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Antibacterial Activity of Cell Suspension Cultures of Castor (*Ricinus communis* L. cv. Roktima)

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Authors' contributions

Both the authors carried out the research work jointly and prepared the manuscript together.

Research Article

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ABSTRACT

Aims: The study was conducted to develop the protocol for callus culture, cell suspension culture and to determine antibacterial activity of *Ricinus communis* L. cv. Roktima in cell extract.
Study Design: Hypocotyl segments used as explants in callus culture and agar disk diffusion method used for antibacterial activity test.
Place and Duration of Study: Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh during the period of 2010-2012.
Methodology: MS medium supplemented with different growth regulators were used for callus induction and cell culture and paper disc diffusion method was used for the determination of antibacterial activities. Growth inhibition was determined against five gram positive bacteria viz., *Sarcina lutea, Staphylococcus aureus, Bacillus megaterium, Bacillus subtillus, Bacillus halodurans,* six gram negative bacteria viz*., Shigella sonnei, Klebsiella species, Proteus species, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* by using disc diffusion and micro broth dilution techniques.
Results: Auxins NAA, 2,4-D and IAA played a great role in callus induction but 2.0 mg/L BAP + 0.5 mg/L NAA and 2.0 mg/L BAP + 0.8 mg/L NAA concentrations proved to be most suitable combinations for induction of callus in *R. communis* L.cv. Roktima. Cells were cultured on the MS medium having 2.0 mg/L BAP + 0.2 mg/L NAA in which the rate of cell growth found highest and the cell continued to grow until 14 days. The peak period of cell

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growth was observed from 4th d to 6th d. Antimicrobial test with eleven bacteria demonstrated that the extracts of cell suspension culture of *R. communis* L .cv. Roktima holds the merit of antimicrobial activity and it was considered to be the potent source of antibacterial compounds and a possible source for obtaining the toxin ricin.
Conclusion: In summary, the results obtained in the present investigation demonstrated that the extracts of cell suspension culture of *R. communis* L. cv. Roktima had the antibacterial activity and considered to be the potent source of antibacterial compounds.

Keywords: Castor; hypocotyl; cell suspension culture and antibacterial activity.

1. INTRODUCTION

Plant tissue and cell culture is an important tool in plant biotechnology that allows for an increase in biomass or metabolite production by utilizing several techniques in callus or morphogenetic cultures [1-2]. Among others, these techniques include bioreactor scale-up, hairy transformed roots, micropropagation, elicitation, precursor compound addition and genetic engineering [3-4,2]. High *in vitro* multiplication was also reported for R. *communis* [5] that produced ricin which is mostly known for its potential as a biological weapon.

Castor oil is a triglyceride of fatty acids and almost 90 percent of its fatty acid content consists of ricinoleic acid. Ricinoleic acid is not found in any other substance except castor oil [6]. The ricin has been used experimentally in medicine to kill cancer cell [5]. Ricin is a secondary metabolite toxic protein abundantly produced from castor bean and it can also be obtained from callus and cell culture of *R. communis* L. [7]. Lethality was investigated of ricinolic acid of castor and its application on the vitellogenesis of Rhipicephalus sanguineus tics that prevented the maturation of oocytes as a results of cytoplasmic changes[8]. As *Calophyllum brasiliense* (Cambes) produces calanolide secondary metabolites that were active against human immunodeficiency virus type 1 reverse transcriptase which was isolated by callus culture [8]. Acridone and furoquinoline alkaloids and coumarins have been isolated from four week old calli in *Ruta* species [9]. The production of the steroidal sapogenin, diosgenin, by callus cultures of *Trigonella foenum-graecum* L. (fenugreek) was also reported [10] .Thus, plant cell culture offers an attractive alternative source to whole plant for the production of high-value secondary metabolites [11-16,2]. Plant cell culture systems represent a potential renewable source of valuable medicinals, flavours, essences and colourants that cannot be produced by microbial cells or chemical syntheses. However, only a few cultures produce these compounds in commercially useful amounts. The low productivities are associated with our poor understanding of the biochemistry of these systems. Recent advances in molecular biology, enzymology, physiology and fermentation technology of plant cell cultures suggest that these systems will become a viable source of important natural products.

Increasing scientific interest was noticed during the past few decades in the growth of plant tissue culture and the commercial development of this technology as means of producing valuable phytochemicals [17]. Callus cultures from medicinal plants have been established under suitable conditions to enable production of antimicrobial substances *in vitro* [18-19]. Papers on investigating the antimicrobial activity of extracts from calli of different medicinal plant species have been published focusing on their potentials for drug development [20-21]. Although very few plant cell processes are operating commercially, the most successful commercial pharmaceuticals produced from undifferentiated cell cultures are antibiotic

compounds [22]. The aim of this study was to determine the *in vitro* antibacterial activity of crude extracts isolation from cell suspension cultures of R. *communis* L.

