International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

DIRECT ROOT REGENERATION AND INDIRECT ORGANOGENESIS IN *SILYBUM MARIANUM* AND PRELIMINARY PHYTOCHEMICAL, ANTIBACTERIAL STUDIES OF ITS CALLUS

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ABSTRACT

The morphogenic potential and free-radical scavenging activity of the medicinal plant, Silybum marianum L. (milk thistle) were investigated. Direct root regeneration and indirect organogenesis and preliminary phytochemical, antibacterial studies of its callus. From leaf explants the highly significance result was found on the NAA at 2 mg/l and KIN at 0.2 mg/l for the direct root induction and indirect organogenesis the 100% callus induction was obtained from leaf explants on 2, 4-D at 2.5 mg/l),and The 100% shoot initiation was obtained from NAA at 2 mg/l and the BAP at 1.5 mg/l and then it was transferred to the shoot elongation medium bearing the hormone concentration of GA3 at 2 mg/l. Finally, it was transferred to root induction medium bearing the hormone concentration of NAA at 2mg/l. the Preliminary Phytochemical showed the Sugar, Saponins, Amino acids, Tannins were occur in the chloroform and ethanol extracts of leaf and hypocotyl callus. More antimicrobial inhibitory activity was recorded for the gram-positive than the gram negative in all extract.

Keywords: Preliminary Phytochemical Screening, Antimicrobial activity, *Silybum Marianum*, Indirect Organogenesis, Direct Root Regeneration.

INTRODUCTION

Plant tissue culture, an important tool to study different aspects of genetic engineering, has been widely used in fundamental and applied fields. It has been proven to be useful in propagation of medicinal plants under controlled conditions. The availability of efficient and long-term plant regeneration systems from callus culture is of prime importance in the application of culture for crop improvement. *In vitro* morphological response in plants is influenced by various factors including initial types of explants such as seed, apical bud, node and internodes, leaf, anther and pollen under culture conditions, genotype and nutrient media composition. These factors modulate cellular differentiation and morphological development using various plant growth regulators to produce callus from any part of the plant. Regeneration of plants through *in vitro* studies is mainly of organogenesis and somatic embryogenesis. Plant tissue culture helps to study somaclonal variations in plants. In *vitro* plants, enhancing precursors of secondary metabolites is to increase the production and amount of particular drug.

***Callus production*:** Callus tissue means it is an unorganized proliferate mass of cells. Callus can be initiated *in vitro* by placing small pieces of the whole plant (explants) onto a growth supporting medium under sterile conditions under the stimulus of endogenous growth substances or regulating chemicals added into the medium. The metabolism of cells which were in a quiescent state is changed, and they begin active division. During this process, cell differentiation and speculation, which may be occurring in the intact plant, is reversed, and the explants give rise to a new tissue which is composed of meristematic and unspecialized cell types. Generally, the callus is of two types, friable and non-friable. Like that, non- friable or nodular callus is

more useful to induce the multiple shoot through organogenesis. The formation of the callus in both friable callus and non-friable depends upon the explants source and the nutrient medium. The morphogenetic response of the explants is greatly influenced by the medium employed for callus induction.[1]

***Phytochemical Analysis*:** The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. Qualitative analysis involves determining the nature of a pure unknown compound or the compounds present in a mixture. Various chemical tests exist for different elements or types of compounds, and systematic analytical procedures of known compounds in a mixture. Quantitative analysis involves measuring the proportions of known components in a mixture. In addition, there are numerous physical methods of qualitative and quantitative analysis, including spectroscopic techniques, mass spectrometry, paleography, different chromatographic techniques, activation analysis, etc. [2]

***Antimicrobial activity*:** Plants have long held a leading place as a source of medicinal drugs, recants, of their use dating as far as 4000 B.C. for centuries. Plants have been in use in various parts of the world for treatment of certain diseases. An antimicrobial is a substance that kills or inhibits the growth of a microorganism such as bacterium, fungus, protozoan, etc..

