International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2020.4.8

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 4 (4): 450-457 (2020)

Callus induction and antibacterial activity of various explants of *Indigofera zollingeriana*

Siti Maesaroh[1,*] **Cigdem Alev Ozel**[2] **Hikmet Katircioglu**[2] **Nurdan Sahin Demirbag**[1]

[1]Department of Field Crops, Faculty of Agriculture, Ankara University, Ankara, Turkey
[2]Department of Biology Education, Faculty of Education, Gazi University, Ankara, Turkey

[*]Corresponding Author: maesaroh@ankara.edu.tr

Abstract

The study explore antibacterial potential of *Indigofera zollingeriana,* a popular medicinal and forage plant of the tropics. Methanol extracts of calli from hypocotyl, cotyledon and leaf excised from *in vitro*-grown plants using different plant growth regulator and their combinations had significant reaction against Gram-positive *Staphylococcus aureus* ATTCC25923 and Gram-negative *Pseudomonas aeruginosa* ATCC25823 bacteria. The best antibacterial activity was noted on the callus extracts of hypocotyl explants with significant inhibition zones on MS medium containing 2 mg/L BAP + 0.1 mg/L NAA. The *P. aeruginosa* had maximum inhibition on 0.5 mg/L BAP + 0.1 mg/L NAA. This antibacterial activity (by the hypocotyl induced calli extracts) was higher compared to the antibacterial activity noted from the extracts of other two explants and non treated control treatments. It was concluded that antibacterial activities were affected by explants source and plant growth regulators. The extracts from *in vitro* induced callus from hypocotyl, cotyledon and leaf explants of *I.zollingeriana* could be used variably and effectively against both type of bacteria used in this study.

Keywords: Callus, Extract, Legume, Medicinical, Phytohormone

Introduction

A huge quantity of antibiotics are being introduced into pharmaceutical industry since last 40-50 years. Inspite all efforts to introduce new drugs, there are number of pouring reports that exhibit pogressive increased antibiotic resistance to the drugs due to natural genetic ability of bacteria to acquire increased resistance (Cohen, 1992). This has lead to the development of increased hospital deaths among people due to development of new types of infections (Nascimento et al., 2000).

A review of literature from 1970s to present times show a large number of researchers, who have reported increased incidence of resistant bacteria all over world (Jansen et al., 1987; Inouye et al., 2001; Chouhan et al., 2017). In view of the present circumstances, the outlook for use of these drugs is obscure. Therefore, there is need to carry out consistent efforts and research to reduce the problem for safe and effective use of antibiotics and understand the genetic and biological mechanisms that induce resistance in bacteria. The ultimate target for each research is to suggest the most appropriate and yielding new antibacterial drugs for the benefit of the people (Jansen et al., 1987; Chouhan et al., 2017).

Indonesia is home to more than 10 percent of the world's known plant species. The genus *Indigofera* among them, comprise around 750 species distributed in tropical regions (Bakasso et al., 2008). *Indigofera zollingeriana* is multiple use crop plant, used for extraction of natural blue dye, as forage crop and as a medicinal plant.

It is believed that *I. zollingeriana* exhibits antibacterial characteristics as they are widely used in folk medicines for

Cite this article as:
Maesaroh, S., Alev Ozel, C., Katircioglu, H., Sahin Demirbag, N. (2020). Callus induction and antibacterial activity of various explants of Indigofera zollingeriana. Int. J. Agric. Environ. Food Sci., 4(4), 450-457
DOI: https://doi.org/10.31015/jaefs.2020.4.8
ORCID: Siti Maesaroh: 0000-0003-1024-284X Cigdem Alev Ozel: 0000-0002-5952-1412 Hikmet Katircioglu: 0000-0002-4866-6106 Nurdan Sahin Demirbag: 0000-0002-8345-1768
Received: 21 August 2020 **Accepted:** 26 October 2020 **Published Online:** 06 December 2020
Year: 2020 **Volume:** 4 **Issue:** 4 (December) **Pages:** 450-457
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

treatment of many health disorders. However, there is no study that reports antibacterial activity/ies of *I. zollingeriana* showing it as a potential phytochemical agent against wide range of bacteria and microrganisms. Discovery of new antibacterial compounds or drug is always desirable.

Antioxidant and antibacterial studies have been carried out in many legume plant species including *Phyllanthus emblica* (Singh and Sharma, 2015), *Ziziphus mauritiana* Lam. (Annathurai et al., 2015), *Alkanna orientalis*'s roots (Petrosyan et al., 2015), *Daucus carota*'s root, stem and petiole explants (Arafa et al., 2016), *Salvia corrugata*'s leaf and stem explants (Bisio et al., 2016), *Pterocarpus santalinu*'s *leaf explants* extracts (Ashrafee et al., 2014), *Trigonella foenum-graecum*'s cotyledons and hypocotyls explants extracts etc. from the callus extracts (ElNour et al., 2015).

Except *I. zollingeriana*, antibacterial studies have been reported from direct leaves extracts of *I. dendroides, I. oblongifoli, I. suffruticosa* and *I. tinctoria* (Dahot, 1999; Esimone et al., 1999; Leite et al., 2006; Ranukadevi and Suhani Sultana, 2011; Santos et al., 2015), crude extract of *I. gerardiana* and *I. trita* (Nisar et al., 2009; Kumar et al., 2013), root extract of *I. lupatana* and *I. aspalathoides* (Ngoci et al., 2012; Rajaperumal et al., 2013), leaves and root extract of *I. glandulosa* (Prabakaran et al., 2011) and leaves and callus leaf extracts under *in vitro* cultures on *I. tinctoria* (Jisha and Benjamin, 2009). These researchers found either negative or positive expressions in response to treatment with extracts obtained from the explants using different solvents.

