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Comparative Antimicrobial Activity of Leaves and Callus of *Gymnema sylvestre* against Pathogenic Bacteria

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Plant tissue culture offers a new way to solve this problem to recover the loss of rare plants and also for production of bioactive components from cultured cells and tissues. In present study *Gymnema sylvestre* leaves and callus was compared for their antimicrobial potentials to assess the feasibility of cell and tissue culture to replace the plants use from natural resources. Callus was induced by using young as explants on MS medium supplemented with 2,4-D and BA. Various solvents s *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus hominis* and *Streptococcus mutans* were tested for antimicrobial activity. Methanolic extract of leaves showed antimicrobial activity against *Candida albicans*, *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* in agar well diffusion assay, but no activity against *Staphylococcus hominis* and *Streptococcus mutans*.

Key words: Callus, pathogenic bacteria, leaves extract.

The treatment of plant cells with biotic and abiotic elicitors has been a useful strategy to enhance secondary metabolite production in cell cultures (Karuppusamy, 2009). The physical (temperature, air, intensity, quality, color of light,) and chemical factors (complex compounds, heavy metals) are known as "elicitors". Elicitation is a process of inducing or enhancing synthesis of secondary metabolites by the plants to ensure their survival, persistence, and competitiveness (Kiong *et al*., 2005). The plants show wide range of chemical components, which make them useful for extraction and production of various pharmaceutical compounds. The *Gymnema sylvestre* is medicinal plant, climbs on bushes and trees in the Western Ghats in South India and to the west of those mountains in the territory around the coastal city of Goa. The primary chemical constituent's peresent in *Gymnema sylvestre* are gymnemic acid, tartaric acid, gurmarin, calcium oxalate, glucose, stigmasterol, betaine and choline. The *Gymnema sylvestre* is used in India and parts of Asia as a natural treatment for diabetes. Its active ingredient, gymnemic acid, can extracted from leaves and roots, which can lower and balance blood sugar levels. The *Gymnema sylvestre* can be used to treat asthma, corneal opacity, parkinsonism, hepatosplenomegaly, dyspepsia, constipation, jaundice, helminthiasis, cardiopathy and amenorrhoea. It is used for many conditions including diabetes, digestion, urinary tract problems, obesity, hypoglycemia, allergies, anemia, cholesterol and hyperactivity. The leaves are used for lowering serum cholesterol and triglycerides (Kuzuyama *et al.*, 2003). Keeping in view of medicinal properties of *Gymnema sylvestre*, our aim in the present study was to investigate the relative examination of antimicrobial activity of *Gymnema sylvestre* and callus produced by the leaves

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MATERIALS AND METHODS

Plant material, chemicals and pathogenic cultures

The seedling of *Gymnema sylvestre* used in the present study was procured from Tau Devi Lal Herbal Park, Yamunanagar, Haryana. All the chemicals and reagents were of high purity and analytical grade. The chemicals and reagents were procured from Hi-media and CDH. The pathogenic microbial cultures [*Candida albicans* (MTCC 3017), *Escherichia coli* (MTCC 43), *Pseudomonas aeruginosa* (MTCC 2295), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermidis* (MTCC 9041), *Staphylococcus hominis* (MTCC 4435) and *Streptococcus mutans* (MTCC 1943)] were procured from Institute of Microbiology and Technology (IMTECH) Chandigarh.

Callus induction

The Murashige & Skoog's (MS) basal medium of pH 5.8 was used and plant growth regulator such as (BA, 2, 4-D) was added prior to autoclaving, because of their heat stable property. The sterilization of *G. sylvestre* leave explants was done by washing with Tween 20 several times to remove the adhered contaminants and 70% v/v ethanol for 1-2 minutes followed by washing with autoclaved distilled water. After complete removal of ethanol, explants were washed with 0.2% mercuric chloride solution for 2-3 minutes followed by 4-5 times washing with autoclaved distilled water. The sterilized explants were transferred to MS medium containing, auxins such as 2, 4-D and cytokinins such as BA in 0.5 to 3.0 mg/l under aseptic conditions for callus induction. The callus was further subcultured for mass proliferation on media having different composition of BAP and 2, 4-D after 2 weeks.

