

# Antibacterial Potentiality of Red Sandalwood Callus Against Pathogenic Isolates of *Aeromonas* and *Pseudomonas*

Tamzida Shamim Ashrafee<sup>1</sup>, MM Rahman<sup>2</sup>, Anindita Chakraborty<sup>1</sup>, Shamsul H. Prodhan<sup>1,\*</sup>

<sup>1</sup>Dept. of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, 3114, Bangladesh

<sup>2</sup>Dept. of Biotechnology, Bangabandhu Sheikh Muzibur Rahman Agricultural University, Salna, Gazipur-1706 Bangladesh

\*Corresponding Author: [shamsulhp@yahoo.com](mailto:shamsulhp@yahoo.com)

Copyright © 2014 Horizon Research Publishing All rights reserved.

**Abstract** The present study is based on the in vitro production of callus, its long period maintenance and antibacterial potentiality. Callus induction and formation occurred by full strength MS media in combination with various concentration (0.5mg/l, 1.0mg/l, 1.5mg/l, 2.0mg/l) of 2, 4-D effective in combination with constant concentration of BA (2.5mg/l) and NAA (0.5mg/ml). The leaf calli were sub cultured and transferred to MS media with various hormonal combinations which were different from callus induction media. Internodes were also subjected to induce callus and callus formation resulted from MS media in combination with Kinetin (0.5mg/l, 1.0mg/l, and 1.5mg/l), BA (1.0mg/ml, 2.5mg/ml), NAA (1.0mg/l, 1.5mg/l, 2.0mg/l and 2.5mg/l) without adding 2, 4 D and another combination of 0.5mg/l 2, 4 D and 2.5 mg/l BA as well as 0.5mg/l NAA . The sustainability rate of total leaf callus for four month was 83.33% and that for inter node callus was 56.65%. Ten strains of *Aeromonas* and *Pseudomonas* were used to antibacterial screening. The crude methanolic extract showed antibacterial activity against four strains of *Aeromonas* (E14, Cok2/2, Cok2/3, Cok2/4) and one strain of *Pseudomonas* (P2F4). Ethanolic extract absorbed filter paper discs showed very low efficiency of inhibition to two strains of *Aeromonas* E17, Cok2/4 and one strain of *Pseudomonas* P2F4. Crude callus extract was effective against the growth of three strains of *Aeromonas* (E14, E17 and Cok2/4). It prohibited the growth of *Pseudomonas* Cla1b10 (avg. 10.71mm). This result implies the potentiality of presence of active compounds or secondary metabolites that have the antibacterial features in Red sandalwood callus.

**Keywords** Callus, Extraction, Antibacterial Potentiality, Red Sandalwood, *Aeromonas*, *Pseudomonas*

*Pterocarpus santalinus*, also known as ‘red sanders’ or ‘red sandalwood’ is a highly valuable forest legume tree. It is locally known as ‘Rakta Chandan’. This species occurs exclusively in a well-defined forest tract of Andhra Pradesh in Southern India [10], now included in red list of endangered plants under IUCN guidelines [3]. It contains many other compounds that have medicinal properties. Since the beginning of civilization in sub-continent, this plant is widely used in ‘Ayurved’ in India. It is considered as an astringent, tonic and diaphoretic, and is useful to cure bilious infections and skin diseases. In recent years different studies showed the antimicrobial activity of the leaf extracts, stem bark extracts’ from this plant. The leaf and stem bark extracts of red sanders showed a wide spectrum of activities against common human pathogenic bacteria, fungus and protozoa [14]. Red sanders has a characteristic anti-inflammatory, analgesic and anti oxidant features. The methanolic wood extract contains flavonoids, essential oils, tannins, phenolic acids and polyphenolic compounds that have multiple biological activities. Phytochemical screening of suggested that presence of these compounds might be responsible for anti-inflammatory and anti oxidant effect. Stem bark extract of *Pterocarpus santalinus* also show hepatoprotective activity. CCl<sub>4</sub> treated male rats’ liver were examined and necrotized liver contained increased amount of serum bilirubin, alanine transaminase and aspartate transaminase etc with a decreased level of total protein. Rats treated with Stem bark extract showed significant recovery in comparison with untreated rats [8]. Through tissue culture, significant rate of callus induction from leaf parts, cotyledon parts, root segments, inter-nodal segments, and nodal segments of red sanders observed [15]. There were many experiments conducted on antimicrobial activity of callus extracts of different plants but study of antibacterial activity of Red sandalwood callus are not noticed. In a recent study, crude extracts of a callus culture from *Alternanthera tenella* Colla (Amaranthaceae) were evaluated for their antibacterial and antifungal activity, against thirty strains of microorganisms