Therefore, this study aimed to find the antibacterial potential of *Staphylococcus aureus* ATTCC25923 Gram-positive and *Pseudomonas aeruginosa* ATCC25823 Gram-negative using respective callus methanol extracts of hypocotyl, cotyledon and leaf explants obtained after culturing them on 10 combinations of BAP + NAA through disc diffusion method.

Material and Methods

The seeds were collected from the Department of Nutrition Sciences and Feed Technology, Bogor Agricultural University, Indonesia.

Preparation of Plant Material

Germination and Micropopagation Studies

The seeds were treated with sandpaper, ensued by washing with 98% H_2SO_4 for 5 minutes. Subsequently, they were rinsed 3×5 minutes with autoclaved distilled water to remove the traces of H_2SO_4. The sterilized seeds were transferred to erlenmeyer flasks containing liquid 0.1 mg/L of GA_3 that was shaked at 120 rpm, at 24°C in the dark using a horizontal shaker. The 4 days old germinated seedlimgs were transferred to agar solidified sterile MS medium (Murashige and Skoog, 1962), pH 5.6-5.8 for 30 days to grow and develop of seedlings to appropriate stage. Thereafter, hypocotyl, cotyledon, leaf and explants were obtained from these seedlings and cultured on 10 different combinations of plant growth regulator (PGR) as BAP + NAA to induce callus on the respective explants for two months. These were dried and powdered to obtain respective methanol extractsand test their antibacterial activities against *Staphylococcus aureus* ATTCC25923 Gram-positive and *Pseudomonas aeruginosa* ATCC25823 Gram-negative bacteria.

The antibacterial tests were performed using agar-well diffusion assay (Perez et al., 1990). The agar plates were prepared using sterile Mueller-Hinton (MH) medium + 0.65 g/l agar. Each plate was inoculated with 100 µL of the bacterial strains with OD600= 0.8 followed by their even spread onto the surface of the agar plates. The 20 µL of each methanol extract (OD600 = 0.5) were added to the discs (5 mm diameter) from the cultured bacterial strains. The plates with bacterial strain and methanol extracts were incubated at 37°C for 24 h.

The liquid extracts obtained from the respective explants obtained after pretreatment with liquid GA_3 were used as control treatment 1, 2, 3, 4, 5 and 6. The Erythromycin (15µg), Gentamycin (30 µg), Penicillin (1IU) and Chloramphenicol (10 µg) containing discs were used as control treatment 7, 8, 9 and 10 respectively.

Determination of Antibacterial Activities

The inhibition of bacterial growth was indicated by a clear zone around the discs that was measured and expressed as an average diameter of the inhibition zone.

Antimicrobial Index

Antimicrobial index of extracts were calculated as formula below (Ghasemi et al., 2003).

Antimicrobial Index = (Extract inhibition zone/ Antibiotic inhibition zone) × 100

Statistical Analysis

The average fresh and dry callus weight and average inhibition zones induced due to methanol extracts of hypocotyl, cotyledon and leaf were measured in triplicate. Callus weight data was analyzed by comparing means using IBM SPSS 24 program for Windows. The significant differences among the means were determined by Duncan's Multiple Range Test.

Results and Discussion

Induction of Callus Fresh and Dry Weight

Fresh Weight

Compact and friable soft calli were induced on all hypocotyl, cotyledon and leaf explants (100%) irrespective of the BAP + NAA treatments excluding control treatments (Table 1). Callus fresh weights ranged 3.39-6.88 g on hypocotyl, 1.18-3.83 g on cotyledon and 1.23-4.91 g on leaf explants. The maximum fresh weight of 6.88 g, 3.83 g and 4.91 g of hypocotyl, cotyledon and leaf based calli was noted on MS medium containing 1.0 mg/L BAP + 0.10 mg/L NAA (Fig. 1a), 2.5 mg/L BAP + 0.10 mg/L NAA (Fig.1b) and 2.5 mg/L BAP + 0.15 mg/L NAA (Fig.1c) in the same order. Inhibited shoots and roots were also observed on callus induced on hypocotyl explants on all explants that were cultured on 1.0 mg/L BAP + any concentration of NAA. No callus was induced on hypocotyls, cotyledon and leaf explants of control 1, 2 and 3 treatments. Therefore, the extracts of the respective explants were obtained directly from the growing seedlings that were not treated with any concentrations of BAP+NAA.

Dry Weight

Callus dry weights ranged 0.24-0.42 g on hypocotyl, 0.11-0.27 g on cotyledon and 0.13-0.32 g on leaf explants.

 DOI: https://doi.org/10.31015/jaefs.2020.4.8

The maximum dry callus weight of 0.42 g, 0.27 g and 0.32 g was induced on each of hypocotyl, cotyledon and leaf based explants on MS medium containing 1.0 mg/L BAP + 0.10 mg/L NAA, 1.0 mg/L BAP + 0.15 mg/L NAA and 2.5 mg/L BAP + 0.10 mg/L NAA in the same order and 2.0 mg/L BAP + 0.15 mg/L NAA, respectively (Table 1).

Table 1. Effects of MS medium containing different concentrations of BAP + NAA on callus induction of fresh and dry weight of *I. zollingeriana* under *in vitro* conditions after two months of culture

Medium		Fresh weight (g)			Dry weight (g)		
BAP (mg/L)	NAA (mg/L)	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf
0.5	0.10	3.39dA	1.18dB	1.25eB	0.26cA	0.11cB	0.13cB
0.5	0.15	4.42cA	1.59dC	2.88dB	0.27cA	0.13cC	0.21bB
1.0	0.10	6.88aA	1.54dB	1.68eC	0.42aA	0.15cB	0.15cB
1.0	0.15	5.16bA	3.56aB	3.96bB	0.25cA	0.27aA	0.25bA
1.5	0.10	4.61cA	2.00cC	2.42dB	0.27cA	0.18cB	0.20bB
1.5	0.15	5.33bA	2.71cB	1.86eB	0.24cA	0.19cB	0.17cB
2.0	0.10	5.82abA	1.03dB	1.23eB	0.35bA	0.10cB	0.13cB
2.0	0.15	5.48bA	3.01bC	4.45aB	0.28cA	0.22bB	0.32aA
2.5	0.10	4.35cA	3.83aB	3.42cB	0.25cA	0.27aA	0.24bA
2.5	0.15	5.73bA	3.19bC	4.91aB	0.30bcA	0.21bB	0.31aA
Control treatments		1	2	3	1	2	3
		0.00	0.00	0.00	0.00	0.00	0.00

[1] Means not followed by the same small letter within a column differ significantly at $p < 0.05$ using Duncan test
[1] Means not followed by the same capital letter within a row differ significantly at $p < 0.05$ using LSD test.

