Synthesis of secondary metabolites by *Cladosporium resinae* (NRL-6437) under different growth media and chemical inducers and their pharmaceutical activity

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Abstract: The role of different growth media and chemical enhancer on synthesis of secondary metabolites *Cladosporium resinae* (NRL-6437) was investigated for their *in vitro* biological activities. *Cladosporium resinae* (NRL-6437) were grown in various nutrient media (Czapek-dox Broth (CB), Czapek Yeast-extract Broth (CYB), Yeast Extract Sucrose (YES), Potato Dextrose Broth (PDB) and Czapek-dox (supplemented with glucose and starch) Broth (CGSB)) for the production of metabolites. Two chemical epigenetic modifiers (suberoyl-anilide hydroxamic acid (SAHA) and 5-azacytidine (5-AZA) were also used for the expression of silent genes for secondary metabolite production. Our results indicated that among different media, Czapek yeast extract broth produced more secondary metabolites. Application of 15mM of both modifiers was effective for the expressions of silent genes resulting in an increased metabolites production. Secondary metabolites extracted in ethyl acetate and fractionized in n-Hexane were also tested for their biological activity. The secondary metabolites revealed varying degrees of growth inhibitions of the tested organisms. Similarly, these metabolites were also active against brine shrimps and *Lemna*.

Keywords: *Cladosporium resinae*, silient genes, chemical enhancers, growth media, pharmaceutical activity.

INTRODUCTION

About 50% of all deaths in the developing countries are caused by different microbial infections. Though therapeutic agents of both natural and synthetic origins are available to cure different microbial infections, however, the injudicious use of these therapeutic drugs has increased resistance in different microbes (Valverde et al., 2008). Therefore, searching for new, safe and more potentially biologically active metabolite is an urgent need of the developing countries (Hadi et al., 2008).

About three quarter population of the world depends on indigenous plants and their materials for treating different kinds of health related problems and about 56% of lower income population of the world use plants medicine for their major health care (Pirzada et al., 2010). A renewed curiosity has occurred in the last two decades to investigate the phytochemicals and antimicrobial activities of native and naturalized plants for pharmaceutical and nutritional purposes (Bakht et al., 2011 a, b, c and d; 2012; 2013 a,b; 2014 a, b,c; 2015; Nasir et al., 2015; Ullah et al., 2015; Zakir et al., 2015; Chaun et al., 2015; Parveen and Bakht, 2015; Bilal et al., 2016; Wajid et al., 2016 a, b; Amjad et al., 2016; Anwar et al., 2016; Anwar et al., 2017). However, fungi cannot be used directly for domestic health care due to its toxic nature, even though fungi are good producer of biologically active metabolites. Majority of the human and animal therapeutic agents have been produced and isolated from fungi through fermentation, or by modification of the product (Montenegro et al., 2004).

Secondary metabolites are low-molecular-weight compounds with often potent physiological activities. Digitalis, morphine and quinine are plant based secondary metabolites, whereas penicillin, cephalosporin, ergotrate and the statins are also well known important fungal secondary metabolites (Keller et al., 2005). Although chemically diverse, all secondary metabolites are produced by a few common biosynthetic pathways, often in conjunction with morphological development. Fungi are remarkable organisms that readily produce a wide range of natural products often called secondary metabolites (Calvo et al., 2002; Abid et al., 2016). The benefits of these secondary metabolites are some times not known for the producing organism (Bu’Lock, 1961). However, these compounds are of considerable interest as many natural products are of medical, industrial and/or agricultural importance. Some natural products are deleterious (e.g., mycotoxins), while others are beneficial (e.g., antibiotics) to humankind (Dorn, 1970). It is well known that natural products are generally related to cell differentiation or development, and in fact most secondary metabolites are produced by organisms that reveal filamentous growth and have a relatively complex
morbidity. Fungi produce variety of metabolites with high therapeutic agent such as antibiotics, cyto-toxins, pesticides etc (Siddhardha et al., 2009). Genetic approaches are useful techniques for estimating the biosynthetic potential of microorganisms. This approach has been remained successfully for the gene(s) involved in the synthesis of polyketides and histone deacetylase inhibitor (Bode and Muller, 2005; Pelzer et al., 2005; Fisch et al., 2009; Cherblanc et al., 2013). Fungi may produce broad spectrum of diverse metabolites depending on the cultivating conditions as well as on additives or chemical modifiers (Knight et al., 2003; Bode et al., 2002).