## 1. Introduction

including Gram-positive and Gram-negative bacteria, yeasts and dermatophytes [5]. Nagarajan et al [6] showed the comparison of natural extracts and callus extracts of *Solanum trilobatum* L. as studied against *E. coli*, *Staphylococcus aureus*, *Aspergillus flavus* and *Aspergillus niger* for their antimicrobial activity using cup diffusion method. As a measure of testing the medicinal properties of *Jatropha curcas*, methanol extract obtained from both *in vivo* leaf and leaf derived callus were subjected to antimicrobial activity against six microorganisms, of the six different concentrations tested, [11]. In this present study, healthy and cell proliferating callus induced from leaf explants of red sanders and two to four months old callus were subjected to antibacterial screening. Pathogenic bacterial isolates of *Aeromonas* and *Pseudomonas* were used as test microorganisms. The isolates of *Aeromonas* are responsible for diarrhea and *Pseudomonas* cause severe skin lesions in human. Therefore, these pathogenic isolates were selected for antibacterial study. The undifferentiated cell layer of red sandalwood callus may accumulate the secondary metabolites as in matured plant and crude callus as well as methanolic extracts of Red sanders showed antibacterial effect against isolates of *Aeromonas* and *Pseudomonas*.

## 2. Materials and Methods

### 2.1. Plant Collection

One to three years old Red sandalwood trees were collected from 'Boishakhi nursery' and Sylhet Forest Office. The plants are authenticated by a government employee of Sylhet forest office.

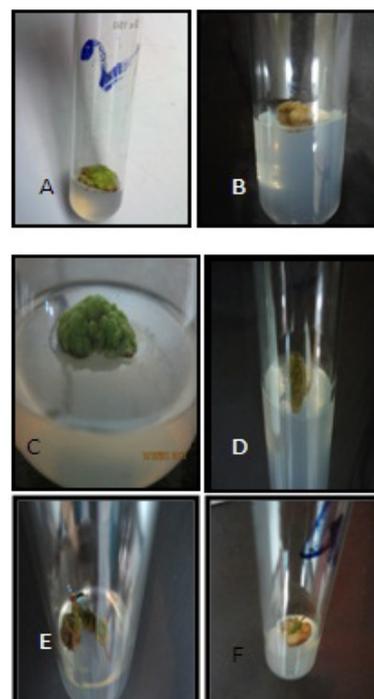
### 2.2. Sterilization of Explants

Leaves were washed with distilled water and then surface sterilized for 30 seconds in 70% ethanol and then treated with 0.1% aqueous  $HgCl_2$  for 3 minutes. Inter nodes were surface sterilized for 2 minutes in 70% ethanol, then subjected to 0.1% aqueous  $HgCl_2$  containing 2 to 3 drops of tween-20 for 7 minutes (Prakash *et. al.*2006). Due to the pliable texture, leaf explants were conducted to less period of time for sterilization.

### 2.3. Callus Induction and Callus Transfer

Full strength MS media with different hormonal conditions was used for callus induction. The concentrations were C1( 0.5mg/L 2, 4-D, 2.5mg/L BA, 0.5mg/L NAA ), C2(1.05mg/L 2, 4-D, 2.5mg/L BA, 0.5mg/L NAA), C3(1.55mg/L 2, 4-D, 2.5mg/L BA, 0.5mg/L NAA), C4 (2.0 5mg/L 2, 4-D, 2.5mg/L BA, 0.5mg/L NAA). Callus induction occurred after two weeks. Subsequent callus transfer was done once in two week. Among healthy and cell