In this case, the best results of fresh and dry callus weight were obtained from hypocotyl explants on MS medium using various combinations and concentrations of plant growth regulators. The results are in agreement with Soorni and Kahrizi (2015), who indicated that adding different plant growth regulators and their concentrations might support variable callus growth leading to variable fresh weight from different explants. This could be related to accumulation of antimicrobial (antibacterial) compounds that in turn activate diffrent responses to bacterial defence mechanisms. Furthermore Sari et al. (2018) showed that concentrations of plant growth regulators influence rapid cell division and stimulate cell division and enlargement of *Myrmecodia tuberosa*'s explants correlated to callus size and protein synthesis.

It is established that playing with the ratio and concentrations of plant gowth reulators, sugars, growth medium, culture environment affects the activity and induction of secondary metabolites in the regenerating explants/callus positively or negatively and accumulation of metablic compounds from them during *in vitro* cultures (Skoog and Miller 1957; Basra, 2000; Spartz and Gray, 2008; Saikia et al., 2013; Murthy et al., 2014). In addition, taking into account the obtained results, it can be stated that the composition of PGRs in the growth medium is important to optimize both extracts and callus induction. These conditions must be optimised to positively increase the biosynthesis of alkaloids and plant metabolites (Verpoorte et al., 1991).

Callus, shoot and root induction was noted on hypocotyl explants (Figure 1.). Other studies conducted by Shrikhande et al. (1993) and Vaezi et al. (2015) also approve influence of BAP and NAA treatments also lead to callusing and rhizogenesis in species like *Azadirachta indica* and *Trigonella foenum-graecum* L respectively. There were no shoot induction and root induction on callus derived from cotyledon and leaf explants of *I. zollingeriana* in agreement with Vaezi et al. (2015), who reported no regeneration on callus derived cotyledon explants of *Trigonella foenum-graecum* L cultured on MS medium supplemented with BAP and NAA. It was implied that the different types of explants with or without organ regeneration had been influenced by auxin and cytokinin ratio in the cultures medium (Skoog and Miller, 1957; Iwase et al., 2011; Vaezi et al., 2015).

Antibacterial Activity Study

Antibacterial activity against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823 bacteria on methanol extracts of hypocotyl, cotyledon and leaf induced calli were variably positive.

***S. aureus* ATTCC25923**

The methanol extracts obtained from hypocotyl induced calli on combinations of BAP + NAA presented the highest antibacterial activity against *S. aureus* ATTCC25923 with inhibition zones in range of 0.9-2.3 cm (Table 2). The antibacterial activity or inhibition zones of the methanol extracts of calli increased when 0.5 or 1.0 mg/L BAP + 0.15 mg/L NAA (two combinations) was used. However, at increased concentrations of 1.5, 2.0 and 2.5 mg/L BAP + 0.15 mg/L NAA (three combinations), the inhibition zones were smaller compared to the inhibition zones noted on methanol

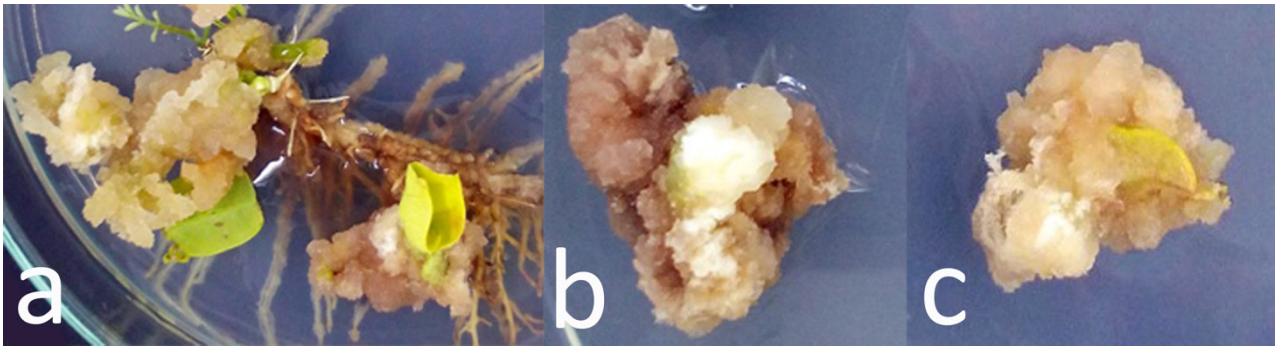

Figure 1. Callus induction on different explants of *Indigofera zollingeriana* (a) hypocotyl with roots on MS medium containing 1 mg/L BAP + 0.10 mg/L NAA (b) cotyledon node on MS medium containing 2.5 mg/L BAP + 0.10 mg/L NAA (c) leaves on MS medium containing 2.5 mg/L BAP + 0.15 mg/L NAA

extracts of 1.5, 2.0 and 2.5 mg/L BAP + 0.10 mg/L NAA (three combinations) induced methanol extracts of hypocotyl based calli. An inhibition zones (1.2 cm) of methanol extracts was obtained from hypocotyl from plants germinated on 0.1 mg/L GA_3 (control treatment No. 1).

