shoot proliferation frequency, number of shoot and shoot length probably due to detrimental effect on the cells predetermined to form vegetative buds.

Among different concentrations and combinations of cytokinin (higher) and auxin (lower) were used, BAP and NAA combinations were also proved to be efficient in axillary shoot proliferation from nodal explants and shoot tips**.** The combined effects of different concentration of Auxin (IBA) and cytokine (BAP) on shoot multiplication of kalmegh were presented Table 3. Among the auxin were used, NAA significantly induced shoot regeneration, number and length of shoots while; IBA did not considerably developed the parameters evaluated. Among all the growth hormone tested, the highest

Table 3. Effects of different concentration and combination of IBA and NAA with BAP on shoot multiplication from both types of explants (Data were collected after 6 weeks of culture)

Treatment (mg/l)	Shoot tips			Nodal segments		
	% of culture responded	Mean No. of shoots per explants	Mean length of longest shoot in cm	% of culture responded	Mean No. of shoots per explants	Mean length of longest shoots in cm
BAP+NAA						
0.2 + 0.1	55.12±1.8	2.8±0.3	2.6±0.3	60.19±2.2	3.0±0.3	2.9±0.4
0.2 + 0.2	30.14±1.1	1.6±0.2	1.0±0.1	40.10±1.1	2.6±0.1	1.4±0.1
0.5 + 0.1*	82.16±2.0	3.4±0.4	3.4±0.6	90.20±1.8	4.2±0.6	3.7±0.3
1.0 + 0.1	70.14±1.2	2.9±0.3	3.0±0.4	80.16±2.5	3.5±0.4	3.2±0.2
1.0 + 0.5	45.1.4±1.7	2.2±0.0	1.8±0.3	51.17±1.3	2.8±0.2	2.0±0.0
2.0 + 0.1	40.0.9±2.1	2.7±0.1	1.5±0.2	45.18±1.6	3.2±0.3	1.8±0.1
BAP+IBA						
0.2 + 0.1	50.21±1.1	2.6±0.3	2.5±0.1	55.15±1.6	3.0±0.3	2.7±0.1
0.5 + 0.1*	70.12±1.8	3.2±0.1	3.0±0.05	80.24±1.9	3.9±0.12	3.3±0.3
0.5 + 0.2	60.22±2.1	2.1±0.0	2.6±0.13	62.22±4.4	3.6±0.08	3.0±0.06
1.0 + 0.1	61.20±1.5	2.3±0.5	2.5±0.2	63.26±1.6	3.3±0.2	2.9±0.04
1.0 + 0.5	45.21±1.9	2.0±0.2	2.3±0.12	50.22±1.6	2.9±0.1	2.7±0.2
2.0 + 0.1	40.14±1.8	2.1±0.1	1.2±0.01	40.24±1.4	2.4±0.04	1.4±0.07

The airstrike mark in the table mentioned best result in the current study. Data were presented as Mean±Standard deviation, n=3

percentage regeneration (90%) and (82%) with the highest (4.2±0.6) and (3.4±0.6) number of shoots were found at 0.5 mg/lBAP+0.1 mg/lNAA from nodal segments and shoot tip explants, respectively. Whereas, 0.5 mg/l BAP + 0.1 mg/l IBA combination showed comperatively lower percentage multiple shoots (80%) then NAA combination from nodal segment. The findings from the observation were in accord with a number of previous literature which indicated that a combination of cytokinin and auxins particularly BAP with low concentration of auxin is essential shoot proliferation and multiplication [35,36]. The efficient combination of BA and NAA for *in vitro* shoot proliferation and multiplication has already been reported by anumber of workers in various medicinal plants such as *Centella asiatica* and *Asteracantha longifolia* [37,38].

3.1.3 Adventitious root induction in microclones

For root induction, successfully grown multiple shoot (4-5 cm in length) were aseptically separated from the primary culture and again cultured on MS media supplemented with IBA and NAA at different concentration 0.1, 0.2, 0.5, 1.0, 2.0 mg/l. The highest recorded rooting (100%) were observed in the media containing 0.2mg/l IBA with mean number of roots per explants was 12.4 and mean length of roots was 3.7 against 90% in the media containing 0.2 mg/l NAA (Table 4). The degree responses of different concentration of IBA and NAA on shoot multiplication and root induction followed by acclimatizing in field condition from nodal segment and shoot tip are shown in Fig. 1. The results from the current study were in agreement with the findings reported by Vijayalaxmi and Hosakatte [39] which demonstrated that maximum rooting was on half strength MS medium supplemented with 2.0 µM IBA. The enhancing effect of NAA over IBA on rooting in microshoots were reported by a range of worker during in vitro micro-propagation of medicinal plant [40], which also contradictory with our present study.