In Bangladesh *R. communis* is widely grown all over the country holding great potential for producing castor oil as a source of green energy and also for commercial production of toxic protein ricin. We have developed the standard protocol for *in vitro* regeneration of *R. communis* and under the present investigation we have made efforts to develop the standard protocol for wide scale callus culture and cell culture of *R. communis* towards isolation of active protein ricin from these cultures.

2. MATERIALS AND METHODS

The hypocotyl explants of *R. communis* L. cv. Roktima were used as experimental materials in the present investigation. Seeds were collected from the research field of the Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh. Seeds were washed throughly under running tap water and then treated with 1% savlon and 2-3 drops of Tween-80 for about 10 min. This was followed by successive three washing with distilled water to make free the seeds from savlon and Tween-80. Surface sterilization was carried out with 0.1% $HgCl_2$ for 6-7 min followed by gentle shaking. After this treatment, the seeds were rinsed 4-5 times in sterile distilled water to make free the seeds from $HgCl_2$. Sterilized seeds were partially decoated and aseptically germinated in glass bottle containing 50 mL of autoclaved (121ºC temperature for 20 min at 1.1 Kg/cm² pressure) MS medium [23], fortified with BAP (1.0 mg/L), sucrose (30 g/L) and agar (8.0 g/L). The pH of the medium was adjusted to 5.7-5.8 before autoclaving and the germinating seeds were kept in a growth room maintained at 25 ± 2ºC temperature and 60ºC RH. The experiment was conducted in the biotechnology laboratory, Institute of Biological Sciences, Rajshahi University, Rajshahi, Bangladesh.

2.1 Callus Culture

Hypocotyl segments, as explants were taken from 12 d old *in vitro* growing seedlings of the plant. The explants were cultured in 9 cm petridish and placed horizontally in the callus induction medium. The MS medium supplemented with 3% sucrose and different concentrations (0.1-1.0 g/L) of NAA (α-Napthalene acetic acid), 2,4-D (2,4-Dichlorophenoxy acetic acid), IAA (Indole-3-acetic acid) and BAP 0.5-3.0 g/L (6-Benzylaminopurine) were compared in combination for the induction of callus.The medium was adjusted to pH 5.7 and autoclaved. The data for callus initiation were scored after 4 weeks of culture.

2.2 Cell Culture

Rapidly proliferating friable calli subcultured for 18 d, were aseptically transferred to MS liquid medium supplemented with 2.0 g/L BAP + 0.05 g/L NAA, 2.0 g/L BAP + 0.2 g/L NAA and 2.0 g/L BAP + 0.5 g/L NAA in three lots in 250 mL flasks. The flasks were placed on a rotary shaker (100 rpm). After 4 d, the liquid medium containing cells and micro calli were filtered through a 500 µm sieve. The filtrate containing cell suspension culture was maintained in the laboratory. To observe the growth efficiency, flasks containing the liquid medium with cell culture were kept in a rotary shaker. The growths of the cells were measured by weighting the cells in 5 mL liquid medium taken in every after two d. On the other hand, to obtain callus, some cells were distributed to petridishes (4 cm) containing the

fresh semi solid medium at 25ºC in dark for 35-42 d of incubation. Micro calli were appeared in the plates initiating induction of callus from cell aggregates.

2.3 Preparation of Extracts

The filtrate containing cell suspension culture was maintained in the laboratory. Every after two d, 5 mL liquid medium with growing cells were centrifuged at 4000 rpm for 10 min. The cells were discarded and the clear 15 µL of the supernatant was applied on the test disc.

2.4 Microorganisms

Five gram positive bacteria viz., *Sarcina lutea, Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus subtillus*, *Bacillus halodurans*; and six gram negative bacteria viz., *Shigella sonnei*, *Klebsiella species*, *Proteus species*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* were employed in this test. These species were obtained from the mother stock of the Molecular Biology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh.

2.5 Media

Nutrient broth and Nutrient agar (Mast Diagnostics, Mast Group Ltd. Merseyside, UK) were used for bacterial culture and conducting antibacterial test.

2.6 Disc Diffusion Assay

Antibacterial activity was determined as diameter of inhibition zone using disc diffusion method [24]. Nutrient agar (NA) was distributed in sterilized petridishes. This was accomplished by placing 15 µl of the extract on a small paper disc. This disc was placed on an agar growth medium containing a confluent lawn of microorganism. The concentration of the organism was also 10 µL/petridish.