The methanol extracts of cotyledon based calli induced on 0.5-1.0 mg/L BAP + 0.10-0.15 mg/L NAA and 1.5 mg/L BAP + 0.10 mg/L NAA did not show any antibacterial activity. However, the methanol extracts of the calli induced on 1.5 mg/L BAP + 0.15 mg/L NAA and 2.0-2.5 mg/L BAP + 0.10-0.15 mg/L NAA induced cotyledon based calli showed antibacterial activity with inhibition zones of 0.8-1.2 cm. The inhibition zones of methanol extracts obtained from cotyledon explants of plant germinated on 0.1 mg/L GA_3 remained 1.0 cm.

In case of leaf based calli, antibacterial activity ranged 1.2-1.5 cm. Antibacterial activities was noted on the methanol extracts noted on leaf based calli induced on 0.5-1.0 mg/L BAP + 0.1 mg/L NAA (two combinations), and 1.5 mg/L BAP + 0.15 mg/L NAA induced leaf based calli. The largest inhibition zone was noted on using methanol extracts obtained from 0.5 mg/L BAP + 0.1 mg/L NAA induced leaf based calli. The inhibition zones of the methanol extracts obtained from leaf explants of plants germinated on 0.1 mg/L GA_3 remained 1.0 cm.

Inhibition zones of 2.1, 1.5, 0.8 and 2.5 cm were noted when the antibiotics Erythromycin, Gentamycin, Penicillin and Chloramphenicol were used in the same order against *S. aureus* ATTCC25923 in control 7, 8, 9 and 10 treatments (Table 3).

***P. aeruginosa* ATCC25823**

The methanol extracts obtained from hypocotyl based calli induced on 0.5 mg/L BAP + 0.10 mg/L NAA presented the highest antibacterial activity against *P. aeruginosa* ATCC 25823 with inhibition zones in range of 2.4 cm (Table 2). It was followed by 1.5 cm long inhibition zones induced on methanol extracts obtained from hypocotyl based calli induced on 2.0 mg/L BAP + 0.10 mg/L NAA. The methanol extracts with 0.1 mg/L GA_3 pretreated plants used as control 4 remained 1.3 cm. The methanol extracts of rest of the treatments failed to induce any inhibition zone.

The methanol extracts obtained from cotyledon based calli induced on 0.5 mg/L BAP + 0.10 mg/L NAA presented the highest antibacterial activity against *P. aeruginosa* ATCC25823 with inhibition zones of 1.2 cm (Table 2). It was followed by 1.1 cm long inhibition zones induced on methanol extracts obtained from cotyledons germinated on 0.1 mg/L GA_3 pretreated plants used as control 5. Methanol extracts of the rest of the treatments failed to induce any inhibition zone.

No inihibition zone was noted on methanol extracts leaf based calli induced on any combination of 10 different BAP+NAA treatment or control treatment. Inhibition zones of 1.9, 1.7, 1.0 and 2.6 cm were noted, when the antibiotics Erythromycin, Gentamycin, Penicillin and Chloramphenicol (Control 7, 8, 9 and 10) were used in the same order against *P. aeruginosa* ATCC25823 (Table 3).

The antibacterial activities both Gram-positive and Gram-negative bacteria against extracts of of hypocotyl explants were comparatively higher compared to the control treatment or the extracts obtained from other explants. Similarly, Ravinder Singh (2011) reported higher inhibitory activities on leaf based callus extracts of *Premna serratifolia* compared to the extracts of leaves growing under natural conditions. Staba (1980) has also reported that rate of cell multiplication and cell division influenced increasing secondary metabolites production. There was no antibacterial activity againts both Gram-positive bacteria except two combination of PGR and Gram-negative bacteria on callus leaf extracts in agrement with Jisha and Benjamin (2009), who studied antibacterial activity on leaves and callus leaf extract of *I. tinctoria*. They reported that callus leaf extract showed no inhibiton zones on all bacterial treatments, while antibacterial activities were noted from leaves extracts.

The study conducted by Jahan et al. (2013) who reported that use of leaf exctract of *in vitro* raised plant on *Tylophora indica* had higher inhibition zone of *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823 than leaf callus extracts, as leaf extract of parent plant was low compared to previous mentioned extracts due to nutritional and hormonal manipulation added to culture medium.

It might be due to secondary metabolite accumulation of callus responsed against antibacterial or human pathogens influenced by plant growth regulator in agreement with Goyal et al. (2008). Jain et al. (2012), who noted that the medium with suitable concentration of individual or combination of auxin and cytokinin controls both callus growth and secondary metabolite production in *in vitro* cultures. The

 DOI: https://doi.org/10.31015/jaefs.2020.4.8

Table 2. Antibacterial activities of callus based methanol extracts obtained from hypocotyl, cotyledon, leaf explants of *I. zollingeriana* against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823

Treatments to to induce calli on explants taken from plants germinated on 0.1 mg/L GA_3 *		Inhibition zone (cm) formed against *S. aureus* ATTCC25923 by callus extracts of			Inhibition zone (cm) formed against *P. aeruginosa* ATCC25823 by callus extracts of		
BAP (mg/L)	NAA (mg/L)	Hypocotyl	Cotyledon	Leaf	Hypocotyl	Cotyledon	Leaf
0.5	0.10	1.6	0.0	1.5	2.4	1.2	0.0
0.5	0.15	2.0	0.0	0.0	0.0	0.0	0.0
1.0	0.10	1.8	0.0	1.3	0.0	0.0	0.0
1.0	0.15	2.2	0.0	0.0	0.0	0.0	0.0
1.5	0.10	2.1	0.0	0.0	0.0	0.0	0.0
1.5	0.15	1.3	1.0	1.2	0.0	0.0	0.0
2.0	0.10	2.3	0.8	0.0	1.5	0.0	0.0
2.0	0.15	2.0	1.2	0.0	0.0	0.0	0.0
2.5	0.10	1.5	1.0	0.0	0.0	0.0	0.0
2.5	0.15	0.9	0.9	0.0	0.0	0.0	0.0
Methanol extracts of pretreated Control treatments plants with 0.1 mg/L GA_3**		**1**	**2**	**3**	**4**	**5**	**6**
		1.2	1.0	1.0	1.3	1.1	0.1

