In vitro evaluation of antibacterial activity of bioactive compound of *Bixa orellana* L. (Seed) against five important species of bacteria

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**Abstract**

*In vitro* evaluation of antibacterial activity of bioactive compound of *Bixa orellana* L. seed were evaluated against five pathogens viz., *Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Bacillus cereus* tested at 100 to 1000 ppm concentration. Maximum inhibition was observed in *E. coli*, and recorded 39.0mm inhibition at 1000ppm concentration, followed by *P. vulgaris* and recorded 36.0mm at 1000ppm concentration. *E. aerogenes* and *B. cereus* recorded 31.0mm and 26.0mm inhibition at 1000ppm concentration and least activity was observed in *K. pneumonia* and recorded 23.0mm at 1000ppm concentration. Compared to standard antibiotic gentamycin at a recommended concentration of 25mg.

**Keywords: Bixa orellana, bioactive compound, antibacterial activity, synthetic antibiotic**

**Introduction**

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradski et al. 1999) [1]. Interest in plant-derived drugs has been increasing, mainly due to the current widespread belief that “green medicine” is safer and more dependable than costly synthetic drugs, many of which have adverse side effects. (Parekh, 2006) [4]. Many potent drugs including anti-malarial, anti-bacterial and anti-diabetic compounds have been purified from medicinal plants (Schmidt et al., 2008) [3]. For centuries, plants have been used by indigenous people to produce medicines that were used to treat different kinds of ailments (Samie et al., 2010) [2]. It contains many chemical compounds such as alkaloids, flavonoids, glycosides, phenols, resins, steroids, saponins, tannins and volatile oils which were deposited in their stilled water. The seeds were air dried at room temperature. Completely air pressure. After complete evaporation, 1 gram of

**Materials and Methods**

**Plant material:** Healthy seeds of *B. orellana* L. were washed with tap water thrice and two to three times with distilled water. The seeds were air dried at room temperature. Completely air dried seeds were powered and preserved until further use.

**Extraction**

**Solvent extraction:** Thoroughly washed seed of *B. orellana* were dried in shade for five days and then powdereded with the help of Waring blender. 25 grams of shade dried powder was filled in the thimble and extracted with methanol in a Soxhlet extractor for 48 hours. Solvent extract was concentrated under reduced pressure. After complete evaporation, 1 gram of concentrated methanol extract was dissolved in 9 ml of methanol and used for antibacterial assay (Lalitha et al., 2011) [9].

**Separation of bioactive compound by Thin Layer Chromatography (TLC)**

**Preparation of TLC plates and separation of bioactive compound:** Five 20cm x 20cm glass plates were taken for coating with silica gel. Plates are thoroughly washed with detergent and water, rinse with distilled water and allow to drain. Plates were wiped with acetone soaked
tissue to remove grease and dirt. Plates were mounted on plate spreader and plates were clamped to provide an even spreading surface. 25 grams of silica gel adsorbant was mixed with 60 to 70ml of distilled water. The gap of the TLC applicator was adjusted to 0.25mm using feeler gauge provided. The applicator was placed on the end. Silica gel slurry was poured into spreader and with a single constant motion, the slurry was drawn along the plates. After spreading, the plates were incubated at 110° to 120°C overnight and cooled in a desiccator before use. On thin layer plates, gently mark the intended positions of samples with a clean pointed glass rod at one horizontal edge of the plate. The obtained concentrated Methanol extract was dissolved in

The obtained cultivations were added. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured in millimeter. The experiments were repeated for five times (Joshi, 1999) [10].

Structural elucidation of the Bioactive compound

The bioactive compound, was subjected to 1H- NMR, 13C- NMR analysis.

1H- NMR data analysis: The 1H- NMR spectrum of the bioactive compound was recorded on a Bruker AM 400 F (400 MHZ) NMR spectrometer using CDCl3 as a solvent and TMS as internal standard. All chemical shift values were expressed in δ scale as s= singlet, d= doublet, t= triplet, m= multiplet (Al-Fatimi et al., 2006) [12].

13C NMR analysis: The 13C-NMR spectra were obtained on a Bruker spectrometer AM 400(400 MHZ) with the solvent signal as internal reference (Al-Fatimi et al., 2006) [12].

Statistical Analysis

The data were subjected to Tukey’s HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey’s HSD (P=0.05).

Result

Determination of Rf value of the bioactive compound: from the observation of TLC, the Rf value of the bioactive compound was 2.0. The 13C and 1H NMR spectral analysis was made for the further elucidation for structural analysis of the bioactive compound (Figure 1).