2.7 Preparation of the Test Discs

Sterile test discs were prepared by punching and saturating filter paper (Whatman no1) discs (each disc is 6 mm in diameter) in cell extracts solution using sprit flame sterilized forceps. These discs were dried in the sterilized petridishes. The name (code) of the plant extract was written at the bottom of the petridishes. Inhibition zone of all extracts was compared to the standard antibiotic (Ciprofloxacin 05 µg/disc).

2.8 Culture Media and Inoculums

Solid media of nutrient agar was prepared by dissolving 28 g/L water. About 25 mL of media was poured into a petridish. The inoculum was prepared by culturing a large number of organisms in a test tube containing 10 mL liquid media for bacterial strains and incubating over night at 37ºC. The agar plates for the assay were prepared by labeling them with the date, the name of the microorganism and the name (code) of the discs. The inoculi of bacteria were transferred into petridish containing solid nutrient media of agar using a sterile swab. The swab was used to spread the bacteria on the media in a confluent lawn. It was done by rotating the petridish at 90ºC and continuing the spread of bacteria. One swab was used for one species of bacteria.

2.9 Placing Test Discs

Dried test discs were transferred on bacterial lawn under aseptic conditions using spirit-flame sterilized forceps each time. Each disc was placed gently on the agar surface and plated with the forceps so that it sticks. The petridish was incubated upside down at 37ºC for 24 h. Resulting zones of inhibition were observed and measured in millimeters.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Callus induction

The surface sterilized seeds exhibited 95% germination after 10 d of inoculation on MS medium supplemented with 1.0 mg/L BAP. For callus induction, hypocotyl explant were taken from *in vitro* grown seedlings and cultured on MS media supplemented with cytokinin (BAP 0.5 mg/L – 3.0 mg/L) in combination with auxins (NAA 0.1mg/L – 1.0 mg/L, 2,4-D 0.1 mg/L – 1.0 mg/L and IAA 0.1 mg/L – 1.0 mg/L). Different concentrations took different time periods for callus induction. Data on response after inoculation, percentage of explants responded and nature of callus was recorded and the results are presented in Table 1. When the explants were cultured on MS media containing different combinations and concentrations of BAP (0.5 mg/L – 3.0 mg/L) and NAA (0.1mg/L – 1.0 mg/L), the percentage of explants responded was the lowest 23.5% in 0.5 mg/L BAP+0.1 mg/L NAA and the induced calli were yellow friable (Fig. 2: A and B) in color. This rate gradually increased with the increasing of hormonal concentrations and combinations of BAP and NAA. The highest rate 100% was obtained in both the combinations of 2.0 mg/L BAP+ 0.5 mg/L NAA and 2.0 mg/L BAP + 0.8 mg/L NAA. The induced calli were pinkish, nodular and compact (Fig. 2: E and F). When the explants were cultured on MS media containing different combinations and concentrations of BAP (0.5 mg/L – 3.0 mg/L) with 2,4-D (0.1mg/L – 1.0 mg/L), the lowest percentage was 18.0% in 0.5 mg/L BAP + 0.1 mg/L 2,4-D and the induced calli were yellow friable. This rate gradually increased with the increasing of hormonal concentrations and combinations of BAP and 2, 4-D. The highest rate 85.7% was obtained in 2.0 mg/L BAP + 0.8 mg/L 2, 4-D and the induced calli were pinkish nodular (Fig.2: C and D). When the explants were cultured on MS medium containing different combinations and concentrations of BAP (0.5 mg/L – 3.0 mg/L) with IAA (0.1mg/L – 1.0 mg/L), the lowest percentage was 10.2% in 0.5 mg/L BAP + 0.1 mg/L IAA. This rate gradually increased with the increasing of hormonal concentrations of BAP and IAA. The highest rate 53.3% was obtained in 2.0 mg/L BAP + 0.5 mg/L IAA and the induced calli were pinkish nodular (Fig. 2: D). The experiments in our investigation also demonstrated that combined effect of BAP and NAA showed the best performance in callus induction in *R. communis* L. cv. Roktima. Embryogenic calli were induced when explants were cultured on MS medium containing auxin (NAA) with cytokinin (BAP). The explant showed highest percentage (100%) of embryogenic callus when NAA was used with BAP in MS medium supplemented with 2.0 mg/L BAP + 0.5 mg/L NAA and 2.0 mg/L BAP + 0.8 mg/L NAA. But when the explants were cultured on MS medium with 2, 4-D and IAA with BAP, the callusing rate was comparatively lower. In comparison the embryogenic calli pinkish nodular and compact while the nonembryogenic calli were large, yellow and friable.