proliferating large calli, 18.75% of total amount of two week age calli were transferred to full strength MS media containing other growth regulators such as NA, Kinetin, BA to observe the survival rate of callus depriving of 2, 4-D in corresponding medium. Calluses were randomly chosen. The concentrations C<sub>0</sub>1 (MS+0.5mg/l Kin, 2.5mg/l BA, 1mg/l NAA ), C<sub>0</sub>2 (MS+ 1mg/l Kin, 2.5mg/l BA, 1.5mg/l NAA ), C<sub>0</sub>3 (MS+1.5mg/l Kin, 2.5mg/l BA, 1.5mg/l 1.5mg/l 2.0mg/l NAA), C<sub>0</sub>4(MS+ 1.5mg/l Kin, 2.5mg/l BA, 2.5mg/l NAA) were used as new concentrations. Different callus induced from these hormonal combinations from leaves and internodes explants are shown in figure 1.



**Figure 1. Callus induced from different hormonal concentrations explant sources A:** 8 week age callus transferred to C<sub>0</sub>2 concentration **B:** Two week age callus **C :** 12 week age sustainable callus **D:** Callus sub cultured to C<sub>2</sub> concentration **E:**Two week age callus induced from node in combination of full strength MS medium with 2.5 mg/l BA and 0.5 mg/l NAA **F:** Six week age callus induced from node in combination of full strength MS medium with 0.5 mg/l 2, 4-D, 2.5 mg/l BA and 0.5 mg/l NAA

### 2.4. Callus Extraction

Calluses were grouped according to their age (4 or 2 month), weight and explants source (Table 1). The 4 month callus group included extract no 3, 4, 5 and 6 as well as the 2 month groups included extract no 1 and 2. Then numbered and added with absolute ethanol (extract no.1 & 4) 50% ethanol (extract no 2, 5, 6) and absolute methanol (extract no. 3). The weights of callus were taken including their moisture content. The weights were 4.08gm, 1.14gm, 1.2 gm, 2.5 gm, 2.6 gm and 0.95 gm for extract no. 1, 2, 3, 4, 5 and 6 respectively as well as the added amount of ethanol/methanol were 6ml, 10ml, 8ml, 10ml, 20ml and 6 ml chronologically. Calluses were extracted for 72 hour in room temperature. Then the foggy upper portion of preparation

was taken to sterilized eppendroff tube and centrifuged for 5000rpm for five minutes. Supernatant was taken for agar well diffusion antimicrobial screening of selected bacterial isolates.

**Table 1.** Preparation of ethanolic and methanolic extract

No. of extract	Concentration no. of callus media;	Explants source
1	C3	leaf
2	C2	leaf
3	C1	leaf
4	C1	Inter node
5	C03	leaf
6	C02	leaf

A four month age cultured in combination of 1.5 mg/l BA, 0.1 mg/l Kinetin and 0.5 mg/l NAA had a weight of 4.00 gm was taken. Then it was grinded in a fine paste by sterilized mortar and pestle. 3 ml sterilized distilled water was added to the paste. Then 1.0 ml of sterilized eppendroff tube was filled with the crude callus substance and subjected to 10000 rpm for five minutes. The supernatants were taken as ethanolic and methanolic extracts for antimicrobial screening of selected bacterial strains.

### 2.5. Test Pathogenic Bacterial Isolates

Ten of pathogenic bacterial isolates of *Aeromonas* and *Pseudomonas* are selected for antibacterial activity screening of red sandalwood. Isolates of *Aeromonas* included E14, E17, Cok2/2, Cok2/3, Cok2/3, Cok2/4 and *Pseudomonas* included PukL<sub>2</sub>, P<sub>2</sub>F<sub>4</sub>, Cla<sub>1</sub>b<sub>10</sub>, Cla<sub>2</sub>b<sub>7</sub>, Cla<sub>2</sub>B<sub>8</sub> respectively. All of the microorganisms were collected from USDA project laboratory of Genetic Engineering and Biotechnology department, Shahjalal University of Science and Technology. The isolates were identified as vital pathogen of different fish and prawns and responsible for economically important diseases.