However, stimulatory action of auxin on root induction has widely been documented by a number of worker on various explants. The rooted plantlets were transferred to plastic pots and then acclimatized to field conditions. It was observed that the prevailing atmospheric conditions (humidity and temperature) of the transplanting season greatly influenced the initial survival of potted plantlets. In this regard, the month of October- November and February – March with moderate temperature and low humidity were found to be more suitable than any other months of the year. Similarly plantlets having 2-3 cm roots at their active elongation

period survived better than those transferred with much elongated and branched root systems. It was observed that 70.0% and 80% plantlets were established in *ex vitro* conditions when transferred on garden soil and compost potting mix and on garden soil, sand and compost potting mix, respectively.

Table 4. Effect of different concentration of auxins on rooting from regenerated shoots (Data were collected after 30 days of culture)

Treatments (mg/l)	No. of explants inoculation	Days of root initiation	% root induction	Mean No. of root per explants	Mean length of longest root in cm
IBA					
0.1	20	10-15	95.12±1.5	7.5±.8	2.8±0.5
0.2*	20	8-10	100.00±0.9	12.4±0.5	3.7±.0.2
0.5	20	7-12	80.20±1.9	6.5±0.3	2.4±0.05
1.0	20	8-12	70.41±2.5	4.2±0.08	2.2±0.09
2.0	20	-	-	-	-
NAA					
0.1	20	8-12	80.64±4.6	6.6±0.6	2.3±0.04
0.2*	20	7-10	90.21±3.2	8.2±0.4	3.1±0.1
0.5	20	10-12	65.26±2.6	4.4±0.02	1.7±0.07
1.0	20	10-12	50.24±1.8	3.0±0.05	1.4±0.06
2.0	20	-	-	-	-

The airstrike mark in the table mentioned best result in the current study. Data were presented as Mean±Standard deviation, n=3

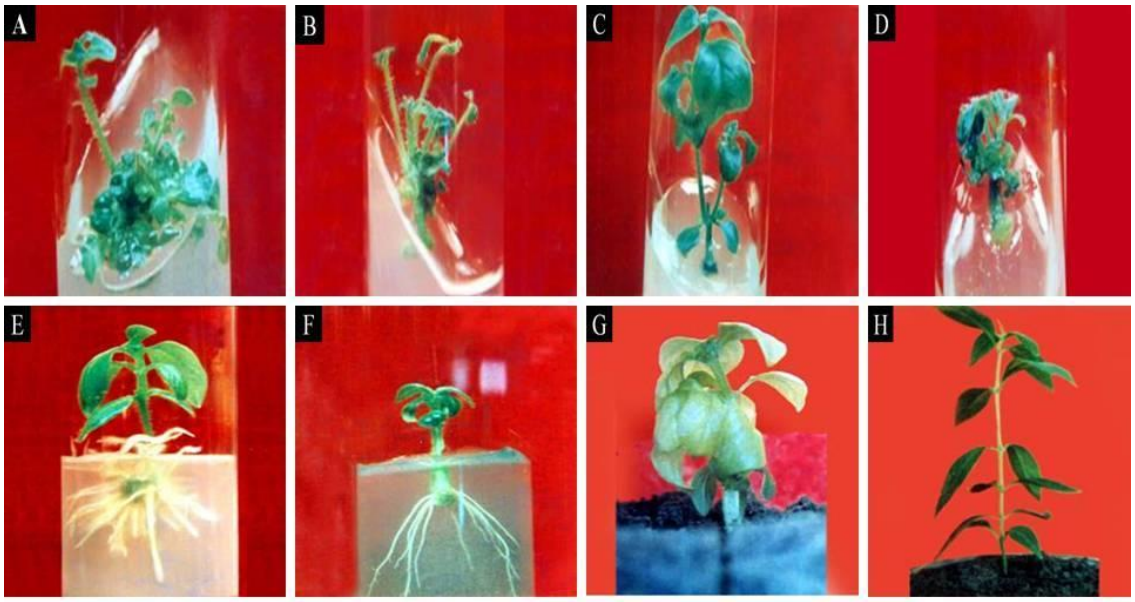

Fig. 1. Shoot regeneration and root induction with subsequent acclimatization from nodal explants and shoot tip. (A) Multiple shoot proliferation from the nodal explants on medium MS + 0.5 mg/l BAP. (B) Shoot proliferation from the shoot tips explants on medium MS + 0.5 mg/l BAP. (C) Shoot proliferation from the nodal explants on medium MS + 0.5 mg/l BAP + 0.1 mg/l NAA. (D) Multiple shoot proliferation from the shoot tips explants on medium MS + 0.5 mg/l BAP + 0.1 mg/l NAA. (E) Root formation in media with MS + 0.2 mg/l IBA. (F) Root induction in media with MS + 0.2 mg/l NAA. (G) Hardening of *in vitro* cultured plant. (H) *In vitro* cultured plant under natural condition after 1 month