Table 1. Effect of different growth regulators on callus induction of *R. communis* L cv. Roktima from hypocotyl explant (Each treatment consisted of 20 explants)

Effect of different concentrations and combinations of BAP and NAA in MS medium on callus induction				Effect of different concentrations and combinations of BAP and 2,4-D in MS medium on callus induction				Effect of different concentrations and combinations of BAP and IAA in MS medium on callus induction			
Concentration of growth regulators (mg/L)	Response after days	% of explants responded	Nature of callus	Concentration of growth regulators (mg/L)	Response after days	% of explants responded	Nature of callus	Concentration of growth regulators mg/L)	Response after days	% of explants responded	Nature of callus
BAP + NAA				BAP+ 2,4-D				BAP + IAA			
0.5+0.1	10	23.5	YF	0.5+0.1	13	18.0	YF	0.5+0.1	15	10.2	YF
0.5+0.2	9	35.6	YF	0.5+0.2	12	28.1	YF	0.5+0.2	14	15.1	YF
0.5+0.5	8	45.7	BF	0.5+0.5	11	35.2	YF	0.5+0.5	13	20.0	YF
0.5+0.8	7	49.8	BF	0.5+0.8	10	41.3	BF	0.5+0.8	12	22.2	BF
0.5+1.0	7	46.3	BF	0.5+1.0	10	37.6	BF	0.5+1.0	12	25.3	BF
1.0+0.1	9	36.4	YF	1.0+0.1	12	21.9	YF	1.0+0.1	14	17.7	YF
1.0+0.2	8	45.5	YF	1.0+0.2	11	37.5	YF	1.0+0.2	13	22.4	YF
1.0+0.5	7	62.2	YF	1.0+0.5	10	45.0	BF	1.0+0.5	12	27.5	BF
1.0+0.8	6	77.5	YC	1.0+0.8	9	64.8	BC	1.0+0.8	11	35.2	BC
1.0+1.0	6	60.6	YC	1.0+1.0	9	56.1	BC	1.0+1.0	11	38.8	BC
1.5+0.1	8	34.7	YF	1.5+0.1	11	29.0	BF	1.5+0.1	13	23.5	BF
1.5+0.2	7	52.8	PB	1.5+0.2	10	40.9	BF	1.5+0.2	12	30.9	BF
1.5+0.5	6	80.9	PB	1.5+0.5	9	55.3	PB	1.5+0.5	11	43.0	PB
1.5+0.8	5	89.0	PB	1.5+0.8	8	69.4	PB	1.5+0.8	10	46.5	PB
1.5+1.0	5	68.3	PB	1.5+1.0	8	61.5	PB	1.5+1.0	10	45.5	PB
2.0+0.1	7	36.4	YF	2.0+0.1	10	30.8	PF	2.0+0.1	12	27.8	PB
2.0+0.2	6	83.2	PN	2.0+0.2	9	39.2	PF	2.0+0.2	11	40.2	PB
2.0+0.5	5	100.0	PNC	2.0+0.5	8	63.1	PN	2.0+0.5	10	53.3	PN
2.0+0.8	4	100.0	PNC	2.0+0.8	7	85.7	PN	2.0+0.8	9	53.1	PN
2.0+1.0	4	73.1	PNC	2.0+1.0	7	77.3	PN	2.0+1.0	9	52.0	PN
2.5+0.1	8	35.2	YF	2.5+0.1	11	30.0	PB	2.5+0.1	13	27.5	PB
2.5+0.2	7	45.3	PN	2.5+0.2	10	44.2	PB	2.5+0.2	12	35.0	PB
2.5+0.5	6	80.4	PN	2.5+0.5	9	57.1	PN	2.5+0.5	11	50.3	PN
2.5+0.8	5	85.5	PN	2.5+0.8	8	77.5	PN	2.5+0.8	10	50.4	PN
2.5+1.0	5	64.8	PN	2.5+1.0	8	68.3	PN	2.5+1.0	10	48.5	PN
3.0+0.1	8	25.5	YF	3.0+0.1	11	24.2	YF	3.0+0.1	13	22.3	PB
3.0+0.2	7	36.6	PB	3.0+0.2	10	39.0	YF	3.0+0.2	12	30.1	YF
3.0+0.5	6	61.2	PB	3.0+0.5	9	51.4	PB	3.0+0.5	11	38.2	PB
3.0+0.8	5	72.3	PB	3.0+0.8	8	65.5	PB	3.0+0.8	10	37.0	PB
3.0+1.0	5	53.4	PB	3.0+1.0	8	54.8	PB	3.0+1.0	10	34.1	PB