Antimicrobial activity of callus and leaves

The 5 g dried leaves powder of *Gymnema sylvestre* was soaked in 20 ml of solvents for 72 hours. The callus was taken and pressed on filter paper to remove the excess moisture and dried in hot air oven at 40º C for 24 hours. The dried callus was grinded to fine powder and 5g of callus powder soaked in 20 ml of solvent for 72 hours The filtrate of leaves and callus powder was evaporated at 45-50º C in water bath and dissolved in DMSO and stored at 4 ºC for further study. The antimicrobial activities of plant leaves and callus were evaluated by agar well diffusion assay (Pereze *et al*., 1990) on Mueller Hinton Agar (MHA) by using leaf and callus extract. The DMSO was used as negative control whereas Ciprofloxacin and Ketokanozole were used as positive controls.

Effect of plant growth regulators, sucrose and callus age on antimicrobial activity

The effect sucrose, PGRs on *Gymnema sylvestre* callus on antimicrobial activity was checked by callus formation at different concentration of sucrose (1.0 %, 1.5 %, 2.0 %, 2.5 %, and 3.0 %), different concentration of PGRs (0.25 mg/L, 0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 3.0 mg/L). The effect of callus age (20, 25, 30, 35, 40, 45, 50 days) on antimicrobial activity was checkek. The callus were taken out; dried, grinded and methanolic extracts were prepared which were used for antimicrobial assay. The zones of inhibition of different callus were observed.

Effects of various elicitors on growth, morphology of callus and its antimicrobial activity:

The production of more metabolite, morphology and growth in callus was checked by using various elicitors such as sodium chloride, yeast extract and heavy metals (cadmium chloride, cobalt chloride, and mercuric chloride). The sodium chloride, yeast extracts and heavy metal salts concentration was (0.1 % to 1%. And 0.001 % to 0.005 %) respectively. These elicitors were mixed with medium containing plant growth regulators and green callus were transferred to medium containing elicitors. The callus was subculture to same medium for mass production. After sufficient mass production of callus, was dried and extract in selected solvent and tested by agar well diffusion assay

Antioxidant activity

The leaves extract, callus extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80 and 100 µg/ml were added to 3 ml of 0.004% methanol solution of DPPH for 30 minutes in the dark and absorbance was recorded at 517 nm, methanol and DPPH act as control. The percentage inhibition activity was calculated from [(A0–A1)/A0] ×100, where A0 was the absorbance of the control and A1 was the absorbance of the extract/standard. The antioxidant activity of the extract was expressed as IC50 (in µg/ml) that inhibited the formation of DPPH radicals by 50 %. All the tests were performed five times and the graph was plotted with the average of five observations (Kumaran, 2007).

RESULTS

Callus induction and proliferation

The callus was induced on MS basal medium atdifferent concentration and combinations of auxins such as 2, 4-D, and cytokinins such as BA as shown in Table 1. The minimum days of callus initiation was observed on MSD4 supplemented with 2, 4-D (2.0 mg/L) and BA (0.5 mg/L), while in other medium it took 23-38 days. After initiation it started rapidly proliferating in MS media and developed green mass with in 25 days. The higher concentration of 2,4-D in MS medium developed fragile and green callus, while higher concentration of BA in MS medium developed compact and bright green callus in MSD9. No callus was observed on control, which was not supplemented with any of plant growth regulator.

Comparative analysis of antimicrobial activity of callus and leaves extract

The antimicrobial activity of leaves extract against pathogenic strain is shown in Table 2. The methanolic extract of leaves showed maximum zone inhibition diameters of 27 mm against *Candida albicans*, 23 mm for *Escherichia coli*, 35 mm for *Pseudomonas aeruginosa*), 22 mm for *Staphylococcus aureus*, 23 mm for *Staphylococcus*, but no activity was observed against *Staphylococcus hominis*) and *Streptococcus*. The methanolic extracts of leaves showed more antimicrobial activity as compare to chloroform. The DMSO was act as negative control in which, no inhibition zone was observed. The ciprofloxacin (0.1 mg/ml) and fuconazole (0.1 mg/ml) act as positive control. The antimicrobial activity of callus and leaves extract against pathogenic strain is shown in Table 3. The