**MATERIALS AND METHODS**

**Fungal strain and growth condition**

The spore suspensions (106 spores/ml) of Cladosporium resinae were transferred to different culture media i.e. Czapek-dox Broth (CB), Czapek Yeast-extract Broth (CYB), Yeast Extract Sucrose (YES), Potato Dextrose Broth (PDB) and Czapek-dox (supplemented with glucose and starch) Broth (CGSB). The culture was then incubated at 28°C in shaking incubator at 200 rpm for 12 days.

**Use of epigenetic modifiers**

Two epigenetic modifier suberoyl anilide hydroxamic acid (SAHA) and 5-azacytidine (5-AZA) were tested in different concentrations of 1, 5, 10, 15 and 20 mM/100 ml in Czapek dextrose broth (CYB) media to activate the silence gene(s) for the production of metabolite (Fisch et al., 2009).

**Extraction of metabolites from liquid culture and fractionation**

After the incubation, 200 to 500 µl of concentrated HCl were added and culture was grinded with blender and filtered with Whatman filter paper using vacuum pump. Equal volume of ethyl acetate was added and mixed thoroughly for half an hour. The organic layer were recovered and washed with 2M brine solution. Anhydrous sodium sulphate (Na2SO4) was used to remove the aqueous components. The metabolites were concentrated by rotary evaporator at 45°C. The crude metabolites were recovered and dried under liquid nitrogen. The metabolites (400 mg) were suspended in 200 ml distilled water and defatted with n-hexane and about 180 mg crude metabolites were reserved for biological screening.

**In vitro antibacterial activity**

The antibacterial activity was carried by disc diffusion assay as described in Bauer et al. (1966) Six bacteria (Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri and Staphylococcus aureus) were incubated in 10 ml x 6 of nutrient broth (different additive) at 37°C for 24 hours for the production of fresh culture. Then 20 ml x 6 of the nutrient agar were taken in sterile petri plates and allowed to cool and 0.2 ml of each experimental organism was taken from broth culture and poured on the agar media. Stock solutions were prepared in sterile di-methyl sulfoxide (DMSO) with 1, 10, 20, 50, 100, 250, 500 µg/ml concentrations and was poured in each well. Carbenicillin was used as standard drug with concentration of 100 µg/ml. The plates were left at room temperature for 2-3 hours for the diffusion of the samples and transferred to incubator for 24 hours incubation at 37°C (Benkeblia, 2004; Gulluce et al., 2003). The experiments were conducted in triplicate and the zone of inhibitions was determined by the following formula:

\[
\text{Inhibition} \% = \frac{\text{Zone of sample}}{\text{Zone of control}} \times 100
\]

**Antifungal activity**

Antifungal activity was carried out as describes in Ramdas et al. (1998). Different fungi (Aspergillus flavus, Candida albicans, Candida glabrata, Fusarium solani, Micsroporum canis and Trichophyton longisusus) were cultured in 25 ml x 6 of potato dextrose agar (PDA-different additive) at 28°C for 7 days for the production of fresh culture. Then 10 ml x 6 of the potato dextrose agar were taken in sterile test tubes and the test samples were added at concentrations of 10, 20, 50, 100, 250, 500 and 1000 µg/ml from stock solution prepared in sterile di-methyl sulfoxide (DMSO) allowed in slanted position to cool and a small piece of about 4mm in diameter was detached from the old culture (7 days old) of fungi and implanted. Meconazol was used as standard drug with concentration of 100 µg/ml. The test tubes were transferred to incubator for 7 days and 28°C. The experiments were conducted in triplicate and inhibition percentage was determined by the following formula.

\[
\text{Inhibition} \% = \frac{\text{Growth of controle}}{\text{Growth of sample}} \times 100
\]