The increasing prevalence of multidrug resistant strains of bacteria, recent appearance of strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics has raised the specter of "untreatable raised" bacterial infections and adds urgency to the search for new infection fighting strategies. In contrast to synthetic drugs, antimicrobials of plant origin usually are not associated with many side-effects and have an enormous anti-infective potential in numerous infectious diseases. Antibiotics are generally used to treat bacterial infections. The toxicity to humans and other animals from antibiotics is generally considered to be low. However, over the past few decades, these health benefits are under threat as many antibiotics have become less and less effective against certain illness. Many of them not only produce toxic reaction but also facilitate emergence of drug-resistant bacteria. Against this background, it is proposed to carry out the following objectives in Milk Thistle, *Silybum marianum*:

MATERIALS AND METHODS

Plant material: *Silybum marianum* (L.) Gaertn. belongs to the family Asteraceae, is also known as Milk Thistle because the leaves of the plant have white veins that look as if milk were spilled upon them, which, according to legend, is the milk of the Virgin Mary. It is a unique herb which contains a natural compound called silymarin.

Culture Medium: Medium was used in the study. Depending upon the culture, different growth regulators were added to the medium. [3] The composition of MS medium (readymade: Product code: PT100).

Preparation of Solid Medium: Suspended 42.41 g of dehydrated medium in 600 ml of distilled water and rinsed media vial with small quantity of distilled water to remove traces of powder. Added desired heat stable supplements prior to autoclaving. Adjusted the medium pH 5.8 using 1N/HCL/1N NaOH/IN KOH and made up to final volume to 1000 ml with distilled water. Boiled the medium to dissolve agar completely and sterilized the medium by autoclaving at 121ºC for 15 minutes.

Growth Regulators: The following growth regulators were used at various concentrations: (Auxins -2, 4-Dichlorophenoxy acetic acid, Naphthalene acetic acid, Indole 3- acetic acid) and (Cytokinins- Benzl aminopurine, Kinetin).

Surface Sterilization

Treatment 1: The explants were washed under the running tap water for 30 min. Then one drop of detergent solution (teepol) was added and kept under the running tap water for 5 min. Then, the explants were washed with distilled water 4 times.

Treatment 2: Under the laminar air flow chamber the explants were sterilized with 70% alcohol for 1 min and washed with sterile distilled water for 3 times. Then 0.1% of mercuric chloride for 4 min and washed with sterile distilled water for 3 times.

Inoculation 1: Before starting inoculation the surface of the chamber was wiped with 95% alcohol and then the door was tightly closed and UV light was switched on for 15 min. After 15 min, all the equipments and materials (sterilized forceps and petri plates, sterile blade, sterile distilled water and sprit

lamp) were transferred to laminar air flow chamber and the door was tightly closed and UV light was switched on for 10 min. The equipments used for aseptic manipulation like forceps, scalpels were sterilized by dipping in 95% alcohol followed by flaming and cooling. Then, the explants were inoculated on the medium. Before starting inoculation, hands were surface-sterilized with 95% alcohol and the inoculation was carried out under the vicinity of the flame. After inoculation, the culture bottles were transferred to the culture room.

Culture conditions: All cultures were maintained in a growth room at 25 ± 1ºC under a 16 h photoperiod with a light intensity provided by cool-white fluorescent tube lights.

Aseptic seed germination: The seeds received for identification purposes were used for experimental work. *In vitro* raised seedlings were used as the source of explants. To raise the seedlings under aseptic condition, seeds were first completely surface sterilized. The disinfected seeds were inoculated into the basal MS medium. After germination, healthy and vigorously grown seedlings were selected and used as the source of explants.

Preparation of Explants: Leaves and roots from 25-30 days old *in vitro* plants were used as explants to callus induction.

Direct root regeneration: The leaflets of initial raised explants were cut into small pieces (2-4 mm) using sterile blade and were cultured on MS solid medium containing 3.0% (w/v) sucrose, 0.8% (w/v) agar along with combination of NAA (2 mg/l) and KIN (0.1 - 2.0 mg/l) as standard for root induction.

Indirect Organogenesis

Initiation, Proliferation and Selection of Organogenic Callus: The initial raised (leaflets) and (hypocotyl) explants were cut into small pieces (2-4 mm) using sterile blade and were cultured on MS solid medium containing 3.0% (w/v) sucrose, 0.8% agar (w/v) along with 2,4D (1.0 – 4.5 mg/l) for callus induction. The callus was transferred to MS supplemented with 2,4-D for proliferation. The culture was maintained on MS medium with same concentration up to 4 weeks interval.