### 2.6. Antibacterial Assay

Tested pathogenic bacterial isolates pure culture were maintained in agar plate containing NA media. A loop of bacterial culture were inoculated in NA broth media and kept at 30°C for 24h in incubator. Muller-Hinton agar was used in agar well diffusion assay for antibacterial assay. After 24h culture of NA broth media, 50µl of broth culture was poured in MH agar plate and spread over on plate by an L shaped glass rod. Then 6 mm diameter sterilized borer were used for preparing well in agar plate. 50µl of methanolic and ethanolic extracts were filled in each well. After 24 h incubation, zone of inhibition was measured in mm. Antibacterial screening through crude callus was done by

applying callus paste within 6 mm diameter range in seeded MH agar plate and then clear zone measured after 24h.

As control test, the seeded agar plates with tested bacterial strains were screened with methanol, absolute ethanol and 50% ethanol. 6 mm well were made in agar plate and 50 µl of each reagent were loaded in each well. After 24 hour, zone of inhibition were measured.

## 3. Results & Discussions

Full strength MS medium in combination with different synthetic Auxins and cytokinin is efficient for induction of sustainable callus. At the present experiment, after four to five days of inoculation of leaves in callus inducing medium, callus formation was noticed in the middle portion of explants. It began swell and light greenish pigmentation occurred. After ten days, creamy white callus tissues were observed. After 14 days, the complete formations of creamy white globular callus were expressed. Induced callus from leaf explants was non fragile and globular in nature and it was hard to cut with surgical blade during sub culture. In case of 1<sup>st</sup> replication of callus induction from leaf explants, 100% callus formation occurred. In 2<sup>nd</sup> replication 7.14% contamination and 10.71% Unresponsive explants were found. However, from both of two replications, total 82.143% callus were induced and successfully formed. The concentration of 2, 4 D was (0.5mg/l, 1.0mg/l, 1.5mg/l, 2.0mg/l) effective in combination with constant concentration of BA (2.5mg/l) and NAA (0.5mg/ml)

Young, pliable internodes were inoculated in combination with 2, 4 D, BA and NAA in full strength MS media. Internodes were also inoculated in full strength MS media in combination with Kinetin (0.5mg/l, 1.0mg/l, and 1.5mg/l), BA (1.0mg/ml, 2.5mg/ml), NAA (1.0mg/l, 1.5mg/l, 2.0mg/l and 2.5mg/l) without adding 2, 4 D. Combination of C1 (0.5mg/l 2, 4 D and 2.5mg/l BA as well as 0.5mg/l NAA) concentration was most efficient for good quality callus induction from node. Both poor and good quality callus were obtained from full strength MS media (with BA, NAA and Kin) without adding 2, 4 D. M. Percentage of callus formation without 2, 4-D was 22.73% and with 2, 4-D was 100%. Percentage of callus formation from all combination was 40.91%.

The methanolic extracts (extracts no.3) of leaf callus showed antibacterial activity against both isolates of *Aeromonas* and *Pseudomonas*. It was more effective against the isolates of *Aeromonas* than *Pseudomonas*. The methanolic extracts created clear zones against four isolates of *Aeromonas* and one isolate of *Pseudomonas*. The crude callus showed antibacterial effect against three isolates of *Aeromonas* and one isolate of *Pseudomonas*. The zone of inhibitions ranged from 9 to 16 mm. Average diameters of replicates were used to evaluate the zone of inhibition. The results were summarized in Table 2 which showed susceptibility of bacterial isolates in 50% ethanol, 100% ethanol, 100% methanol.

**Table 2.** effects of different extracts and control reagents against selected bacterial isolates

Extracts & control reagents	Bacterial Isolates									
	E <sub>14</sub>	E <sub>17</sub>	Cok <sub>2/2</sub>	Cok <sub>2/3</sub>	Cok <sub>2/4</sub>	PukL <sub>2</sub>	P <sub>2</sub> F <sub>4</sub>	Cl <sub>a1</sub> b <sub>10</sub>	Cl <sub>a2</sub> b <sub>7</sub>	Cl <sub>a2</sub> B <sub>8</sub>
Methanol	10mm	-	12mm	-	-	-	-	-	-	-
50% ethanol	-	-	-	10mm	-	-	-	-	-	-
Absolute ethanol	15mm	20mm	-	10mm	12mm	-	-	-	-	-
extract. no 3	16mm	-	10.67mm	12.5mm	12.5mm	-	-	-	-	-
Crude extract	9.57mm	12.71mm	-	-	11mm	-	-	10.71mm	-	-