**Brine shrimp lethality assay**

Artificial hatching media or sea water was made by dissolving water and then filtered. One milligram (1mg) of brine-shrimp eggs were transferred to the artificial hatching media (3.8gm of sea salt in 1000ml of de-ionized water) in to a small tank and left for 24hours at 25°C for hatching. The test samples were transferred to Sea water with concentration of 1000, 100 and 10 µl/ml. Ten (10) shrimps were transferred to each vial and left for 24 hours, the surviving shrimps were recorded and value of LD50 were calculated as described by Meyer et al. (1982).

\[
\text{Mortality} \% = \frac{\text{Shrimps in sample}}{\text{Shrimps in -ve control}} \times 100
\]

**Phyto-toxic activity**

The test samples were transferred to E-medium with concentration of 1000, 100 and 10 µl/ml. The solution was allowed for 24hours to evaporate the excessive solvent under aseptic condition. After 24hours 20ml of the medium with slightly basic pH was added to sterilized
flasks having ten healthy plants of *Lemna acquinoctialis* with three fronds each and kept in growth cabinet/chamber for seven days (at 30 °C; light intensity of 9000 lux and 60% humidity). On eighth day the fronds was measured and parquet was used as positive control (De Almeida et al., 2010).

**STATISTICAL ANALYSIS**

Data are presented as mean values of three replicates. MSTATC computer software was used to carry out statistical analysis (Russel and Eisensmith, 1983).

**RESULTS**

**Optimization of growth media**

Optimization of media is an important factor for the growth of any microbe and metabolites production. For this purpose five different media were used for the growth and maximum production of metabolites (fig. 1). Metabolites production in Czapek Yeast Extract Broth started at day 2 (9mg) with gradual increase and maximum production was achieved at day 9 (62mg). Czapek-dox Broth, Yeast Extract Sucrose, Potato Dextrose Broth Czapek-dox (supplemented with glucose and starch) Broth were not effective to induce maximum production of metabolite as evident from low yield by these media (29mg at day 8).

**Effect of epigenetic modifiers on metabolites**

Two epigenetic modifiers i.e. suberoyl anilide hydroxamic acid (SAHA) and 5-azacytidine (5-AZA) in different concentrations (1 to 20 µM/100ml) were tested in Czapek Yeast Extract Broth to investigate their role in secondary metabolite production (fig. 2). The medium containing 10mM concentration of SAHA showed an increase of 17 mg by swapping the production from 25 to 42mg on day 4. The highest production of 105mg was observed on day 8 with an increase of 20mg and afterward a gradual decrease was noted in metabolite production. These results suggested that both the lower and higher concentrations of SAHA (1, 5, 10 and 20 µM/100ml) were ineffective to induce salient genes for significant production of metabolites in the optimized media. In case of 5-AZA, addition of 15µM/100ml resulted in maximum production (30mg by swapping the production from 25 to 55mg) of metabolites on day 4. After days 4, a rapid increase of 28, 58 and 28mg by swapping the production from 40 to 68, 52 to 110 and 72 to 100mg on day 5, 6 and 7 respectively was observed. Highest production of 115mg was observed on day 8 with an increase of 30mg and then a gradual decrease was observed.

**Fig. 2:** Epigenetic modifier (suberoyl anilide hydroxamic acid (SAHA) and 5-azacytidine (5-AZA)) were used in (CGSB) media for the expression of the silent genes for their metabolites production. The graph shows that 10mM of SAHA and 15mM of 5-AZA is effective for activation of the genes.

**Fig. 3:** Antibacterial activities of ethyl acetate extract of *C. resinae* (NRL-6437), the graph shows that 500 µg/ml concentrations of the metabolites in ethyl acetate are active against all the pathogenic bacteria.

