INTRODUCTION

Bacteria are common inhabitants of both the surface and internal tissues of most plants. An endophyte is a bacterial or fungal organism, which spends the whole or part of its life-cycle by colonizing inter and/or intracellularly inside the healthy tissues of the host plant typically causing no symptoms of disease [1,2]. Endophytic bacteria colonize the internal tissues of their host plants and can form a range of relationships including symbiotic, mutualistic, commensalistic and trophobiotic. Recently, endophytic bacteria have gained attention due to their interesting features related to plant growth and health. Some of the bacteria are known to increase nutrient availability, produce growth hormones, convey stress tolerance, induce systemic resistance, or deter plant pathogens [3,4]. Endophyte infected plants often grow faster than bacteria are known to increase nutrient availability, produce growth hormones, convey stress tolerance, induce systemic resistance, or deter plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. Endophyte infected plants often grow faster than plant pathogens [3,4]. 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prepared by mixing 0.5 ml and 1 ml of methanolic solution of extract, 2.5 ml of 10% Folin–Ciocalteu’s reagent dissolved in water and 2.5 ml 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin–Ciocalteu’s reagent dissolved in water and 2.5 ml of 7.5% of NaHCO₃. The samples were thereafter incubated in a thermostat at 45°C for 45 minutes. The absorbance was determined using spectrophotometer at λₘ₉=765 nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. Gallic acid was employed as the standard and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was expressed as gallic acid equivalents (mg GAE/mg of dry weight of the crude extract) from the calibration graph Milan 2011 [9] with modifications.

**Antioxidant assay**

The free radical scavenging activity was tested as bleaching of stable 1,1-diphenyl -2-picylhydrazyl radical (DPPH). The endophytic bacterial extract was diluted to obtain concentrations of 335, 167.5, 83.7, 41.8, 20.9, 10.4, 5.2 µg/ml. 100 ml of 0.002% DPPH solution was prepared in 82% methanol. Diluted samples (1 ml each) were mixed with 2 ml of methanolic solution of DPPH. Mixture of DPPH and each fraction was shaken well and kept in dark at controlled temperature (25-28°C) for 1 hr. After incubation changes in color was measured at 517 nm. Mixture of 2 ml of 82% methanol and 1 ml of methanol was used as blank. Control sample contained all the reagents except the extract. 1 ml methanol and 2 ml of DPPH solution was taken as control. Percentage inhibition was calculated using the following equation, while the inhibitory concentration (IC₅₀) value was estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm [10] (with modifications).

\[
\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of fraction}}{\text{Absorbance of control}} \times 100.
\]

**RESULTS**

A preliminary analysis of the bioactive compounds revealed the presence of carbohydrates, tannins, saponins, alkaloids, glycosides, proteins, amino acids, and saponins. Flavonoids and anthocyanins were showed negative results.

One species of bacteria was isolated from the A. beddomei and it was identified as Lactobacillus species. The bacterial extract was evaluated for its free radical scavenging activity using DPPH. The purple colored DPPH is a free radical molecule that can be change into a stable yellow compound with reaction with antioxidant. The bioactive compounds are antioxidant in nature give a single electron in DPPH resulting in reduction in free radical DPPH [11]. The bioactive compounds present in the endophytic extract showed a strong activity with IC₅₀ values of 35 µg/ml.

**Preliminary analysis of bioactive compounds in the endophytic bacterial extract**

Thus, from Table 1 it can be inferred that in the present study, the endophytic bacterial extract was rich in carbohydrates, tannins, glycosides, proteins and amino acids and steroids. Saponins may be present in trace amount as there was formation of bubbles and not froth upon shaking lengthwise after addition of distilled water to the endophytic bacterial extract. Alkaloids may be present in trace amount as there was appearance of very less white precipitate. However, there was absence of flavonoids, anthocyanin, betacianin and phytosterols in the endophytic bacterial extract.

**Determination of total phenolic content in the endophytic bacterial extract**

In the present study, the concentration of total phenolics in the endophytic bacterial extract was determined to be 0.67 mg/ml (Graph 1).

**Graph 1: Standard graph for determination of total phenolics of endophytic bacterial extract**

**Graph 2: Percentage inhibition by endophytic bacterial extract**

**DISCUSSION**

An enormous variety of plants have been studied for new sources of natural antioxidants. Phenolic compounds derived from plants were identified as gallic acid equivalents (mg GAE/mg of dry weight of the crude extract) from the calibration graph Milan 2011 [9] with modifications.

**Table 1: Preliminary analysis of bioactive compounds in the endophytic bacterial extract**

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for carbohydrate</td>
<td>Appearance of reddish ring</td>
<td>+</td>
</tr>
<tr>
<td>Test for tannins</td>
<td>Appearance of greenish black colour</td>
<td>+</td>
</tr>
<tr>
<td>Test for saponins</td>
<td>Formation of bubbles</td>
<td>+</td>
</tr>
<tr>
<td>Test for flavonoids</td>
<td>No observation</td>
<td>-</td>
</tr>
<tr>
<td>Test for alkaloids</td>
<td>Appearance of white precipitate</td>
<td>+</td>
</tr>
<tr>
<td>Test for anthocyanin and betacianin</td>
<td>No color change</td>
<td>-</td>
</tr>
<tr>
<td>Test for glycosides</td>
<td>Appearance of yellow color</td>
<td>+</td>
</tr>
<tr>
<td>Test for proteins and amino acids</td>
<td>Formation of blue color</td>
<td>+</td>
</tr>
<tr>
<td>Test for steroids and phytosterols</td>
<td>Reddish brown ring</td>
<td>+</td>
</tr>
</tbody>
</table>

*: Positive, -: Negative
proved to be potent antioxidants and free radical scavengers. Significant correlations between phenolic compounds and antioxidant properties of medicinal plants were noted [12,13]. However there are literature studies on the antioxidant potential of the endophytes like Phoma, Cladosporium, and Chaetomium fungi. Chaetomium was showed a greater activity among all the above fungi accompanied by a higher proportion of the phenolic contents also. The same was observed in the endophytic fungus Alternaria alternata [14]. Furthermore, ethylacetate is often used as an extraction solvent with a significant selectivity in the extraction of low-molecular-weight phenolic compounds and high-molecular-weight polyphenols [15]. On the other hand [16], have reported that ethylacetate allowed that highest phenolic content and the selective removal of non-phenolic compounds. Therefore, the antioxidant activity of endophytic ethyl acetate extract could be due to the presence of phenolic compounds.

The present results lead to the conclusion that endophytes are considered to be a potent source for bioactive products [17]. The current study demonstrates that extracts of endophyte bacterial isolated from A. beddomei have significant antioxidant property. Endophytic bacteria might also represent an alternative source for the production of the therapeutic agents and bioactive metabolites that are not easily obtained by chemical synthesis, which are high antioxidant agents. Hence, this work will serve as a source to more comprehensive studies on the chemistry and biology of the bioactive natural products produced by these endophytes. Further examination can be done to learn about endophytes may have the potential to serve as a biological or as a new pharmacological agents.

REFERENCES