### 3.1. Result of Zone of Inhibition Occurred Through Methanolic Extract of Four Month Old Callus

In this study, among ten strains of both *Pseudomonas* and *Aeromonas*, aqueous methanolic extract is effective against four strains of *Aeromonas* (E<sub>14</sub>, Cok<sub>2/2</sub>, Cok<sub>2/3</sub>, Cok<sub>2/4</sub>) and one strain of *Pseudomonas* (P<sub>2</sub>F<sub>4</sub>). The zone of inhibition in strains of *Aeromonas* ranges from 10 -16 mm and it showed 9mm in P<sub>2</sub>F<sub>4</sub>. The average zone of inhibition was 16mm observed in E<sub>14</sub> and 10.67mm 12.5mm, 12.5mm were observed in Cok<sub>2/2</sub>, Cok<sub>2/3</sub> and Cok<sub>2/4</sub> chronologically. The measurements of zone of inhibition were studied after 24 hour and 36 hour. In both of periods, the zone of inhibition remained same, which expressed that no.2 methanolic extract are bactericidal for these five strains. On the other hand, the ethanolic extracts (no.1,2,4,5,6) did not show any type of inhibition through agar well diffusion method to all these ten strains.

### 3.2. Zone of Inhibition Occurred in *Pseudomonas* and *Aeromonas* from Crude Callus Extract

The crude callus extract is directly applied on muller hinton agar plate inoculated with all the ten bacterial strains. Among the ten pathogenic isolates, crude callus extract was effective against the growth of three strains of *Aeromonas* (E<sub>14</sub>, E<sub>17</sub> and Cok<sub>2/4</sub>). The zone of inhibition in strains of *Aeromonas* ranges from 9 to 13 mm. It created zone of inhibition ranges on *Aeromonas* E<sub>14</sub>, 9-10 mm (avg.9.57mm). It prohibited the growth of *Pseudomonas* Cl<sub>a1</sub>b<sub>10</sub> (avg. 10.71mm). The E<sub>17</sub> of *Aeromonas* strain E<sub>17</sub> ranges 12 to 13 mm (avg. 12.71mm) and it was measured avg. 11mm for strain Cok<sub>2/4</sub>.

### 3.3. Zone of Inhibition Occurred in *Pseudomonas* and *Aeromonas* from Methanol, 50% Ethanol and Absolute Ethanol

Most of the bacterial strains were survived in methanol, 50% ethanol and absolute ethanol. Among the strains of *Pseudomonas*, 12mm zone of inhibition occurred in PukL<sub>2</sub> through absolute ethanol. Among the strains of *Aeromonas*, 12mm, 10mm and 15mm zone of inhibition occurred in Cok<sub>2/2</sub>, Cok<sub>2/3</sub> and E<sub>14</sub> respectively were resulted due to methanol. 10mm, 12mm, 15mm and 20mm zone of inhibition were created in strains of Cok<sub>2/2</sub>, Cok<sub>2/3</sub>, E<sub>14</sub> and E<sub>17</sub> respectively. 50% ethanol was not effective against any of the selected strains.

To test the antimicrobial activity of Red sandalwood callus, callus of four and two month age were selected. Solvents, such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used for the extraction of phenolics from plant materials, often with different proportions of water. Selecting the right solvent affects the amount and rate of polyphenols extracted. Gami *et. al.* 2011 [9] suggested that, extractive value of methanol is higher than other reagents' extractive value such as acetone. Due to the high rate of secondary metabolite extraction and solubilize them into aqueous phase of methanol, methanol aqueous crude extract were subjected to Phytochemical screening through UV absorption and column chromatography [1]. In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols. Ethanol is another good solvent for polyphenol extraction such as anthocyanin-rich phenolic extracts from plant materials most commonly methanol or ethanol, is used. This solvent system denatures the cell membranes, simultaneously dissolves the anthocyanins and stabilizes them. Therefore in this study ethanol and methanol were used to extract out the soluble active compounds which probably caused the antibacterial activity against selected microflora.