Adventitious Shoot Proliferation from the Callus: Fresh organogenic callus obtained from hypocotyl were transferred to shoot initiation medium. The plant growth regulators, in particularly, the auxin and cytokinin were tested to induce the adventitious shoot from the callus cultures. The individual effect of NAA (0.2 - 2.0 mg/l) and BAP (1.5 mg/l) was tested for proliferation of shoot induction.

Phytochemical Screening, *Sample Preparation:* About 300 mg of *in vitro* callus (leaf and hypocotyl callus) material taken from 2,4-D medium. The material was extracted with the help of mortar and pestle using different solvents such as chloroform and ethanol. The dried substance was dissolved in suitable solvents and stored in cold room for future use.[4]

Preliminary Phytochemical Screening: The solvent extracts were subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present in them.[5] Steroids: A 3 ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Purple color thus formed changes into blue or green color indicating the presence of steroids. Triterpenoids: A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids. Reducing Sugars: A 3 ml of test solution was added with a 2 ml of Fehling's reagent and 2 ml of water. Formation of reddish orange color indicates the presence of reducing sugar. Sugars: A 3 ml of the test solution was added with very small quantity of anthrone reagent and a few drops of concentrated H_2SO_4 and heated. Formation of green or purple color indicates the presence of sugars. Alkaloids: A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids. Phenols: A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols. Flavonoids: A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids. Saponins: A 3 ml of test solution was added with H_2O and shacked. Formation of foamy lather indicates the presence of Saponins. Tannins: A 3 ml of test solution was added with H_2O and lead acetate. Formation of white precipitate indicates the presence of tannins. Anthroquinones: A 3 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of anthroquinones. Amino Acids: A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet

color indicates the presence of amino acids. Catechins: A 3 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink color indicate the presence of catechins.

Determination of Antimicrobial Activity

Microbial Strains: The extracts were tested for the antimicrobial activity. The microbial strains employed in the biological assays were Gram-Positive bacteria: *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435); Gram-Negative bacteria: *Aeromonas hydrophila* (MTCC 646), *Escherichia coli* (MTCC 724), *Klebsiella pneumoniae* (MTCC 432), *Proteus mirabilis* (MTCC 425), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 741), *Salmonella* paratyphi (MTCC 735), *Salmonella typhi* (MTCC 733). Fungal Strains: *Aspergillus flavus* (MTCC 277), *Aspergillus fumigatus* (MTCC 343), *Aspergillus niger* (MTCC 1344), *Candida albicans* (MTCC 227), *Microsporum canis* (MTCC 2820), *Microsporum gypseum* (MTCC 2819), *Trichophyton rubrum* (MTCC 296), obtained from Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh.

Sample Preparation*:* About 200 mg of *in vitro* callus (leaf and hypocotyl callus) material taken from 2, 4-D medium. The material was extracted with the help of mortar and pestle using different solvents such as chloroform and ethanol.

Inoculation*:* Inoculums of each bacterial strain were suspended in nutrient broth and incubated for 8 h at 37°C. Agar Well-Diffusion Assay: Agar well-diffusion method was followed.[6] Mueller-Hinton Agar (MHA) plates were swabbed (sterile cotton swabs) with 8 -12 h old broth cultures of the respective bacteria. Sterile circular steel was used to make wells, each measuring 8 mm diameter, in each of the plates. About 0.3 ml each of 100% and 50% aqueous extracts and different concentrations of the solvent extracts were added into the wells using micropipettes and allowed for diffusion at room temperature for 2 h. The plates were incubated at 37°C for 24 h. The solvent without extracts served as negative control. Standard antibiotics of ampicillin-10µ/disc, erythromycin-10µ/disc, kanamycin-30µ/disc, methicillin-5µ/disc, Nalidixic acid-30µ/disc, rifampicin-30µ/disc, and trimethoprin-10µ/disc were used as positive controls. After 24 h of incubation, diameter of the inhibition zone was recorded in mm. The experiment was repeated thrice and the average values were calculated for antibacterial activity.