Table 1. Callus initiation in MS medium with different concentration of 2, 4-D and BA

Medium Code	2,4-D (mg/L)	BA (mg/L)	Days of callusinitiation
MSD1	0.25	0.5	38
MSD2	0.50	0.5	28
MSD3	1.0	0.5	25
MSD4	2.0	0.5	18
MSD5	3.0	0.5	23
MSD6	0.5	0.25	28
MSD7	0.5	1.0	23
MSD8	0.5	1.50	23
MSD9	0.5	2.0	24
MSD10	0.5	3.0	25
Control	-	-	No callus

Table 2. The antimicrobial activity (mm) of *Gymnema sylvestre* leaf extract against pathogenic strains

Solvent	*Candida albicans*	*E.coli*	*P.aeruginosa*	*S.aureus*	*S.epidermidis*	*S.hominis*	*S.mutans*
Methanol	27	23	35	22	23	-	-
Dichloro methane	20	21	27	18	20	-	-
Distilled water	15	15	20	18	13	-	-
Ethanol	18	18	28	21	18	-	-
Ethyl acetate	17	22	28	19	18	-	-
Petroleum ether	19	20	17	16	14	-	23
Propanol	25	21	22	22	18	-	21
Chloroform	23	23	20	20	18	-	23
Benzene	23	23	21	17	16	-	19
Hexane	22	21	18	16	13	-	23
(+) control	16	20	15	22	23	16	20
(-) control	-	-	-	-	-	-	-

methanolic extract of callus showed inhibition zones diameters of 16 mm against *Candida albicans*, 15 mm for *Escherichia coli*, 22 mm for *Pseudomonas aeruginosa*, 16 mm for *Staphylococcus aureus*, 17 mm for *Staphylococcus epidermidis* but no activity was observed against *Staphylococcus hominis* and *Streptococcus mutans*. The methanolic extract of callus showed better antimicrobial activity as compare to chloroform.

Effect of callus age and PGRs and sucrose on antimicrobial activity

The callus of 35 days showed maximum inhibition zone of (18, 19, 28, 17 and 20 mm) for (*Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) respectively, but no activity was observed against *Staphylococcus hominis* and *Streptococcus mutans* as shown in Table 4. The green callus turned into dark brown mass in 45-50 days. The antimicrobial activity of callus start decreasing, after 50 days (12, 12, 19, 11 and 13 mm) for (*Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) respectively, but no activity was observed against *Staphylococcus hominis* and *Streptococcus mutans*. The antimicrobial activity was not affected by different concentration of PGR. The effect of different concenteration of sucrose on callus morphology and growth is shown in Table 5. In media containing sucrose concentration more than 3% callus was unable to grow. The antimicrobial activity of callus was affected by sucrose concentration in MS medium. The antimicrobial activity was not shown, when callus proliferated was on medium containing 1% sucrose. The antimicrobial activity increased with increase in concentration of sucrose in medium. The inhibition zone diameters were best measured at sucrose concentration 3% in MS medium. The antimicrobial activity (inhibition zones) of callus (methanolic extract) on MS medium containing 3% sucrose was measured (18, 19, 28,

Table 3. The antimicrobial activity (mm) of *Gymnema sylvestre* callus extract against pathogenic strains

Solvent	*Candida albicans*	*E.coli*	*P.aeruginosa*	*S.aureus*	*S.epidermidis*	*S.hominis*	*S.mutans*
Methanol	16	15	22	16	17	0	0
Chloroform	17	14	18	16	13	0	14

Table 4. The inhibition zone (mm) of methanolic extract of *G. sylvestre* callus against pathogenic strains

Callus Age	*C. albicans*	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
20 day	10	12	17	12	11
25 day	13	13	20	13	13
30 day	16	15	22	16	17
35 day	18	19	28	17	20
40 day	17	19	25	14	19
45 day	16	15	22	16	17
50 day	12	12	19	11	13

Table 5. Growth and morphology callus supplemented with different sucrose concentration

Medium	Sucrose (%)	Growth	Morphology
MSS1	1.0	+	White, very fragile
MSS2	1.5	+	Light brown, very fragile
MSS3 MSS4 MSS5	2.0, 2.5, 3.0	++	Green, Fragile

17 and 19 mm) for (*Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) respectively, but there was no activity against *Staphylococcus hominis* and *Streptococcus mutans*.