**Antibacterial activity**

Figs. 3 and 4 show antibacterial activities of the crude metabolites of *Cladosporium resinae* (NRL-369) against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri* and *Staphylococcus aureus*. Different concentrations of metabolites extracted with ethyl acetate revealed variable inhibition of the test micro-organisms. The data suggested that slight growth inhibition was noted against *B. subtilis* (7.5%) and *S. aureus* (13%) at 50µg/ml concentrations. The growth of *S. flexneri* (26%) and *S. aureus* (25%) was reduced by 26% and 25% respectively at 100µg/ml concentrations. Similarly, at 250µg/ml concentration growth of *B. subtilis* and *S. aureus* was inhibited by 36% and 31% respectively.
The same concentration reduced the growth of *E. coli*, *K. pneumonia*, *S. flexneri* and *S. typhi* by 25%, 25%, 27% and 15% respectively. When concentration was increased up to 500 µg/ml, high activity was noted against *B. subtilis*, *S. flexneri* and *K. pneumoniae* (64.5%, 59.5%, 51% respectively. Similarly, the same concentration reduced the growth of *E. coli*, *S. typhi* and *S. aureus* by (35% 38.5% and 36% respectively. The first four concentrations of *n*-Hexane fraction (1 to 50 µg/ml) showed no inhibition activity against any pathogenic bacteria. The results suggested that hundred and 250 µg/ml concentration of the same fraction showed 10% and 32% growth inhibition respectively against *E. coli*. Good activity was observed for *E. coli* (57.5%) at 500 µg/ml concentration.

**Fig. 4**: Antibacterial activities of *n*-Hexane fraction of *C. resinae* (NRL-6437), the graph shows that 500 µg/ml concentrations of the metabolites in *n*-Hexane were active against *E. coli* and *S. aureus*.

**Fig. 5**: Antifungal activities of ethyl acetate extract of *C. resinae* (NRL-6437), the graph shows that 1000 µg/ml concentrations of the metabolites in ethyl acetate are active against all the pathogenic fungi.

**Antifungal activities**

Antifungal activities of the crude metabolites of Aspergillus carbonarius (NRL-369) against the test organisms (*Aspergillus flavus, Candida albicans, Candida glabrata, Fusarium solani, Microsporum canis* and *Trichophyton longifusus*) are shown in fig. 5 and 6. Different concentrations of metabolites extracted with ethyl acetate have shown variable inhibition. All the pathogenic fungi survived and no zone of inhibition was observed at 10, 20 & 50 µg/ml concentration of metabolites in ethyl acetate fraction. Low linear inhibitions were recorded against all the tested fungi except *M. canis* using 100 µg/ml concentrations. Similarly, low activities were recorded against *F. solani, A. flavus, C. albicans, C. glabrata, and T. longifusus* (6%, 10%, 15.5%, 14.5% and 16% respectively) and no activity against *M. canis* at 250 µg/ml concentrations. The data further suggested the growth of *C. Albicans, C. glabrata, and T. Longifusus, A. Flavus, F. solani and M. canis* was 35%, 28.5%, 24.5%, 15%, 13.5% and 5.5% at 500 µg/ml concentrations. When concentration was increased to 1000 µg/ml concentration, good activity was observed against *C. albicans* (72%), *C. glabrata* and *T. longifusus* (57% 57%) and again low activity was against *M. canis* (10%) at the same concentration. The first five concentrations of *n*-Hexane fraction (10 to 250 µg/ml) did not show any inhibition activity against any of the pathogenic fungi. At 500 µg/ml concentration, low activity was noted against *M. canis* (13%). The results also indicated that 1000 µg/ml concentration reduced the growth of *C. albicans* by 14% and *M. canis* by 35.5%.

**Fig. 6**: Antifungal activities of *n*-Hexane fraction of *C. resinae* (NRL-6437), the graph shows that 1000 µg/ml concentrations of the metabolites in *n*-Hexane were active against *C. albicans* and *A. flavus*.

**Fig. 7**: Cytotoxic activities of ethyl acetate extract & *n*-Hexane fraction of *C. resinae* (NRL-6437), the graph shows that 1000 µg/ml concentrations of the metabolites are highly active against the brine shrimps.