The recovery of phenolic compounds from plant materials is also influenced by the extraction time and temperature, which reflects the conflicting actions of solubilization and analyte degradation by oxidation. An increase in the

extraction temperature can promote higher analyte solubility by increasing both solubility and mass transfer rate. In addition, the viscosity and the surface tension of the solvents are decreased at higher temperature, which helps the solvents to reach the sample matrices, improving the extraction rate [7]. However, many phenolic compounds are easily hydrolyzed and oxidized. Long extraction times and high temperature increase the chance of oxidation of phenolics which decrease the yield of phenolics in the extracts. Therefore the extraction period 72 hour and room temperature maintained.

Another study revealed that, crude methanolic callus extracts of *Nigella* had antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [13]. Kavitha *et al.* [12] revealed that root callus extracts of different solvents *viz.*, petroleum ether, chloroform, ethyl acetate and ethanol were tested against both Gram positive and Gram negative bacteria and also against *Fusarium* spp. Root callus extract of chloroform and ethanol showed significant activity against *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Staph. epidermidis* and also against the other spp. of Gram negative bacteria *viz.*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Alcaligenes faecalis*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella paratyphi A* and *Salmonella enterica* subsp. Agar disk diffusion is also used to test antimicrobial activity of leaf callus. Another study showed that ibrahmi, a traditional medicinal plant in subcontinent had antibacterial potentiality against *Bacillus subtilis*, *Klebsiella pneumonia*, *Staphylococcus aureus* etc [2].

In this study, among ten isolates of both *Pseudomonas* and *Aeromonas*, methanolic extract was effective against four isolates of *Aeromonas* (E<sub>14</sub>, Cok<sub>2/2</sub>, Cok<sub>2/3</sub>, Cok<sub>2/4</sub>) and one isolate of *Pseudomonas* (P<sub>2</sub>F<sub>4</sub>). The zone of inhibition in strains of *Aeromonas* ranges from 10 -16 mm and it showed 9mm in P<sub>2</sub>F<sub>4</sub>. The average zone of inhibition was 16mm observed in E<sub>14</sub> and 10.67mm 12.5mm, 12.5mm were observed in Cok<sub>2/2</sub>, Cok<sub>2/3</sub> and Cok<sub>2/4</sub> chronologically. The measurements of zone of inhibition were studied after 24 hour. On the other hand, the ethanolic extracts (no.1, 2, 4, 5, 6) of did not show any type of inhibition through agar well diffusion method to all these ten isolates.

The antimicrobial activity of methanolic supernatant would be increased by soluble active compounds of grinded callus. But all type of alcohol has antimicrobial properties therefore question may be raised that the zone of inhibition may be caused through methanol. To find out the actual antimicrobial potentiality of callus, crude callus paste was prepared and bacterial strains were subjected to screened through methanol and zone of inhibition occurred in Cok<sub>2/2</sub>, Cok<sub>2/3</sub> and E<sub>14</sub> respectively. Methanol is not effective against Cok<sub>2/4</sub> and P<sub>2</sub>F<sub>4</sub>, which implied the antibacterial property of red sandalwood callus.

The crude callus extract is directly applied on Muller Hinton agar plate inoculated with bacterial isolates. After 24 hour, the zone of inhibition was measured. Crude callus

extract was effective against the growth of three isolates of *Aeromonas* (E<sub>14</sub>, E<sub>17</sub> and Cok<sub>2/4</sub>). The zone of inhibition in isolates of *Aeromonas* ranges from 9 to 13 mm. It was bactericidal to *Aeromonas* E<sub>14</sub>, created zone of inhibition ranges 9-10 mm (avg.9.57mm). It prohibited the growth of *Pseudomonas* Cl<sub>1</sub>b<sub>10</sub> (avg. 10.71mm). The E<sub>17</sub> of *Aeromonas* strain E<sub>17</sub> ranges 12 to 13 mm (avg. 12.71mm) and it was measured avg. 11mm for strain Cok<sub>2/4</sub>. Observation after 36 hour determined the type of inhibition by crude callus.