**Liquid extracts obtained after pretreatment with liquid GA_3 followed by culture on MS medium for 30 days and post treatment with 10 combinations of BAP+NAA followed by bacterial culture on agar solidified Mueller-Hinton (MH) medium*

*** Liquid extracts obtained after pretreatment with liquid GA_3 followed by culture on MS medium for 30 days and no post treatment followed by bacterial culture on agar solidified Mueller-Hinton (MH) medium*

Table 3. Antibacterial activities of erythromycin, gentamycin, penicillin and chloramphenicol against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823

Control treatment	Inhibition zone (cm) formed against S. aureus ATTCC25923	Inhibition zone (cm) formed against P. aeruginosa ATCC25823	Antimicrobial index (%)
7- Erythromycin	2.1	1.9	100
8- Gentamycin	1.5	1.7	100
9- Penicillin	0.8	1.0	100
10-Chloramphenicol	2.5	2.6	92-88

results of this study are fully supported by literature and it is confirmed that the plant extracts are effective against Gram-positive and Gram-negative bacteria. However, the extracts from different explants under the influence of variable PGRs' combinations have variable capability to kill the two type of bacteria (Mohajer et al. 2012). In addition, Soorni and Kahrizi (2015) reported higher amounts of secondary metabolites sometimes could be produced by callus cultures excised from plant tissues. These results also indicated the significance and superiority of *I. zollingeriana* hypocotyl plant extracts with induction of variable inhibition zones formed against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823.

Different inhibition zones on various explants could vary depending on the source of explant and the characteristics of extraction solvents. Johnson and Babu (2010) has observed that some extraction solvents may act as potential inhibitors to both Gram-positive and Gram-negative bacteria. Praveen and Nair (2014) confirm that the increased concentration of extraction solvents act linearly with the increased antibacterial activity of *Myxopyrum smilacifolium* Blume. Other studies also show that the different antibacterial potentials of the callus and leaf methanol extracts on *Melaleuca alternifolia* could be due to changing composition of the bioactive compounds in part of plant used as source. This could improve and reduce ability of the solvent extracts to extract in their antibacterial activities (Jeyakani Santosh and Rajalaksmi, 2016). The results of the present study are in line with these studies reported earlier.

The results of this study were very encouraging and and showed the presence of biological active compounds in methanolic extracts of *I. zollengriana.* The antibacterial activity of the tested extracts of *I. zolingeriana* showed significant reduction in bacterial growth in terms of zone of inhibition in relation to the type of explant and treatments. All callus formation seemed to be dependent to explant and phytohormone concentrations with related antibacterial activity in agreement with Frank et al. (2000). Maximum antibacterial activities in terms of inhibition zones was noted using extracts from callus induced on hypocotyls. Varied but very similar inhibition zones were noted on the extracts obtained from calli of cotyledon and leaf origin. The antibacterial activities showed that callus extracts had higher variability inhibition zone than extracts obtained from the control treatments of

respective explants. This confirms that their occured chemical changes in the explants under the influence of hormones that influenced their antibacterial activities. Control treatments 1, 2, 3, 4, 5, 6 had minimum or poor inhibition zones. Inhibition zones due to Erythromycin, Gentamycin, Penicillin, and Chloramphenicol (control treatment 7, 8, 9, and 10) were comparable to the inhibition zones from the extracts of the 3 explants in agreement with (Borgatti,1998). According to the antimicrobial index, highest inhibition zones of hypocotyl extracts on both Gram-negative and Gram-positive bacteria (Table 2) showed 100% activity compared to Erythromycin, Gentamycin and Penicillin, when Chloramphenicol showed 88-92% activity, respectively (Table 3). It could be determined that the mentioned results had high antibacterial activity againts compared to Erythromycin, Gentamycin, Penicillin and Chloramphenicol.

There are many reports on the production of secondary metabolites and phenolic acids and antibacterial in many plant species including the plants belonging to the genus Indigofera; however, there are no reports about antibacterial activities of the extracts of *I. zollingeriana* cells or organ culture. Esimone et al. (1999), Ngoci et al. (2012) and Santos et al. (2015) have also reported antibacterial activities leaf, root extracts of *I. suffruticosa, I. lupatana, I. dendroides* against Gram-positive bacteria (*S. aureus*, *P. aeruginosa, Bacillus subtilis*) and Gram-negative bacteria (*K. pneumoniae, E. coli*).

Previous study by Christie et al. (1969) showing naturally occurring amino acid indospicine that is also present in aqueous extracts of *I. linnaei* (Hoffman and Gallaher 2007; Medeiros et al., 2011) as a natural toxin of the plant (Terras et al., 1995). Similar toxic peptide or protein fractions have been reported in aquous extracts of leaves of of *I. oblongifolia* by Dahot (1999) and change variably under the influence of PGRs. Contrarily, Leite et al. (2002) presume that biological activity of aqueous leaf extracts of *I. suffruticosa* could be due to the presence of lectins. The results indicated that *I. zollengriana* extracts in this study also have significant but variable antibacterial activity against both Gram-positive and Gram-negative bacteria depending on the type of explant used.

Conclusions

The results of this study signified that antibacterial activity had affected explants source (type of tissue or organ), extracts source and use of plant growth regulator. The methanol callus extracts of hypocotyl explant of *I. zollingeriana* have great potential as antibacterial compounds against *S. aureus* ATTCC25923 and *P. aeruginosa* ATCC25823 and can be used in the treatment of infectious diseases due to these bacteria. The understanding that *I. zollingeriana* plant cells, tissues, and organs carry distinguished compounds of medicinal importance is very important. In conclusion, further and more specific research is needed to establish and determine secondary metabolites as well as accumulation of antibacterial compounds with phytochemical screening on these and other callus extracts of this plant.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

The authors are grateful to Prof. Luki Abdullah, Bogor Agricultural University, for help in providing seeds for the study.