RESULTS

Direct Root Regeneration from Leaves: Leaf explants of *Silybum marianum* were tested for root induction medium along with growth regulators such as NAA and KIN at the ranges of KIN (0.1-2 mg/l) and NAA (2 mg/l) for standard. The best result was observed on NAA at 2 mg/l and KIN at 0.2 mg/l for root induction (Table1).

Indirect Organogenesis

Callus Induction from leaf explants*:* Leaf explants of *Silybum marianum* were tested for callus induction in MS medium with different concentrations of 2, 4-D (1.0 to 3.0 mg/l). Callus induction was observed in all the concentrations but the percentage and yields have significantly varied. The best result of 100% callus induction was obtained from leaf explants on 2, 4-D at 2.5 mg/l. (Table 2a).

Callus Induction from Hypocotyls explants*:* Hypocotyls explants of *Silybum marianum* were tested for callus induction in MS medium with different concentrations of 2, 4-D (1 to 4.5 mg/l). Callus induction was observed in all the concentrations but the percentage and yields have significantly varied. The best result of 100% callus was obtained from hypocotyl explants on 2, 4-D at 4.5 mg/l (Table 2b).

Shoot Initiation and Elongation*:* Matured hypocotyl callus was transferred to the shoot initiation medium. The hormone concentration was NAA (0.2 – 2 mg/l) and BAP (1.5 mg/l). The best result of 100% shoot initiation was obtained from NAA at 2 mg/l and the BAP at 1.5 mg/l and then it was transferred to shoot elongation medium bearing the hormone concentration of GA3 at 2 mg/l. Finally, it was transferred to root induction medium bearing the hormone concentration of NAA at 2mg/l. (Table 3). Preliminary

Phytochemical Analysis: Preliminary phytochemical analysis of chloroform and ethanol extracts of the hypocotyl callus and leaf callus were indicated the presence of secondary metabolites in a different manner (Table 4). Sugar was present in chloroform and ethanol extracts of leaf callus and hypocotyl callus. Saponins were present in chloroform extract of leaf callus. Tannins were present in ethanol extract of hypocotyl callus. Amino acids were present in ethanol extract of leaf callus.

Antibacterial Activity: In different extracts of *Silybum marianum* were tested for antibacterial activity. Chloroform and ethanol extracts of the hypocotyl callus, and leaf callus in triplicates and the average values were calculated and recorded. *Leaf Callus Extract:* Chloroform and ethanol extracts of leaf callus were tested (Table 5). Inhibitory activity was recorded against both gram-negative and gram-positive bacteria. Significant inhibitory activity of ethanol extract and chloroform extract was recorded at the range of 7 - 13 mm against *Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis* (gram-positive) and 8 - 14 mm against gram-negative bacteria of *Salmonella paratyphi* (A). Zone of inhibition ranging from 8 - 12 mm to the ethanol extract against gram-negative bacteria such as *Escherichia coli, Aeromonas hydrophila, Proteus mirabilis, Pseudomonas aeruginosa* and 8 - 10 mm in chloroform extract against gram-negative bacterium of *Staphylococcus epidermidis*. More inhibitory activity recorded for gram-positive than gram negative. No inhibition recorded against *Klebsiella pneumoniae* and *Proteus vulgaris.* Hypocotyl Callus Extract: Chloroform and ethanol extracts of leaf callus were tested (Table 6, Fig 8, 9, 10). Hypocotyl callus extraction also showed more activity to *Escherichia coli, Aeromonas hydrophila, Bacillus subtilis, Bacillus cereus*, and *Staphylococcus epidermidis*. Ethanol extract showed activity ranging from 11 – 16 mm against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* and *Proteus mirabilis*. *Pseudomonas aeruginos Staphylococcus epidermidis, Bacillus subtilis, Proteus mirabilis* and *Salmonella paratyphi* (A) were recorded 8–12 mm in chloroform extract. No inhibitory activity was recorded against *Bacillus cereus, Staphylococcus aureus* and *Proteus vulgaris* in chloroform extract and *Salmonella typhi* in both the extracts.