## 4. Conclusions

From this result, the antibacterial effect of methanol extracts can be explained. The crude callus paste did not contain any antibacterial reagents like ethanol or methanol, but despite of any chemical reagents for extraction or making solution with these reagents, antibacterial effect against four isolates of assayed pathogens. Therefore, this study showed the probability of presence of antibacterial active compounds in long period cultured globular callus of red sandalwood.

## Acknowledgements

The Present study was conducted in Plant genetic Engineering and USDA microbiology laboratories of department of Genetic Engineering and Biotechnology. We are thankful to these two laboratories and also to all teachers.

## REFERENCES

- [1] Ahmed I, Asghar I, Choudhary , Hussain H, Rehman H, Muhammad I , Rahman A , Shahid A, Syeda F.; 2009; Secondary Metabolites Isolated from *Iris germanica*; Rec. Nat. Prod. 3:3 139-152
- [2] Alam K, Hwisa N, Sharif M A, Molvi K, Murti Y, Parvez N, Pathak D S, Yadav S, and Zafar R; 2011; Antimicrobial activity of leaf callus of *Bacopa Monnieri* L.; Der Pharmacia Lettre, , 3(1): 287-291
- [3] Chaturani G.D.G, Jyatileke M P Subasinghe S.; 2006; In vitro establishment, germination and growth performance of Red sandalwood (*Pterocarpus santalinus* L.); Tropical agricultural research and extension 9,2006 p. no.116-130
- [4] Candido RC, Dias DA, França SC, Ito IY, Pereira PS, Salvador MJ, 2009 Bioactive chemical constituents and comparative antimicrobial activity of callus culture and adult plant extracts from *Alternanthera tenella*. Z Naturforsch C. May-Jun;64(5-6):373-81.
- [5] Candido RC, Dias DA, França SC, Ito IY, Pereira PS, Salvador MJ, 2009 Bioactive chemical constituents and comparative antimicrobial activity of callus culture and adult plant extracts from *Alternanthera tenella*. Z Naturforsch C. May-Jun;64(5-6):373-81.
- [6] Chinnappa R, Kandasamy S, Nagarajan S M, 2009

- Comparative antimicrobial activity of callus and natural plant extracts of *Solanum trilobatum* L.; *Ancient Science of Life*, Vol 28, pp 3-5
- [7] Dai J and Mumper R J; 2010; Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties; *Molecules*, 15, 7313-7352;
- [8] Devi N S, Devi RS, Shyamala C S, Srinivasan P; 2005; *Pterocarpus santalinus*: a traditional herbal drug as a protectant against ibuprofen induced gastric ulcers; *Phytother Res. Nov*;19(11):958-62
- [9] Gami B, Patel J P, Patel K and Solanki R; 2011; Antibacterial activity of methanolic and acetone extract of some medicinal plants used in indian folklore; *International Journal of Phytomedicine* 3, 261-269
- [10] Khan P S SV, Prakash E, Rao T J VS, Singh E M; 2006; Micropropagation of red sanders (*Pterocarpus santalinus* L.) using mature nodal explants; *J For Res* 11:329-335
- [11] Kalimuthu K, Senthilkumar R, Vijayakumar S; 2010; Antimicrobial activity of the biodiesel plant *Jatropha curcas* L. *International Journal of Pharma and Bio Sciences*, Vol.1/Issue-3/Jul-Sep
- [12] Kavitha H U, Radfar M, Niranjana M H, Sudarshana M S, Satish S 2012; Evaluation of antibacterial and antifungal activity of root and root callus extracts of *Trianthema decandra* L.; *African Journal of Biotechnology* Vol. 11(2), pp. 510-515, 5 January,
- [13] Kokoška L, Landa P., Maršík P, Rada V, Vaněk T; 2006; In vitro anti-microbial activity of extracts from the callus cultures of some *Nigella* species; *Biologia* 61 [3]: 285-288,
- [14] Manjunatha BK; 2006; Antibacterial activity of *Pterocarpus santalinus*; *Indian Journal of Pharmaceutical science* Volume : 68 | Issue : 1 | Page : 115-116
- [15] Thesis: Chaturani G D G, Jayatilleke M P & Subasinghe S ; 2005 In vitro callus formation of Red Sandalwood; *Forestry and Environment Symposium*, Sri Lanka.