References

Annathurai, K., Palaniyandi, K. and Raamaiah R. (2015) Callus induction and antimicrobial activity from *Ziziphus mauritiana*. Lam cotyledon kernels. Indian Journal of Applied Microbiology, 18, 2, 32-39. Retrieved from http://ijamicro.com/18-2-2015/05.pdf

Arafa, N.M., Mohamed, S.S. and Aly, U.I. (2016) *In vitro* antimicrobial activity of carrot callus extracts as affected by tyrosine and tryptophan precursor. International Journal of PharmTech Research 9, 9, 121-129. Retrieved from https://www.researchgate.net/publication/316888805_In_vitro_antimicrobial_activity_of_carrot_callus_extracts_as_affected_by_tyrosine_and_tryptophan_precursor

Ashrafee, T.S., Rahman, M.M., Chakraborty, A. and Prodhan, S.H. (2014) Antibacterial potentiality of red sandalwood callus against pathogenic isolates of aeromonas and pseudomonas. Universal Journal of Plant Science, 2, 4, 86-91. Doi: https://doi.org/10.13189/ujps.2014.020402

Bakasso, S.A., Lamien-Meda, C.E., Lamien, M., Kiendrebeogo, J., Millogo, A.G. and Ouedraogo Nacoulma, O.G. (2008) Polyphenol contents and antioxidant activities of five *Indigofera* species (Fabaceae) from Burkina Faso. Pakistan Journal of Biological Sciences, 11, 1429-1435. Doi: https://doi.org/10.3923/pjbs.2008.1429.1435

Basra, A. (2000). Plant Growth Regulators in Agriculture and Horticulture: Their Role and Commercial Uses. Taylor & Francis, CRC Press, USA, 264 pages.

Bisio, A., Fraternale, D., Schito, A.M., et al. (2016) Establishment and analysis of *in vitro* biomass from *Salvia corrugate* Vahl. and evaluation of antimicrobial activity. Phytochemistry, 122, 276-285. Doi: https://doi.org/10.1016/j.phytochem.2015.12.017

Borgatti, S.P. (1998). Elicitation techniques for cultural domain analysis. In: Ethnographer's Toolkit, Schensul, J., LeCompte, M. (Eds). Altimira Press, Walnut Creek, CA, 1-2.

Chouhan, S., Sharma, K. and Guleria, S. (2017) Antimicrobial activity of some essential oils-present status and future

perspectives. Medicines, 4, 3, 58. Doi: https://doi.org/10.3390/medicines4030058

Christie, G.S., Madsen, N.P. and Hegarty, M.P. (1969) Acute biochemical changes in rat liver induced by the naturally occurring amino acid indospicine. Biochemical Pharmacology, 18, 693-700. Doi: https://doi.org/10.1016/0006-2952(69)90039-2

Cohen, M.L. (1992) Epidemiology of drug resistance: implications for a post-antimicrobial era. Science, 257, 1050-1055. Doi: https://doi.org/10.1126/science.257.5073.1050

Dahot, M.U. (1999) Antibacterial and antifungal activity of small protein of *Indifogera oblongifolia* leaves. Journal of Ethnopharmacology, 64, 277-282. Doi: https://doi.org/10.1016/S0378-8741(98)00136-6

ElNour, M.E.M., Ali, A.M.A. and Saeed, B.E.A.E. (2015) Antimicrobial activities and phytochemical screening of callus and seeds extracts of fenugreek (*Trigonella foenum-graecum*). International Journal of Current Microbiology and Applied Sciences, 4, 2, 147-157. Retrieved from *https://www.researchgate.net/publication/324648029_Antimicrobial_Activities_and_Phytochemical_Screening_of_Callus_and_Seeds_Extracts_of_Fenugreek_Trigonella_foenum-graecum*

Esimone, C.O., Adikwu, M.U. and Muko, K.N. (1999) Antimicrobial properties of *Indigofera dendroides* leaves. Fitoterapia, 70, 517-520. Doi: https://doi.org/10.1016/S0367-326X(99)00072-6

Frank, M., Rupp, H.M, Prinsen, E., Motyka, V., Van Onckelen, H. and Schmülling, T. (2000) Hormone autotrophic growth and differentiation identifies mutant lines of Arabidopsis with altered cytokinin and auxin content or signaling. Plant Physiology, 122, 721-729. Doi: https://doi.org/10.1104/pp.122.3.721

Ghasemi, Y., Yazdi, M.T., Shokravi, S., et al. (2003) Antifungal and antibacterial activity of paddy fields Cyanobacteria from the North of Iran. Journal of Science, Islamic Republic of Iran, 14, 203-209. Retrieved from https://www.researchgate.net/publication/242615525_Antifungal_and_antibacterial_activity_of_paddy-fields_cyanobacteria_from_the_north_of_Iran

Goyal, S. and Ramawat, K.G. (2008) Synergistic effect of morphactin on cytokinin-induced production of isoflavonoids in cell cultures of *Pueraria tuberosa* (Roxb. ex. Willd.) DC. Plant Growth Regulation, 55, 175-181. Doi: https://doi.org/10.1007/s10725-008-9271-x

Hoffman, B. and Gallaher, T. (2007) Importance indices in Ethnobotany. Ethnobotany Research & Applications, 5, 201-218. Retrieved from https://pdfs.semanticscholar.org/9cfc/d09cc02d0a71213af42636fc23d22e5c4b3e.pdf

Inouye, S., Takizawa, T. and Yamaguchi, H. (2001) Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. The *Journal of Antimicrobial Chemotherapy,* 47, 5, 565-573. Doi: https://doi.org/10.1093/jac/47.5.565

Iwase, A., Mitsuda, N., Koyama, T., et al. (2011) The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis*. Current Biology, 21, 508-514. Doi: https://doi.org/10.1016/j.cub.2011.02.020