Effect of yeast extract, NaCl, and heavy metals on growth, morphology of callus and its antimicrobial activity

The callus was developed on MSD4 medium containing 2, 4-D (2mg/L) and BA (0.5mg/L) and transferred aseptically to medium containing yeast extract. The callus blackness was observed in medium containing yeast extract at initial stages, within 10-15days new callus mass started proliferating from this blackened callus. The callus produced in YE1 medium was light green and compact as shown in Table 6. In medium (YE3, YE2, YE3 and YE4) green and compact callus was develop. The increase in concentration of yeast extracts callus become dark and growth was decrease. The yeast extract supplementation in MS media positively affects the antimicrobial activity of *Gymnema sylvestre* callus. The 0.8 % yeast extract showed inhibition zones of (26, 22, 32, 20 and 22 mm) for *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* respectively, but no activity was observed against *Staphylococcus hominis* and *Streptococcus mutans*. The 1% concentration of yeast extract was not found to increase the antimicrobial activity as

Table 6. The growth and morphology of *Gymnema sylvestre* callus at different concentration of yeast extracts in MS Medium

MediumCode	Yeast Extract	Callus Growth	Callus morphology
YE1, YE2, YE3	0.1, 0.2, 0.3 %	+++	Green Callus
YE4, YE5	0.4, 0.5 %	++	Dark Callus
YE6, YE7	0.6, 0.7 %	+	Dark Green
YE8, YE9, YE10	0.8, 0.9, 1.0 %	+	Greyish Green

Table 7. The inhibition zone (mm) of methanolic extract of *G. sylvestre* callus proliferated on different concentrations of yeast extract, against various pathogenic strains

Mediumcode	*C.albicans*	*E.coli*	*P.aeruginosa*	*S.aureus*	*S.epidermidis*
YE1	17	16	28	17	18
YE2	17	16	28	18	18
YE3	18	19	29	18	19
YE4	20	19	29	19	19
YE5	21	19	30	19	20
YE6	24	20	30	19	20
YE7	24	20	31	19	20
YE8	26	22	32	20	22
YE9	26	22	32	20	22
YE10	26	22	32	20	22

Table 8. The growth and morphology of *Gymnema sylvestre* callus proliferated on different concentration of NaCl in MS Medium

Mediumcode	NaCl(%)	Callus Growth	Callus morphology
NC1, NC2, NC3, NC4, NC5	0.1, 0.2, 0.3, 0.4, 0.5 %	+	green, compact
NC6	0.6 %	_	callus brown within 7-14 days
NC7	0.7 %	_	callus brown within 7-10 days
NC8, NC9 NC10	0.8, 0.9 and 1.0 %	_	callus brown within 5-7 days

shown in Table 7. The callus was developed on MSD4 supplemented with 2, 4-D (2 mg/L) and BA (0.5 mg/L). After sufficient growth of callus on MSD4 medium it was transferred aseptically to medium containing NaCl. The bright green and compact callus proliferated on NC1 and NC2 as shown in Table 8. The green and compact callus developed on NC5. The callus developed on NC1 to NC10 showed different morphology. The callus on medium NC6 to NC10 turned brown within 10-15 days and no new callus developed from those. The antimicrobial activity of callus proliferated on different concentrations of NaCl in MS medium (methanolic extract) was observed, no significant variation in zone observed. The effect of various concentrations of heavy metals on callus growth and morphology is shown in Table 9. Callus was initiated and developed on MSD4 containing 2, 4-D (2mg/L) and BA (0.5mg/L). The MS medium contain 0.004% cadmium chloride showed maximum inhibition zones (24, 21, 30, 19, and 21mm) for (*Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively, but no antimicrobial activity was observed against *Staphylococcus hominis* and *Streptococcus mutans* as shown in Table 10. The increase in concentration of cadmium chloride in

Table 9. The growth and morphology of *Gymnema sylvestre* callus proliferated on different concentrations of heavy metal salts in MS Medium