**Cytotoxic activity**

Fig. 7 presents cytotoxic activities of three different concentrations (10, 100 and 1000 µg/ml) of the crude metabolites of *Aspergillus carbonarius* (NRL-369) against the test organism (brine shrimps). The ethyl acetate extract showed 50%, 77% and 94% mortality at 10, 100 and 1000 µg/ml concentration respectively, whereas the same concentrations of *n*-hexane fraction showed 37%, 73% and 90% mortality respectively of the same organism. The data also indicated that ethyl acetate
The graph shows that 1000 µmetabolites are highly active against Lemna. Hexane fraction of 159.25 and 43.37 respectively (table 2). 136.29 respectively (table 1). metabolites production by Cladosporium resinae and epigenetic modifiers on the growth and secondary compounds.

**DISCUSSION**

The present study investigates the effect of growth media and epigenetic modifiers on the growth and secondary metabolites production by Cladosporium resinae (NRL-6437). Our results revealed that metabolites production started at day 2 in Czapek Yeast Extract Broth with gradual increase and maximum production was achieved at day 9. The other growth media under study were not effective to induce maximum production of metabolite. Similar results are also reported by Sunesson et al. (1995), Hestbjerg et al. (2002) and Bragulat et al. (2011). To assess the effect of epigenetic modifiers on metabolite production, suberyl anilide hydroxamic acid (SAHA) and 5-azacytidine (5-AZA) in different concentrations were used in Czapek Yeast Extract Broth. The data indicated that 10mM of SAHA produced maximum metabolites on day 8 after which a gradual decrease was noted. These results suggested that both the lower and higher concentrations of SAHA were not effective for significant production of metabolites in the optimized media. These results agree with Fisch et al. (2009).

The antibacterial activities of the crude metabolites of C. resinae (NRL-6437) against B. subtilis, E. coli, K. pneumoniae, S. typhi, S. flexneri and S. aureus were also investigated. Different concentrations of metabolites extracted with ethyl acetate measured variable zone of inhibition against the studied micro-organisms. The data suggested that when concentration was increased up to 500 µg/ml, high activity was noted against B. subtilis, S. flexneri and K. pneumoniae. Similarly, the same concentration inhibited the activity of E. coli, S. typhi and S. aureus. The first four concentrations of n-Hexane fraction (1 to 50 µg/ml) did not reduce the growth any pathogenic bacteria. However, good activity was observed for E. coli at the highest concentration. Our results are supported by Christophersen et al. (1998) and Rabteb and Rainer (2011). Our results regarding the antifungal activity of the crude metabolites of C. resinae (NRL-6437) against the test organisms (A. flavus, C. albicans, C. glabrata, F. solani, M. canis and Trichophyton longifusus) revealed that no activity was recorded up to 50 µg/ml concentration of metabolites in ethyl acetate.

**Table 1:** Cytotoxic activities of crude and n-Hexane of C. resinae (NRL-6437) showing low LD₅₀. Abid Ali Khan et al.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dose, ug/ml</th>
<th>No of Shrimps Survived</th>
<th>Shrimps Dead</th>
<th>Death Ratio</th>
<th>log₁₀ (Dose)</th>
<th>% Probability</th>
<th>LD₅₀</th>
<th>% Probability</th>
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<tbody>
<tr>
<td>Crude</td>
<td>10</td>
<td>30</td>
<td>24</td>
<td>6</td>
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<td>1</td>
<td>2.122</td>
<td>Intercept 1.917</td>
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<tr>
<td>Crude</td>
<td>100</td>
<td>30</td>
<td>8</td>
<td>22</td>
<td>0.733</td>
<td>2</td>
<td>2.559</td>
<td>Slope 0.263</td>
</tr>
<tr>
<td>Crude</td>
<td>1000</td>
<td>30</td>
<td>2</td>
<td>28</td>
<td>0.933</td>
<td>3</td>
<td>2.648</td>
<td>R-Square 0.872</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>10</td>
<td>30</td>
<td>26</td>
<td>4</td>
<td>0.133</td>
<td>1</td>
<td>1.996</td>
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</tr>
<tr>
<td>n-Hexane</td>
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<td>30</td>
<td>9</td>
<td>21</td>
<td>0.700</td>
<td>2</td>
<td>2.543</td>
<td>Slope 0.319</td>
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<tr>
<td>n-Hexane</td>
<td>1000</td>
<td>30</td>
<td>3</td>
<td>27</td>
<td>0.900</td>
<td>3</td>
<td>2.634</td>
<td>R-Square 0.855</td>
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</table>