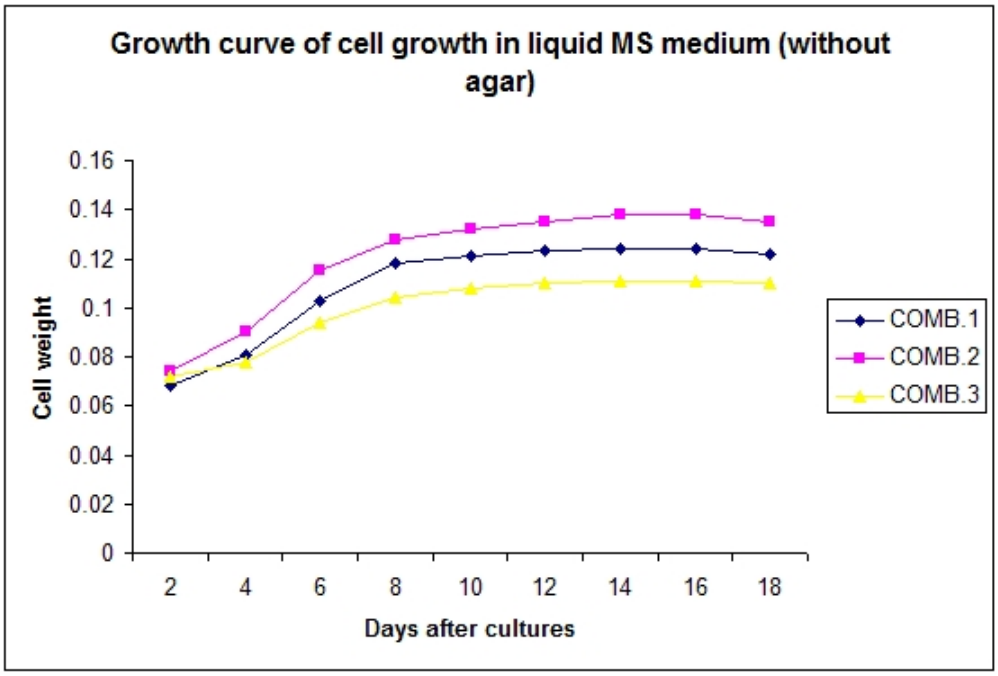
YF – yellow friable, BF – brown friable, YC – yellow compact, PB – pinkish brown, PN – pinkish nodular, PCN – pinkish compact nodular. BC – brown compact. PF – pinkish friable.

3.1.2 Cell culture

Rapidly proliferating friable embryonic calli (about 1-2 g fresh weight) growing in solid medium were considered as the better candidate for initiation of cell suspension culture in castor genotypes. Callus grown in solid media containing 2.0 mg/L BAP + 0.5 mg/L NAA were used to initiate cell suspension in liquid medium. Cell culture experiment was started in three separate lots in three 250 mL flasks in liquid MS medium (medium without agar) contained 2.0 mg/L BAP + 0.05 mg/L NAA, 2.0 mg/L BAP+ 0.2 mg/L NAA and 2.0 mg/L BAP+ 0.5 mg/L NAA. The flasks were placed on a rotary shaker (100 rpm) in dark and within 4 d cells in the liquid medium appeared distinctly visible. Cell weight was recorded nine times with every 2 d intervals. The summarized results are shown in the Table 2.

Table 2. Cell weight of *R. communis* L. cv. Roktima after different periods

Times	Combination of growth regulators											
	2.0 mg/L BAP + 0.05 mg/L NAA				2.0 mg/L BAP + 0.2 mg/L NAA				2.0 mg/L BAP + 0.5 mg/L NAA			
	Flask.1 (g)	Flask.2 (g)	Flask.3 (g)	Mean (g)	Flask.1 (g)	Flask.2 (g)	Flask.3 (g)	Mean (g)	Flask.1 (g)	Flask.2 (g)	Flask.3 (g)	Mean (g)
After 2 d	0.052	0.078	0.074	0.068	0.092	0.051	0.079	0.074	0.062	0.084	0.070	0.072
After 4 d	0.064	0.091	0.088	0.081	0.107	0.070	0.093	0.090	0.067	0.091	0.076	0.078
After 6 d	0.088	0.112	0.109	0.103	0.133	0.095	0.117	0.115	0.081	0.109	0.092	0.094
After 8 d	0.105	0.126	0.123	0.118	0.142	0.109	0.129	0.128	0.093	0.117	0.102	0.104
After 10 d	0.108	0.129	0.126	0.121	0.144	0.119	0.133	0.132	0.097	0.121	0.106	0.108
After 12 d	0.111	0.130	0.128	0.123	0.145	0.125	0.135	0.135	0.101	0.121	0.108	0.110
After 14 d	0.113	0.130	0.129	0.124	0.146	0.131	0.137	0.138	0.103	0.121	0.109	0.111
After 16 d	0.113	0.130	0.129	0.124	0.148	0.131	0.137	0.138	0.103	0.121	0.109	0.111
After 18 d	0.111	0.128	0.127	0.122	0.142	0.128	0.135	0.135	0.103	0.119	0.108	0.110