DISCUSSION

Callus and shoot formation in *Silybum marianum* cultured on MS medium supplemented with BAP (0.25 mg/l and 10 mg/l). This composition was effective in promoting callus induction and transferred callus to MS medium containing similar combination of PGRs for shoot organogenesis. The callus was yellow and friable.[7] But in the present study, the callus induction on the leaf explants was achieved at low concentration of 2,4-D (1.0- 3.0 mg/l) in higher rate and the nature of the callus is also found similar as yellow and friable and then the callus was transferred to MS medium containing different concentrations of NAA and BAP. The results are similar but the growth regulators are different. Even the nature of callus is also similar. Callus induction in *Silybum marianum*.

They used cotyledon explants cultured on B5 medium supplemented with 0.05 mg/l of BAP and 0.5 mg/l of 2,4-D and solidified with 2.5 mg/l of phytagel. This combination was effective in promoting callus induction.[8] In the present study, the callus induction on hypocotyle was with 2,4- D (1.0- 4.5 mg/l) and the nature of the callus was yellow and friable and brown in color. They further reported (2006) that maximum callusing was obtained from leaf segments when B5 medium supplemented with combination of BAP and 2,4-D. In the present study, maximum callusing was obtained in MS medium supplemented with 2, 4-D (2.0 mg/l) without combination of cytokinins.

Acetone and methanolic extract of *Silybum marianum* seeds were tested for their antibacterial activity. In the present study, *in vitro* callus of *Silybum marianum* was tested for antibacterial activity against gram-positive and gram-negative bacteria.[9] *Silybin* has potent antibacterial activity that is more potent than s*ilymarin* against gram-positive bacteria without hemolytic activity. In gram-negative bacteria no activity compared to gram-positive bacteria. In the present study, the antibacterial activity of the plant material was higher against gram-positive bacteria compared to gram-negative bacteria. So, the results of the present study corroborate the same. [10]

CONCLUSION

Direct root regeneration was carried out using different concentrations of auxin and cytokinin such as NAA and KIN. The best results were observed in the range of NAA at 2 mg/l and KIN at 0.2 mg/l. After 20 days, the callus was transferred to shoot induction medium. Callus induction was carried out using different concentrations of auxin such as 2, 4-D. The best result was observed to the leaflets at 2.5 mg/l. The nature of callus was yellow in colour. The best result was observed to the hypocotyl at 4.5 mg/l. Nature of the callus was yellow in color and friable. Within 15 days, the callus was changed into compact mass in nature. The colour was brown. Callus was transferred to shoot induction medium. The best results were observed at the concentration range of NAA at 2 mg/l and BAP at 1.5 mg/l. Then, it was transferred to shoot elongation medium. The best results were observed at the concentration of 2.0mg/l GA3. After shoot elongation, it was transferred to root induction medium. Preliminary phytochemical analysis of the callus showed the presence of sugar, amino acids, saponins, tannins and the absence of

steroids, triterpenoids, reducing sugar (A), reducing sugar (B), alkaloids, phenolic compounds, catechins, flavonoids, anthroquinones and amino acids.

The results revealed the presence of different types of compounds in the callus. Antibacterial activity of the induced callus was estimated against gram- positive and gram-negative bacteria. Different solvents were used to extract leaf callus and hypocotyl callus. Antibacterial activity of ethanol extract is more potent than chloroform extract. More inhibition recorded is for gram-positive than gram-negative bacteria.

Chart.1: Antibacterial activity of the callus from leaf explants in Silybum marianum.

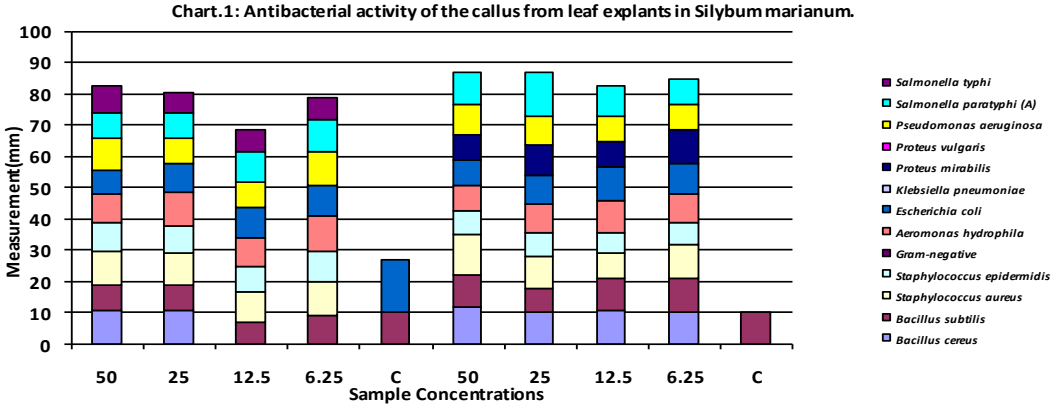

Chart.2: Antibacterial activity of the callus from leaf explants in Silybum marianum.