Antibacterial assay: Among the five bacteria tested, E.coli recorded a maximum inhibition of 39.0mm in 1000ppm concentration. At 700ppm concentration it was recorded 36.0mm, 37.0mm at 800ppm, 38.0 mm at 900ppm respectively. E.coli was followed by P.vulgaris and recorded 30.0mm, 30.0mm, 31.0mm, 32.0mm, 33.0mm, 34.0mm and 36.0mm at 400, 500, 600, 700, 800, 900 and 1000ppm respectively. Moderate activity was observed in E.aerogenes and recorded 30.0 and 31.0mm at 900ppm and 1000ppm concentration. B.cereus recorded 25.0, 26.0 and 26.0mm at 800, 900 and 1000ppm concentration. Least activity was observed in K.pneumonia and recorded 20.0mm inhibition at 800, 900 and 1000ppm concentration. Compared to synthetic antibiotic gentamycin at 25mg concentration, E.coli recorded 28.0mm, E.aerogenes recorded 26.0mm, P.vulgaris recorded 31.0mm, B.cereus recorded 24.0mm and K.pneumonia recorded 20.0mm inhibition respectively (Table 1).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Concentration of the Bioactive compound</th>
<th>Gentamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 ppm</td>
<td>200 ppm</td>
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<tr>
<td>E.coli</td>
<td>23.0±0.0</td>
<td>26.0±0.0</td>
</tr>
<tr>
<td>E.aerogenes</td>
<td>19.0±0.1</td>
<td>20.0±0.1</td>
</tr>
<tr>
<td>P.vulgaris</td>
<td>20.0±0.0</td>
<td>26.0±0.0</td>
</tr>
<tr>
<td>B.cereus</td>
<td>13.0±0.0</td>
<td>17.0±0.0</td>
</tr>
<tr>
<td>K.pneumonia</td>
<td>9.0±0.1</td>
<td>16.0±0.0</td>
</tr>
</tbody>
</table>

Values are the mean of five replicates, ±standard error.

The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey’s HSD.

Pattern of percentage inhibition increase is not uniform for all the microorganisms.

Test organisms: Five pathogenic bacteria viz., Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Klebsiella pneumonia and Bacillus cereus were collected from research center, Pooja Bhagavat Memorial Mahajana P.G. Centre, K.R.S. Road, Metagalli, Mysore. The obtained cultures were sub-cultured on nutrient agar medium. After 24 hours of incubation at 37°C the cultures were preserved aseptically in lower temperature until further use.

Preparation of Inoculum: A loopful of all the test bacteria were taken and sub-cultured in test tube containing 10 ml of nutrient broth. The test tubes were incubated at 37°C for 24 hours. The broth was standardized using sterile normal saline to obtain a population of 10 cfu/ml.

Antibacterial assay

Agar Cup Diffusion Method: An overnight culture of E.coli, E. aerogenes, P. vulgaris, K. pneumonia and B. cereus were inoculated into petri plates containing nutrient agar medium. A sterile cork borer (5.0 mm diameter) was used to punch wells in the nutrient agar. Five wells were made in the petriplate containing media (One in center and four at the border), the agar plugs were removed with a sterilized wire loop. For each well 50 µl of different concentrations (100 to 1000 ppm) of the bioactive compound was added. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured in millimeter. The experiments were repeated for five times (Joshi, 1999) [10].

Table 1: Antibacterial activity of bioactive compound of Bixa orellana L. (Seed) against five important species of bacteria
**Structural elucidation of the bioactive compound**

![13C and 1H NMR spectra of Bioactive compound isolated from the seeds of Bixa orellana L.](image)

**Discussion**

In the present time multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide (Peng *et al.*, 2006) [7]. It is aroused due to indiscriminate and repetitive use of antimicrobial drugs by inadequate disease treatment (Shariff, 2001) [8]. Various medicinal plants have been used for years in daily life to treat disease all over the world. The use of traditional plant extracts as well as other alternative forms of medical treatments have been getting momentum since the 1990s (Abbas Ali, 2008: Cowan, 1999) [13]. It has been observed that, bioactive compound of seeds of *Bixa orellana* showed a promising
result in managing all the five test pathogens tested from 100 to 1000 ppm concentration. Hence seeds of *B. orellana* is an alternative medicinal source for the management of pathogenic bacteria.

**Conclusion**

From the above result, it can be concluded that, further investigation is necessary to elucidate the structure of the bioactive compound isolated from the seeds of *B. orellana* which will be needed for future drug formulation.

**Acknowledgement**

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**References**

8. Shariff ZU. Modern Herbal Therapy for common Ailments, United Kingdom, 2001, 9-84.