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

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Abstract

The study explores 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* ATCC25923 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 types of bacteria used in this study.

Keywords: Callus, Extract, Legume, Medicinal, Phytohormone

Introduction

A huge quantity of antibiotics are being introduced into pharmaceutical industry since last 40-50 years. Despite all efforts to introduce new drugs, there are numerous reports of emerging antibiotic resistance to the drugs due to natural genetic ability of bacteria to acquire increased resistance (Cohen, 1992). This has led 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 shows a large number of researchers, who have reported increased incidence of resistant bacteria all over the 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 medical purposes. This study investigated the antibacterial potential of various explants of *I. zollingeriana*, one of the popular leguminous crop with potential medicinal use.
treatment of many health disorders. However, there is no study that reports antibacterial activity/ies of \textit{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 \textit{Phyllanthus emblica} (Singh and Sharma, 2015), \textit{Ziziphus mauritiana} Lam. (Annathurai et al., 2015), \textit{Alkanna orientalis}'s roots (Petrosyan et al., 2015), \textit{Daucus carota}'s root, stem and petirole explants (Arafa et al., 2016), \textit{Salvia corregata}'s leaf and stem explants (Bisio et al., 2016), \textit{Pterocarpus santalinus}'s leaf explants extracts (Ashrafie et al., 2014), \textit{Trigonella foenum-graecum}'s cotyledons and hypotocyl explants extracts etc. from the callus extracts (ElNour et al., 2015).

Except \textit{I. zollingeriana}, antibacterial studies have been reported from direct leaves extracts of \textit{I. dendroides}, \textit{I. oblongifoli}, \textit{I. suffraticosa} and \textit{I. tinctoria} ( Dahot, 1999; Esimone et al., 1999; Leite et al., 2006; Ranukadevi and Suhani Sultana, 2011; Santos et al., 2015), crude extract of \textit{I. gerardiana} and \textit{I. tria} (Nisar et al., 2009; Kumar et al., 2013), root extract of \textit{I. lupatana} and \textit{I. aspalathoides} (Ngoci et al., 2012; Rajaperumal et al., 2013), leaves and root extract of \textit{I. glandulosa} (Prabakaran et al., 2011) and leaves and callus leaf extracts under \textit{in vitro} cultures on \textit{I. tinctoria} (Jisha and Benjami, 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 \textit{Staphylococcus aureus} ATTTCC25923 Gram-positive and \textit{Pseudomonas aeruginosa} ATTTCC25823 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$_2$SO$_4$ for 5 minutes. Subsequently, they were rinsed 3×5 minutes with autoclaved distilled water to remove the traces of H$_2$SO$_4$. 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 seedlings were transferred to agar solidifed 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 extract and test their antibacterial activities against \textit{Staphylococcus aureus} ATTTCC25923 Gram-positive and \textit{Pseudomonas aeruginosa} ATTTCC25823 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 Antimicrobial 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).

\[
\text{Antimicrobial Index} = \left( \frac{\text{Extract inhibition zone}}{\text{Antibiotic inhibition zone}} \right) \times 100
\]

#### 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.
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

<table>
<thead>
<tr>
<th>Medium</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypocotyl</td>
<td>Cotyledon</td>
</tr>
<tr>
<td>BAP (mg/L)</td>
<td>NAA (mg/L)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.10</td>
<td>3.39dA</td>
</tr>
<tr>
<td>0.5</td>
<td>0.15</td>
<td>4.42cA</td>
</tr>
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<td>1.0</td>
<td>0.10</td>
<td>6.88aA</td>
</tr>
<tr>
<td>1.0</td>
<td>0.15</td>
<td>5.16bA</td>
</tr>
<tr>
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<td>0.10</td>
<td>4.61cA</td>
</tr>
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<td>5.33bA</td>
</tr>
<tr>
<td>2.0</td>
<td>0.10</td>
<td>5.82abA</td>
</tr>
<tr>
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<td>0.15</td>
<td>5.48bA</td>
</tr>
<tr>
<td>2.5</td>
<td>0.10</td>
<td>4.35aA</td>
</tr>
<tr>
<td>2.5</td>
<td>0.15</td>
<td>5.73bA</td>
</tr>
</tbody>
</table>

Control treatments

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>0.00</td>
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</tbody>
</table>

Means not followed by the same small letter within a column differ significantly at \( p < 0.05 \) using Duncan test.

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 different 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 growth regulators, sugars, growth medium, culture environment affects the activity and induction of secondary metabolites in the regenerating explants/callus positively or negatively and accumulation of metabolic 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.
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 GA₃ (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 GA₃ 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 GA₃ 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 ATCC25923 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 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 GA₃ pretreated plants used as control 5. Methanol extracts of the rest of the treatments failed to induce any inhibition zone.

No inhibition 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 against 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 antibacteral activity on leaves and callus leaf extract of I. tinctoria. They reported that callus leaf extract showed no inhibition 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 extract of in vitro raised plant on Tylophora indica had higher inhibition zone of S. aureus ATCC25923 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 responded 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
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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 ATCC25923 and P. aeruginosa ATCC25823

<table>
<thead>
<tr>
<th>Treatments to to induce calli on explants taken from plants germinated on 0.1 mg/L GA, *</th>
<th>Inhibition zone (cm) formed against S. aureus ATCC25923 by callus extracts of</th>
<th>Inhibition zone (cm) formed against P. aeruginosa ATCC25823 by callus extracts of</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP (mg/L)</td>
<td>NAA (mg/L)</td>
<td>Hypocotyl</td>
</tr>
<tr>
<td>0.5</td>
<td>0.10</td>
<td>1.6</td>
</tr>
<tr>
<td>0.5</td>
<td>0.15</td>
<td>2.0</td>
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<td>1.0</td>
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<td>1.8</td>
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<td>1.5</td>
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<tr>
<td>2.5</td>
<td>0.15</td>
<td>0.9</td>
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</table>

Methanol extracts of pretreated Control treatments plants with 0.1 mg/L GA, **

<table>
<thead>
<tr>
<th></th>
<th>Inhibition zone (cm) formed against P. aeruginosa ATCC25823</th>
<th>Antimicrobial index (%)</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>1.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Liquid extracts obtained after pretreatment with liquid GA, 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, 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 ATCC25923 and P. aeruginosa ATCC25823

<table>
<thead>
<tr>
<th>Control treatment</th>
<th>Inhibition zone (cm) formed against S. aureus ATCC25923</th>
<th>Inhibition zone (cm) formed against P. aeruginosa ATCC25823</th>
<th>Antimicrobial index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7- Erythromycin</td>
<td>2.1</td>
<td>1.9</td>
<td>100</td>
</tr>
<tr>
<td>8- Gentamycin</td>
<td>1.5</td>
<td>1.7</td>
<td>100</td>
</tr>
<tr>
<td>9- Penicillin</td>
<td>0.8</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>10-Chloramphenicol</td>
<td>2.5</td>
<td>2.6</td>
<td>92-88</td>
</tr>
</tbody>
</table>

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 ATCC25923 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) confirmed 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 showed the presence of biological active compounds in methanolic extracts of I. zollingeriana. The antibacterial activity of the tested extracts of I. zollingeriana 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 treatments. This could improve and reduce ability of the solvent extracts to extract in their antibacterial activities 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 occurred 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 against 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 normally occurring amino acid indosipicine 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 aqueous 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 ATCC25923 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
Conflicts 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.

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