3.2 Antibacterial Screening Asssy

The antibacterial activity of crude protein extracts from young leaf of kalmegh against five pathogenic bacteria was presented in Table 5 and showing various degrees of dose and species dependent antagonoistic activitiy. The crude protein exhibited strong antimicrobial activity against all the test organism by showing inhibition zone ranging between 7.9-17.5 mm. Among the test organism *E. coli* showed highest sensitive to the extracts and presented highest 17.5 mm inhibition zone and *P. Aeruginosa* produced 15.01 mm at the same concentration, indicating comparatively resistant strain among the test organism used in the present study. However, all the test species were subjected to considerable inhibition in their growth comparing with the standard drug Gentamicin (30 µg/ disc).

The observed antimicrobial activity of crude protein extracts of *A. paniculata* was mainly attributed to the interaction of some important phytochemical (andrographolide) as well as potent antibacterial polypeptide "arabinogalactan protein" present in the cell wall, playing a significant biological function. *A. paniculata* possess a range of bioactive secondary metabolites (Andrographolide, echiodinin) responsible for inhibitory action againsta rang of infectious microorganism. The results of the current study were in agreement with the findings reported by Singh et al. [41] which demonstrated that aqueous extracts from the leaves of kalmegh containing arabinogalactan proteins exhibited strong activity against a number of bacteria. Among the extraction techniques, methanolic extracts from leaves showed highest antibacterial potentiality against a number of pathogenic bacteria [20]. The methanolic extract (75%) from the leaves of *Andrographis paniculata*at dose of 3 mg/disk produced 26 mm inhibition zone [20] whereas crude protein extract in the current study showed 16 mm zone at dose of 4 mg/disk against *Staphylococcus aureus.* The higher antimicrobial potential of methanolic extract probably due to existence of higher andrographolide compound then crude protein extract. Test microorganisms were used in the present study, all are pathogenic and associated with a range of infectious disease by targeting multiple organ of human body [42,43]. Both types of test organisms (gram positive and gram negative) were subjected to inhibition because of the inimical action of potential bioactive compound including along with inimical polypeptide which probably capable of disrupting the cell membrane by interfering with lipid molecule on the cell surface [44]. The molecules of antimicrobial peptides interfere with bacterial cell membrane leading to the disruption of transmembrane protein (probably, an ion channel) resulting the reduction of the membrane potential and subsequent cytolysis [45]. The fluctuations of antibacterial activity among the test organisms were attributed to difference in morphological structure of the cell membrane components of test organism, extraction method and dose of bioactive components [46]. All the extraction method along with crude protein extract may contain all the bioactive compounds but the quantity depending upon extraction technique itself. However, considering the antibacterial activity of *A. paniculata* based on previously reported extraction techniques, the present study has strongly supported for crude protein extract of kalmegh as valuable natural raw material against tested bacterial isolates.

Table 5. Inhibition zone (mm) of crude protein extracts of *Andrographis paniculata* measured on five human pathogenic bacteria

Test organisms	Inhibition zone (mm)				Gentamicine (30 µg/ disc)
	0.5mg/disc	1 mg/disc	2 mg/disc	4 mg/disc	
Escheria coli	10.82±0.22	12.42±0.1	15.6±0.8	17.5±0.4	22.6±1.08
Staphylococcus aureus	8.88±0.41	9.32±0.25	12.1±0.40	16.01±0.56	21.6±0.82
Pseudomonas aeruginosa	7.91± 0.33	9.01±0.23	12.2±0.81	15.01±0.55	20.3±1.4
Bacillus subtilis	9.22±0.66	11.21±0.66	14.01±0.1	17.02±0.91	22.1±1.2
Mycobacterium smegmatis	9.01±0.88	10.56±0.91	14.02±0.66	16.56±1.02	16.8±0.55

Data were presented as Mean±Standard deviation, n=3

4. CONCLUSION

In conclusion, the present study describes an efficient, cost effective and successful tissue culture system from shoot tip and nodal explants of *Androgra phispaniculata* with great potentiality for use in the pharmaceutical industry for large scale *in vitro* propagation and acclimatization in field condition. Additionally, rigorous study needs to be carried out to isolate the potent antimicrobial polypeptide along with other inimical bioactive compound from the mixture of different samples with subsequent development of novel drugs to treat of infectious diseases with more safety.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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