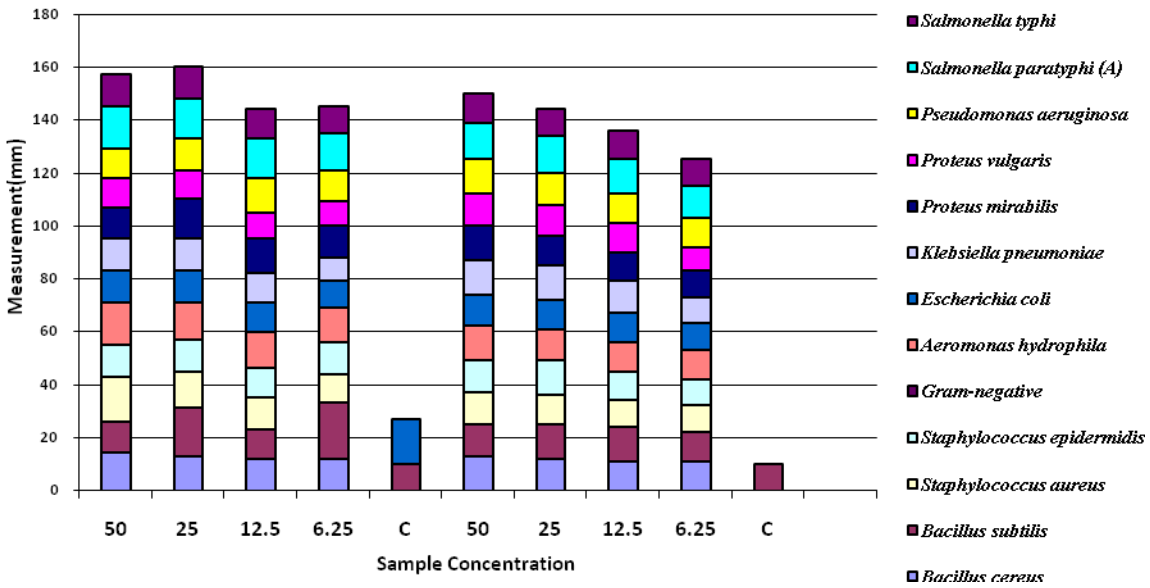

Table 1: Direct Root Regeneration from Leaves

Plant growth regulators (mg/l)		Root induction
NAA	KIN	
2.0	0.1	No root
2.0	0.2	Better root
2.0	0.5	No root
2.0	1.0	No root
2.0	2.0	No root

Table 2a: Effect of 2, 4-Dichlrophenoxy acetic acid on callus formation of leaf explants with full strength MS medium of *Silybum marianum*

2, 4-Dichlrophenoxy acetic acid (mg/l)	Degree of callus formation	Morphology of callus
1.0	-	-
1.5	++	Yellow and friable
2.0	+	Yellow and friable
2.5	+++	Yellow and friable
3.0	+	Yellow and friable

- No callus formation; + slight callus; ++ moderate callus; +++massive callus

Table 2b: Effect of 2, 4-Dichlrophenoxy acetic acid on callus formation of hypocotyle explants with full strength MS medium of *Silybum marianum*

2, 4-Dichlrophenoxy acetic acid (mg/l)	Degree of callus formation	Morphology of callus
1.0	-	-
1.5	-	Yellow and friable
2.0	-	Yellow and friable
2.5	-	Yellow and friable
3.0	+	Yellow and friable

Table 3: Effect of NAA and BAP on shoot formation of hypocotyl callus with full strength medium