COMB.1= 2.0 mg/L BAP+0.05 mg/L NAA
COMB.2= 2.0 mg/L BAP+0.2mg/L NAA
COMB.3= 2.0 mg/L BAP+0.5 mg/L NAA

Fig. 1. Growth curve of cell growth / division in liquid MS medium (without agar)

Under this investigation cells were found to grow continuously in thee flasks with three media combinations of BAP and NAA. But when the cells were cultured on the MS media having 2.0 mg/L BAP + 0.2 mg/L NAA , the rate of cell growth found the highest than that of MS media having 2.0 mg/L BAP + 0.05 mg/L NAA and 2.0 mg/L BAP+ 0.5 mg/L NAA. But in all cases, the cells continued to grow until 14 d. The peak period of cell growth was observed from 4th d to 6th d. Cell weights were measured and the values were 0.081 g to 0.103 g, 0.090 g to 0.115 g and 0.078 g to 0.094 g in media combinations of 2.0 mg/L BAP+0.05 mg/L NAA, 2.0 mg/L BAP+0.2 mg/L NAA and 2.0 mg/L BAP + 0.5 mg/L NAA respectively. The mean values of three combinations were calculated and plotted in a graph and

presented in Fig. 1. The results presented in the graph indicated that three media combinations showed the similar trend of cell growth and their growths were found highest in between 4-6 d. After 14 d their growth found to be stationary in the artificial medium and under given environmental condition of the laboratory.

3.1.3 Antibacterial activities

All the cell extracts isolated from cell suspension culture taken from different periods of growth in *R. communis* L. cv Roktima were subjected for screening against five gram positive bacteria and six gram negative bacteria. It was clear from inhibition zones (Table 3) that most of the cell extracts were taken after different periods were effective against all bacteria. Under bacterial treatment, when 4 d old cell suspension culture was taken, the highest value of inhibition zone was 17 mm in diameter against *Bacillus halodurans* and the lowest value was 9 mm in diameter against *Sarcina lutea* against their corresponding standard Ciprofloxacin values were 30 mm and 29 mm respectively. When cell extracts taken from 6 d, 8 d, 10 d, 12 d, 14 d and 16 d old cell suspension cultures, the highest inhibition zones were 19 mm, 21 mm, 22 mm, 23 mm, 24 mm, and 23 mm in diameter respectively against *Bacillus halodurans* and the lowest values were 6 mm,7 mm, 8 mm, 8mm and 7 mm in diameter respectively against *Klebsiella species,* while the inhibition zones of the standard Ciprofloxacin in all cases were 30 mm and 25 mm respectively.

Table 3. Antibacterial activity of the cell extract isolated after different d of cell suspension culture of *R. communis* L. cv. Roktima (15 µL/disc) and standard ciprofloxacin (5µg/disc)

Test organisms	4 d	6 d	8 d	10 d	12 d	14 d	16 d	Cipro.
	Diameter of zone of inhibition (mm)							
Gram positive								
Sarcina lutea	9	10	11	12	13	14	13	29
Staphylococcus aureus	13	16	17	18	19	19	18	34
Bacillus megaterium	11	13	14	15	16	17	16	32
Bacillus subtillus	12	14	15	17	18	18	17	26
Bacillus halodurans	17	19	21	22	23	24	23	30
Gram negative								
Shigella sonnei	12	14	15	16	16	17	16	22
Klebsiella species	11	6	7	7	8	8	7	25
Proteus species	13	16	17	18	19	20	19	25
Escherichia coli	13	16	17	18	19	20	19	26
Pseudomonas aeruginosa	14	16	17	18	19	20	19	31
Salmonella typhi	13	15	16	17	18	18	17	22