Mediumcode	Heavy Metal (%)	Callus growth	CallusMorphology
CD1, CD2, CD3 and CD4	Cadmium chloride (0.001, 0.002, 0.003 and 0.004 %)	+	Brownish green and fragile
CD5	Cadmium chloride (0.005 %)	_	Browning
CB1, CB2 and CB3	Cobalt chloride (0.001, 0.002 and 0.001 %)	+	Pale green, Less compact
CB4 and CB5	Cobalt chloride (0.004 , 0.005 %)	_	Browning
MC1, MC2 and MC3	Mercuric chloride (0.001%)	+	Brown, Fragile
MC4 and MC5	Mercuric chloride (0.004, 0.005 %)	_	Browning

Table 10. The inhibition zone (mm) of *G. sylvestre* callus (methanolic extract) proliferated on different concentration of cadmium chloride in MS medium against pathogenic strains

Cadmium Chloride (%)	*C.albicans*	*E.coli*	*P.aeruginosa*	*S.aureus*	*S.epidermidis*
0.001%	18	16	28	16	17
0.002%	21	19	29	18	19
0.003%	23	19	29	19	19
0.004%	24	21	30	19	21

MS medium was found toxic and callus failed to grow. The presence of mercuric chloride and cobalt chloride in MS medium was not increase antimicrobial activity of callus.

Antioxidant activity of leaves and callus extract

The maximum scavenging activity (56.675%) was observed at 100 µg/ml is shown in Fig.1. The IC_{50} value of *Gymnema* leaves extract was found 60.28µg/ml. The DPPH radicals due to the scavenging ability of *Gymnema* callus extract. The maximum DPPH scavenging activity (54.589%) of *Gymnema* callus was observed at 100 µg/ml concentration. The IC_{50} value of *Gymnema* callus extract was observed 78.28 µg/ml.

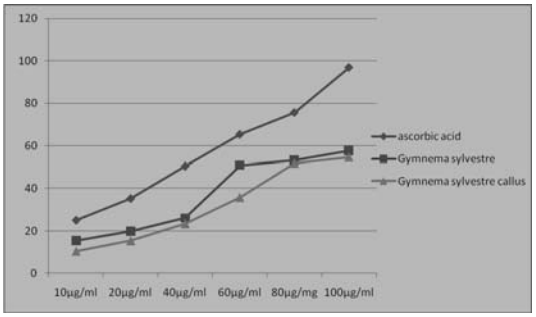

Fig. 1. The DPPH radical scavenging activity of ascorbic acid leaves and callus extract

DISCUSSION

Gymnema sylvestre commonly known as "Gurmar" (destroyer of sugar) and it is well-known to the Indian people since ancient days as a source of antidiabetic drugs. In recent years, it became one of the better known names in the world of herbal medicine. It is a rich source of many bioactive secondary compounds such as gymnemic acid (GA-I-X) quercitol, lupeol, ²-amyrin, stigmasterol, gymnemin, gymnemagenin, gurmarin, etc. which are mainly effective in lowering of blood sugar. Apart from this, getting seeds from the plant is difficult and moreover the chance of getting a disease free plant is less. Also, in order to obtain bioactive compounds, one need not regenerate several complete plants (Jha and Ghosh, 2005). *In vitro* propagation medicinal plants are found to be highly useful for commercial production of medicinally important compounds. Extracts from pathogen free cell culture if generated *in vitro* will prove beneficial. So, in present study callus was used to assess its antimicrobial potential and an attempt to increase its activity using various elicitors for production of antimicrobial metabolite in callus. The increased use of plant cell culture systems can improve understanding of the secondary metabolite pathway in economically important plants. Advances in plant cell cultures could provide new means for the cost-effective, commercial production of even rare or exotic plants, their cells, and the chemicals that they will produce (Vanishree *et al.,* 2004).