Jahan, N., Khatoon, R., Shahzad, A., Shahid, M. and Ahmad, S. 2013. Comparison of antibacterial activity of parent plant of *Tylophora indica* Merr. with its *in vitro* raised plant and leaf callus. African Journal of Biotechnology, 12, 31, 4891-4896. Doi: https://doi.org/10.5897/AJB2013.12488

Jain, S.C., Pancholi, B. and Jain, R. (2012) *In-vitro* callus propagation and secondary metabolite quantification in *Sericostoma pauciflorum.* Iranian Journal of Pharmaceutical Research, 11, 4, 1103-1109. Doi: https://doi.org/10.22037/IJPR.2012.1202

Jansen, A.M., Cheffer, J.J.C. and Svendsen, A.B. (1987) Antimicrobial activity of essencial oils: a 1976-1986 literature review: Aspects of test methods. Planta Medica, 40, 395-398. Doi: https://doi.org/10.1055/s-2006-962755

Jeyakani Santhosh, M. and Rajalakshmi, M. (2016) Antimicrobial activities screening of callus and leaf extracts of tea tree (*Melaluca alternifolia*). World Journal of Pharmacy Pharmaceutical Sciences, 5, 9, 1155-1162. Retrieved from https://www.wjpps.com/Wjpps_controller/abstract_id/5797

Jisha, V.N. and Benjamin, S. (2009) Micropropogation and anti microbial activity studies on *Indigofera tinctoria*. Journal of Tropical Medicinal Plants, 10, 1, 73-79. Retrieved from https://www.researchgate.net/publication/291344658_Micropropogation_and_anti_microbial_activity_studies_on_Indigofera_tinctoria

Johnson, M. and Babu, A. (2010) Somoclonal variation studies on *Passiflora mollussima* (H.B.K.) Bailey using phytochemical methods. Natural Products An Indian Journal, 6, 1, 5-10. Retrieved from https://www.tsijournals.com/articles/somoclonal-variation-studies-on-passiflora-mollussima-hbk-bailey-using-phytochemical-methods.pdf

Kumar, R.S., Moorthy, K., Vinodhini, R. and Punitha, T. (2013) Antimicrobial efficacy and phytochemical analysis of *Indigofera trita* Linn. African Journal of Traditional Complementary and Alternative Medicines, 10, 3, 518-525. Doi: https://doi.org/10.4314/ajtcam.v10i3.20

Leite, S.P., Leite, A.C.R., Paiva, P.M.G., Coelho, L.C.B.B., Lima, E.O. and Lima, V.L.M. 2002. Atividade Lectínica em folhas de *Indigofera suffruticosa* (Lectin activity in leaves from Indigofera suffruticosa). VI Reunião Regional da SBBq, Nordeste, Fortaleza Ceará Resumo Completo. Retrieved from http://sbbq.iq.usp.br/arquivos/regional/2002/cdresumo/Estendido/009.pdf

Leite, S.P., Vieira, J.R.C., Medeiros, P.L., Leite, R.M.P., Lima, V.L.M., Xavier, H.S. and Lima, E.O. (2006) Antimicrobial activity of *Indigofera suffruticosa*. Evidence-based Complementary and Alternative Medicine, 3, 2, 261-265. Doi: https://doi.org/10.1093/ecam/nel010

Medeiros, M.F.T., Silva, P.S. and Albuquerque, U.P. (2011) Quantification in ethnobotanical research: an overview of indices used from 1995 to 2009. Sitientibus série Ciências Biologicas, 11, 2, 211-230. Retrieved from https://pdfs.semanticscholar.org/2628/b72422523a7c4d5f6c32d8638864138476de.pdf

Mohajer, S., Taha, R.M., Khorasani, A. and Yaacob, J.S. (2012) Induction of different types of callus and somatic embryogenesis in various explants of Sainfoin (*Onobrychis sativa*). Australian Journal of Crop Science, 6, 8, 1305-1313. Retrieved from http://www.

cropj.com/mohajer_6_8_2012_1305_1313.pdf

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15, 3, 473-497. Doi: https://doi.org/10.1111/j.1399-3054.1962.tn08052.x

Murthy, H.N., Lee, E.J. and Peak, K.Y. (2014) Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. Plant Cell, Tissue and Organ Culture, 118, 1-16. Doi: https://doi.org/10.1007/s11240-014-0467-7

Nascimento, G.G.F., Locatelli, J., Freitas, P.C. and Silva, G.L. (2000) Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian Journal of Microbiology, 31, 4, 247-256. Doi: https://doi.org/10.1590/S1517-83822000000400003

Ngoci, S.N., Matasyoh, J.C., Mwaniki, C.G. and Mwendia, C.M. (2012) Antibacterial activity of methanol root extract of *Indigofera lupatana* Baker F. Eastern Journal of Medicine, 17, 1, 11-16. Retrieved from https://www.journalagent.com/ejm/pdfs/EJM_17_1_11_16.pdf

Nisar, M., Tariq, S.A., Marwat, I.K., Shah, M.R. and Khan, I.A. (2009) Antibacterial, antifungal, insecticidal, cytotoxicity and phytotoxicity studies on *Indigofera gerardiana*. Journal of Enzyme Inhibition and Medicinal Chemistry, 24, 1, 224-229. Doi: https://doi.org/10.1080/14756360802051313

Perez, C., Paul, M. and Bazerque, P. (1990) An antibiotic assay by agar-well diffusion method. Acta Biologiae et Medicinae Experimentalis 15: 113-115.