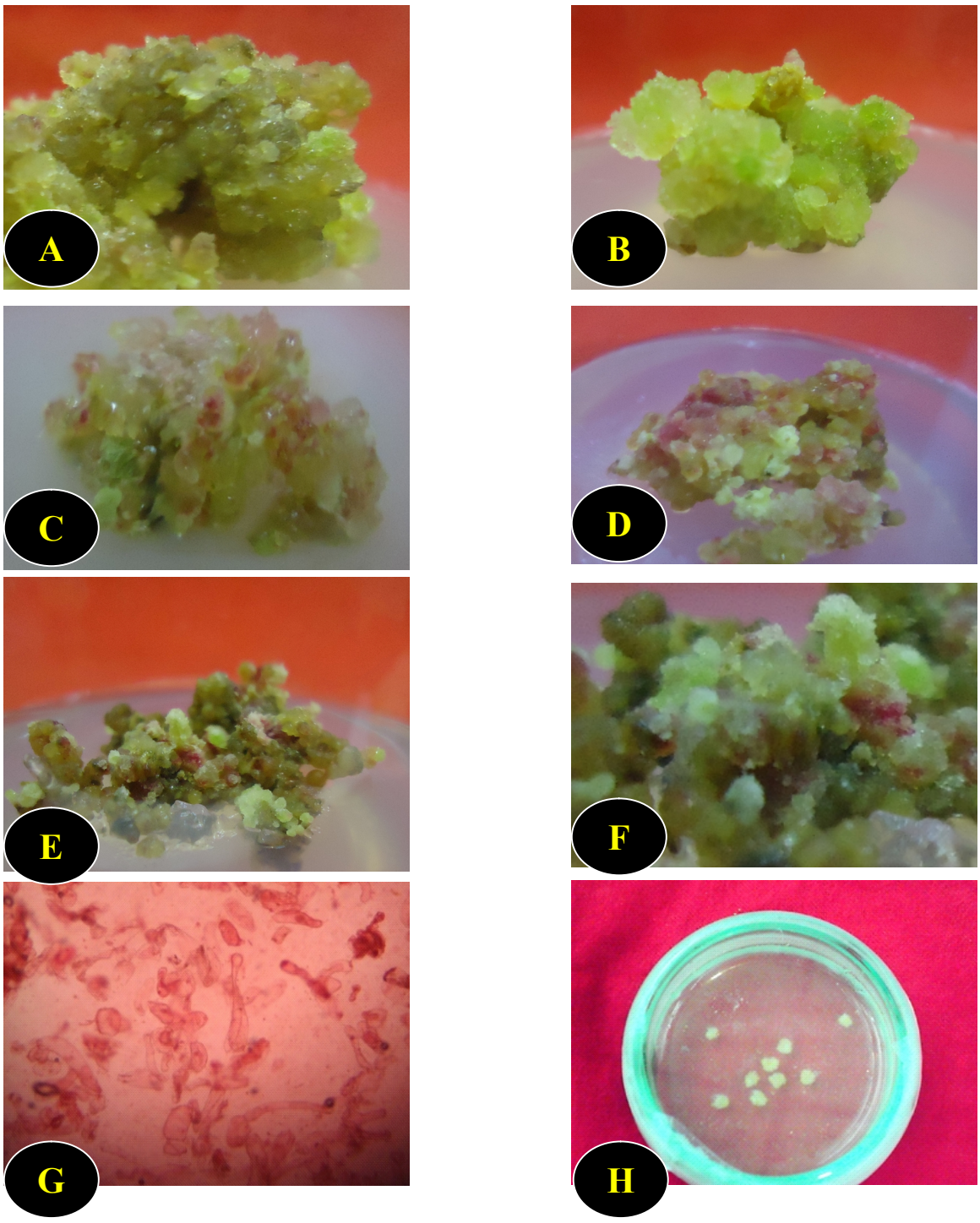


Fig. 2. A. Four weeks old yellow friable callus. B. Six weeks old yellow compact callus. C. Six weeks old pinkish brown callus. D. Six weeks old pinkish nodular callus. E. Six weeks old pinkish nodular compact callus. F. Seven weeks old pinkish nodular compact callus. G. Cell division. H. Callus induction from single cells

3.2 Discussion

Under the present investigation the calli were induced when explants were cultured on MS medium containing auxin (NAA) with cytokinin (BAP). The explant showed highest percentage (100%) of callus when NAA was used with BAP in MS medium supplemented with 2.0 mg/L BAP + 0.5 mg/L NAA and 2.0 mg/L BAP + 0.8 mg/L NAA. Similar results were also reported [25] in *R. communis* L. and obtained callus induction from cotyledon explants using combination of BAP 2.0 mg/L + 0.8 mg/L NAA. Similar results in *Melia azedarach* [26] was also observed in callus cultures using combination of BAP (4.4 µM) and NAA (0.46 µM) and successful regeneration of plantlets was obtained by using these callus cultures. [27] Combination of 2, 4-D and IAA found most effective for the callus culture in *R. communis. C*allus culture from roots, hypocotyl and cotyledonary leaves of *Ricinus communis* was reported [28] and tested for antibacterial activities by cell extract. Callus culture in *R. communis* from different varieties was obtained [29] and efficient regeneration protocol in *R. communis* has also been reported by other workers [30-31] but callus culture was not mentioned.