**Table 2:** Phytotoxic activities of crude and n-Hexane of C. resinae (NRL-6437) showing deq4e./low LD₅₀

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dose, ug/ml</th>
<th>No of Fronds Survived</th>
<th>Fronds Dead</th>
<th>Death Ratio</th>
<th>log₁₀ (Dose)</th>
<th>% Probability</th>
<th>LD₅₀</th>
<th>% Probability</th>
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<tbody>
<tr>
<td>Crude</td>
<td>10</td>
<td>30</td>
<td>27</td>
<td>3</td>
<td>0.100</td>
<td>1</td>
<td>1.910</td>
<td>Intercept 1.641</td>
</tr>
<tr>
<td>Crude</td>
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<td>10</td>
<td>20</td>
<td>0.667</td>
<td>2</td>
<td>2.525</td>
<td>Slope 0.355</td>
</tr>
<tr>
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<td>0.867</td>
<td>3</td>
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</tr>
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<td>n-Hexane</td>
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<td>30</td>
<td>21</td>
<td>9</td>
<td>0.300</td>
<td>1</td>
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<td>Intercept 2.101</td>
</tr>
<tr>
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<td>100</td>
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<td>24</td>
<td>0.800</td>
<td>2</td>
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<td>Slope 0.198</td>
</tr>
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</table>

and n-hexane displayed very low LD₅₀ value of 61.18 and 136.29 respectively (table 1).

**Phytotoxic activity**

Phytotoxic activities of three concentrations (10, 100 and 1000 µg/ml) of the crude metabolites of Cladosporium resinae (NRL-6437) against the test organism (Lemna) were measured (fig. 8). Our results suggested that ethyl acetate extract resulted in 40%, 64% and 90 % mortality at 10, 100 and 1000µg/ml respectively, whereas the same concentrations of n-hexane fraction revealed 54%, 80% and 94 % mortality respectively with very low LD₅₀ value of 159.25 and 43.37 respectively (table 2).
extracted fraction. Low linear inhibitions were recorded against all the tested fungi except *M. canis* at 100µg/ml concentrations. Similarly, low activities were recorded against *F. solani*, *A. flavus*, *C. albicans*, *C. glabrata*, and *T. longifusus* and no activity against *M. canis* at 250µg/ml concentrations. The data further suggested at 1000µg/ml concentration, good activity was observed against *C. albicans*, *C. glabrata* and *T. Longifusus* and low activity was against *M. canis*. The first five concentrations of *n*-Hexane fraction were ineffective to control the growth of any pathogenic fungi, however, highest concentration of the same extract reduced the growth of *C. albicans* and *M. canis*.

The cytotoxic activity of the ethyl acetate and *n*-hexane extracted crude metabolites of *Cladosporium resinae* (NRL-6437) against the test organism (*brine shrimps*) revealed high mortality at 1000µg/ml concentration. The data also showed that ethyl acetate and *n*-hexane displayed very low LD$_{50}$ value of 61.18 and 136.29 respectively. Our findings are in accordance with Wu et al. (2012). Ethyl acetate and *n*-hexane extracted metabolites were also screened for their phytotoxic activity at three concentrations against the test organism (*Lemna*). Our results showed that ethyl acetate and *n*-hexane extract samples measured good phytotoxic activity at highest concentration with very low LD$_{50}$ value. Similar results are also revealed by Jiao et al. (2004).

**CONCLUSION**

It can be concluded from these results that Czapek yeast extract broth was good medium for the production of secondary metabolites compared with other media. Application of 15mM of both modifiers was effective to express silent genes for maximum metabolites production. The secondary metabolites revealed different levels of growth inhibitions of the tested organisms and were active against brine shrimps and *Lemna*.

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**REFERENCES**