Plant growth regulators (mg/l)		Shoot formation
NAA	BAP	
0.2	1.5	No shoot
0.5	1.5	No shoot
1.0	1.5	No shoot
1.5	1.5	No shoot
2.0	1.5	Shoot induction

Table 4: Preliminary Phytochemical screening of the various solvent extracts of different plant parts in *Silybum marianum*

Test	Chloroform extract		Ethanol extract	
	Leaf callus	Hypocotyl callus	Leaf callus	Hypocotyl callus
Steroid	-	-	-	-
Triterpenoids	-	-	-	-
Reducing Sugar (A)	-	-	-	-
Reducing Sugar (B)	-	-	-	-
Sugars	+	+	+	+
Alkaloids	-	-	-	-
Phenolic compounds	-	-	-	-
Catechins	-	-	-	–
Flavonoids	-	-	-	-
Saponins	+	-	-	-
Tannins	-	-	-	+
Anthroquinones	-	-	-	-
Amino acids	-	-	+	-

+ = present; - =absent

Table 5: Antibacterial activity of the callus from leaf explants in *Silybum marianum*

Microorganisms	Chloroform (mg/l)					Ethanol (mg/l)					Standard
	50	25	12.5	6.25	C	50	25	12.5	6.25	C	
Gram-positive											
Bacillus cereus	11	11	-	-	-	12	10	11	10	-	33 (T)
Bacillus subtilis	8	8	7	9	10	10	8	10	11	10	26 (A)
Staphylococcus aureus	11	10	10	11	-	13	10	8	11	-	45 (M)
Staphylococcus epidermidis	9	9	8	10	-	8	8	7	7	-	29 (T)
Gram-negative											
Aeromonas hydrophila	9	11	9	11	-	8	9	10	9	-	20 (Tr)
Escherichia coli	8	9	10	10	17	8	9	11	10	-	30 (K)
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-	30 (K)
Proteus mirabilis	-	-	-	-	-	8	10	8	11	-	25 (E)
Proteus vulgaris	-	-	-	-		-	-	-	-		30 (T)
Pseudomonas aeruginosa	10	8	8	11	-	10	9	8	8	-	20 (K)
Salmonella paratyphi (A)	8	8	10	10	-	10	14	10	8	-	30 (G)
Salmonella typhi	9	7	7	7		-	-	-	-		20 (Na)

- No activity; C = DMSO (dimethyl sulphoxide); measurements are given in mm; Ampicillin (A); Erythromycin (E); Kanamycin (K); Methicillin (M); Nalidixic acid (Na); Trimethoprin (Tr); Tetracycline (T); Gentamicin (G).

Table 6: Antibacterial activity of the callus from hypocotyle explants in *Silybum marianum*

Microorganisms	Chloroform (mg/l)					Ethanol (mg/l)					Standard
	50	25	12.5	6.25	C	50	25	12.5	6.25	C	
Gram-positive											
Bacillus cereus	14	13	12	12	-	13	12	11	11	-	33 (T)
Bacillus subtilis	12	18	11	21	10	12	13	13	11	10	26 (A)
Staphylococcus aureus	17	14	12	11	-	12	11	10	10	-	45 (M)
Staphylococcus epidermidis	12	12	11	12	-	12	13	11	10	-	29 (T)
Gram-negative											
Aeromonas hydrophila	16	14	14	13	-	13	12	11	11	-	20 (Tr)
Escherichia coli	12	12	11	10	17	12	11	11	10	-	30 (K)
Klebsiella pneumoniae	12	12	11	9	-	13	13	12	10	-	30 (K)
Proteus mirabilis	12	15	13	12	-	13	11	11	10	-	25 (E)
Proteus vulgaris	11	11	10	9	-	12	12	11	9	-	30 (T)
Pseudomonas aeruginosa	11	12	13	12	-	13	12	11	11	-	20 (K)
Salmonella paratyphi (A)	16	15	15	14	-	14	14	13	12	-	30 (G)
Salmonella typhi	12	12	11	10	-	11	10	11	10	-	20 (Na)

- No activity; C = DMSO (dimethyl sulphoxide); measurements are given in mm; Ampicillin (A); Erythromycin (E); Kanamycin (K); Methicillin (M); Nalidixic acid (Na); Trimethoprin (Tr); Tetracycline (T); Gentamicin (G).

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