The callus induction and proliferation of many important medicinal plant species has been done by using Murashige and Skoog medium. The rate of callus induction and proliferation is affected by many factors including nature of explants, composition of medium used for callus induction and proliferation. Callus induction needed the presence of auxins or cytokinins or both in the nutrient media depending on the source of explants. In present investigation, the callus induction from leaf in minimum time was observed in MSD4 medium supplemented with 2, 4-D (2.0 mg/L) and BA (0.5 mg/L). The increase in concentration of 2, 4-D produced fragile callus. The increase in concentration of BA produced compact callus. Gopi and Vatsala (2006) observed callus induction in 0.5 mg/l of 2, 4-D supplemented medium for leaf. At the initial stage, some parts of explants enlarged and gave raise to pale yellowish callus after 2-3 weeks of incubation. Roy *et al.,* (2008) reported that the best callus initiation was observed with 2, 4-D (1 – 5 mg/L) in combination with KIN (1 mg l/L) that showed fluorescent green friable callus in 20 - 25 days. In *Gymnema sylvestre* callus induction from leaf observed within 18 days on MSD4. Roy *et al.,* (2010) reported antimicrobial potential of *Gymnema Sylvestre* leaves. Antibacterial activities of different extracts were evaluated by paper disc diffusion method. Sinha *et al.*, (2010) reported antibacterial activity of petroleum ether, chloroform and ethanol extracts of *Gymnema sylvestre* against three Gram positive bacteria and five Gram negative bacteria. Devi *et al*. (2010) reported antibacterial activity of *Gymnema sylvestre* extracts using petroleum ether, methanol and chloroform by solvent extraction techniques then screened for antimicrobial activity by agar well diffusion method against *Streptococcus mutans, Staphylococcus aureus*, *Streptococcus mitis* and *Candida albicans*. The methanol extract showed strong antimicrobial activity. The antimicrobial activity of leaf extracts of *Gymnema sylvestre* was done by (Wani *et al*., 2012; Jyothi and Rao 2012; Murugan *et al*., 2012). Antimicrobial activity of various extracts of *Gymnema sylvestre* leaves was done by agar well diffusion assay in present study. Methanolic extract showed good results with maximum diameter of inhibition zones. Hence present work favored investigations of Jyothi and Rao (2012) and Wani *et al*., (2012). Methanol extract showed inhibition zones of 27 mm for *Candida albicans*, 23 mm for *Escherichia coli*, 35mm for *Pseudomonas aeruginosa*, 21 for *Staphylococcus aureus*, 23mm for *Staphylococcus epidermidis*, but no activity against *Staphylococcus hominis* and *Streptococcus mutans*. In our study, methanolic extracts showed antimicrobial activity by agar well diffusion method against *Streptococcus mutans*. *Gymnema sylvestre* methanolic extract of callus showed inhibition zones against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, for *Staphylococcus epidermidis*, but no activity against *Staphylococcus hominis* and *Streptococcus mutans*. It was clear that callus has less antimicrobial potential than leaves of intact plant.

The PGRs, sucrose concentration and age of callus was found to affect the growth of callus. So, it was necessary to study their effects on production of desired compound in callus culture. The antimicrobial potential of callus age was affected by age and sucrose concentration in medium. The MS medium with 3% sucrose concentration showed maximum antimicrobial potential. PGRs used in present study (BA and 2, 4-D) in MS medium showed no effect on production of desired compound in callus culture. The callus below 30 days showed very less accumulation of bioactive compound. The callus of 35 days showed maximum accumulation of bioactive compound, activity decreased after 40-45 days and completely lost after 90 days. In the present study yeast extract, NaCl and heavy metal were used to investigate their effect on callus growth and its antimicrobial activity. The cadmium chloride and yeast extract supplementation in MS medium increased antimicrobial activity of callus. The Yeast extract was found to increase production of secondary metabolite in callus. Veerashree (2012) reported increased production of gymnemic acid by yeast extracts supplementation in the medium. The maximum increase in activity was observed at 0.8% yeast extract, further increase of yeast extract failed to alter antimicrobial activity of callus. The 0.004 % cadmium chloride showed maximum antimicrobial activity of callus but it decreased the growth of callus. The increase in callus secondary metabolite due to cadmium chloride was reported Bhuwaneshwari *et al.,* (2012). The NaCl addition in MS medium decreased the growth of callus. Further research work is needed for identification, isolation and purification of antimicrobial secondary metabolite present in leaves and callus of *Gymnema sylvestre.* Quantification of elicitor-induced accumulation of responsible secondary metabolite in callus culture is also needed to interpret best elicitor for maximum increase in production of responsible secondary metabolite in callus culture.

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