Petrosyan, M., Shcherbakova, Y., Sahakyan, N., Vardanyan, Z., Poladyan, A., Popov, Y. and Trchounian, A. (2015) *Alkanna orientalis* (L.) Boiss. plant isolated cultures and antimicrobial activity of their extracts: phenomenon, dependence on different factors and effects on some membraneassociated properties of bacteria. Plant Cell, Tissue and Organ Culture, 122, 727-738. Doi: https://doi.org/10.1007/s11240-015-0806-3

Prabakaran, M., Chandrakala, N. and Panneerselvam, A. (2011) Antimicrobial activity of *Indigofera glandulosa* (wild). Asian Journal of Plant Science and Research, 1, 2, 18-25. Retrieved from https://www.imedpub.com/articles/antimicrobial-activity-of-indigofera-glandulosa-wild.pdf

Praveen, R.P. and Nair, A. (2014) Comparative stufy on antimicrobial efficacy of methanolic extract of root, callusand fruit extracts of *Myxopyrum Smilacifolium* Blume. International Journal of Applied Sciences and Biotechnology, 2, 4, 521-524. Doi: https://doi.org/10.3126/ijasbt.v2i4.11362

Rajaperumal, S., Nimmi, M. and Ranjitha Kumari, B.D. (2013) *In vitro* studies on antimicrobial and antioxidant effect of methanolic extract of *Indigofera aspalathoides* (Vahl ex DC) and its cytotoxic property against human lung cancer cell line NCI H460. European Journal of Experimental Biology, 3, 3, 18-29. Retrieved from https://www.imedpub.com/articles/iin-vitroi-studies-on-antimicrobial-and-antioxidant-effect-of-methanolic-extractof-iindigofera-aspalathoidesi-vahl-ex-dc-and-its-c.pdf

Ranukadevi, K.P. and Suhani Sultana, S. (2011) Determination of antibacterial, antioxidant and cytotoxicity effect of Indigofera tinctoria on Lung Cancer Cell Line NCI-h69. International Journal of Pharmacology, 7, 3, 356-362. Doi: https://doi.org/10.3923/ijp.2011.356.362

Ravinder Singh, C. (2011) Antimicrobial effect of callus and natural plant extracts of *Premna serratifolia* L. International Journal of Pharmaceutical and Biomedical Research, 2, 1, 17-20. Retrieved from https://www.semanticscholar.org/paper/Antimicrobial-effect-of-Callus-and-Natural-plant-of-Singh/f99be0881bc783486c128269a94c9d438b9de92d

Saikia, M., Shrivastava, K. and Singh, S.S. (2013) Effect of culture media and growth hormones on callus ınduction in *Aquilaria malaccensis* Lam., a medicinally and commercially ımportant tree species of North East India. Asian Journal of Biological Sciences, 6, 96-105. Doi: https://doi.org/10.3923/ajbs.2013.96.105

Santos, I.P., Silva, L.C.N., Silva, M.V., Araújo, J.M., Cavalcanti, M.S., and Lima, V.L.M. (2015) Antibacterial activity of endophytic fungi from leaves of *Indigofera suffruticosa* Miller (Fabaceae). Frontiers in Microbiology, 6, 350. Doi: https://doi.org/10.3389/fmicb.2015.00350

Sari, Y.P., Kusumawati, E., Saleh, C., Kustiawan, W. and Sukarningsih. 2018. Effect of sucrose and plant growth regulators on callogenesis and preliminary secondary metabolic of different explant *Myrmecodia tuberosa*. Nusantara Bioscience, 10, 3, 183-192. Doi: https://doi.org/10.13057/nusbiosci/n100309

Shrikhande, M., Thengane, S.R. and Mascarenhas, A.F. (1993) Somatic embryogenesis and plant regeneration in Azadirachta indica A. Juss. In Vitro Cellular & Developmental Biology-Plany, 29, 1, 38-42. Doi: https://doi.org/10.1007/BF02632237

Singh, B. and Sharma, R.A. (2015) Antioxidant and antimicrobial activities of callus culture and fruits of *Phyllanthus emblica* L. Journal of Herbs, Spices and Medicinal Plants, 21, 3, 230-242. Doi: https://doi.org/10.1080/10496475.2014.949998

Skoog, F. and Miller, C.O. (1957) Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Retrieved from Symposia of the Society for Experimental Biology, 11, 118-130. https://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55))/reference/ReferencesPapers.aspx?ReferenceID=1583255

Soorni, J. and Kahrizi, D. (2015) Effect of genotype, explant type and 2,4 D on cell dedifferentiation and callus induction in cumin (*Cuminum cyminum* L.) medical plant. Journal of Applied Biotechnology Reports, 2, 3, 265-270. Retrieved from https://www.researchgate.net/publication/284579672_Effect_of_Genotype_Explant_Type_and_24-D_on_Cell_Dedifferentiation_and_Callus_Induction_in_Cumin_Cuminum_cyminum_L_Medicinal_Plant

Staba, E.J. (1980). Plant tissue culture as a source of biochemicals. CRS Press, Boca Raton, Florida, 285 pages.

Spartz, A.K. and Gray, W.M. (2008) Plant hormone receptors: new perceptions. Genes and Development, 22, 16, 2139-2148. Doi: https://doi.org/10.1101/gad.1693208

Terras, F.R.G., Eggermont, K., Kovaleva, V. et al. (1995) Small cysteine rich antifungal proteins from radish: their role in host defence. Plant Cell, 7, 5, 573–588. Doi: https://doi.org/10.1105/tpc.7.5.573

Vaezi, Z., Daneshvar, M.H., Heidari, M. and Chehrazi, M. (2015) Indirect regeneration plant Fenugreek (*Trigonella foenumgraecum* L), with the use of plant growth regulators *in vitro*. Bulletin of Environment, Pharmacology and Life Sciences, 4, 5, 103-108. Retrieved from http://bepls.com/beplsapril2015/14f.pdf

Verpoorte, R., van der Heijden, R., van Gulik, W.M. and ten Hoopen, H.J.G. (1991). Plant biotechnology for the production of alkaloids: present status and prospects. In: The alkaloids: chemistry and pharmacology, Brossi, A. (ed), Vol 40. Academic Press, San Diego, 1-187