Under the present investigation in castor, cells were cultured on the MS media having 2.0 mg/L BAP + 0.2 mg/L NAA , the rate of cell growth found highest than that of MS media having 2.0 mg/L BAP + 0.05 mg/L NAA and 2.0 mg/L BAP+ 0.5 mg/L NAA. But in all cases, the cell continued to grow until 14 d. The peak period of cell growth was observed from 4^{th} d to 6^{th} d. Similar result was obtained in brinjal and cell growth showed the highest peak within 4-6 d of incubation [32]. Similar peak period of cell growth was obtained in *Abrus precatorius* where cells attained their highest peak within 6 – 8 d of growth [33]. Similar results was also reported in sugar beets, using different concentrations and combinations of BAP and 2,4-D. The growth patterns of cell suspension cultures were examined during a range of culture periods (0, 3, 5, 7, 9, 11, 13 and 15 d). In all lines, the growth rates of cells were initially slow but as the culture proceeded, they increased significantly and accumulated great amounts of biomass over a period of 15 d in Sugar Beet [34]. Plant cell growth and their growth measurement procedure were also very clearly described in PROTOCOL [35]. In some countries endeavours are being made to design the cell culture biorector for commercial production of cell product in laboratory [36]. In banana cell suspension growth is absolutely different from castor, in banana, most of the cultivars showed the highest performance within 60-70 d of culture in liquid medium and the cell growth became stationary after 80 d of culture [37]. The time required to establish the cell suspension culture varied greatly and depends on the tissue of the plant species and the medium composition. The use of fine suspension culture offers the opportunity to confirm single cell origin of somatic embryos and the asymmetry of the first cell division, which starts the process. The other advantage of cell suspension culture is the use of single embryogenic cells and somatic embryos as a source for direct gene transfer via particle bombardment, transit gene expression and confocal microscopy observation. Cell suspension cultures are particularly suitable for physiological, biochemical and molecular studies of the process of somatic embryogenesis and its different stages. The development of a genome and proteome database of model *Medicago truncatula* species together with the presence of protocol of cell suspension provided the opportunity to identify and characterize genes involved in the whole process of somatic embryogenesis [38]. From the foregoing discussion it may be concluded that the auxins NAA, 2,4-D and IAA have great role in callus induction but 2.0 mg/L BAP + 0.5 mg/L NAA and 2.0 mg/L BAP + 0.8 mg/L NAA proved to be most suitable combinations for induction of embryonic calli of *R. communis* L. cv. Roktima. Under this investigation NAA in combination with BAP proved to be suitable media composition for enhancing cell growth of *R. communis* L. cv. Roktima in artificial medium. The present experiments clearly demonstrated that in *R.*

communis L. cv. Roktima, cell begins to grow only within 4 d of culture proving its potential for developing cell culture industry for production of important toxic protein ricin as secondary metabolite. In regards of other medicinal plants *R. communis* L. cv. Roktima proved suitable plant species for developing cell suspension culture in artificial medium and the present findings open up a new potential venture for obtaining secondary metabolite ricin as a alternative source from its cell culture. For any drug development, antibacterial screening is obligatory and in the present investigation antibacterial screening against cell extract was performed and the results are shown in Table- 3. The extracts from cell suspension cultures, which were collected from different periods showed significant antibacterial activities. Especially the extract which was taken after 14 d from cell suspension culture showed the best result. Significant inhibitory activity was observed from callus culture extract of *Nigella* species [39]. Since variations in chemical composition of callus tissues and intact plants have been described for number of plant species [40-42]. According to the 2007 edition of the Guinness Book of world Records, the castor plant was mentioned as the most poisonous in the world [43]. The present investigation demonstrated that the extracts of cell suspension culture of *R. communis* L. cv. Roktima considered to be the potent source of antibacterial compounds and proved as an alternative source for production toxic compound ricin.

4. CONCLUSION

Isolation of secondary metabolites from cell culture offers a new alternative for drug development from medicinal plants that needs protocol establishment for cell culture and performance of antibacterial activity of the cell extract. In our investigation, we have developed the protocol for cell culture of an important toxic plant, castor, and we tested their antibacterial activity against gram positive and gram negative bacterial strains. A very significant antibacterial effect was observed in the cell extract of castor plant, indicating their potential for drug development against bacterial infection. Ricin in castor plant is a very toxic and medicinally important compound which is also postulated to be present in the cell extract of castor; therefore, it may be the active ingredient for bacterial death in our antibacterial test, though its content is to be determined. Further research is needed to ascertain the presence and content of ricin in callus and cell suspension culture in *R. communis*. Cell lines can be developed from the promising castor plant that could be alternative sources of having ricin from these cell lines in artificial medium.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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