ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 1, 2015

ISSN - 0974-2441

Research Article

BIOACTIVE COMPOUND ANALYSIS AND ANTIOXIDANT ACTIVITY OF ENDOPHYTIC BACTERIAL EXTRACT FROM *ADHATHODA BEDDOMEI*

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Received: 16 October 2014, Revised and Accepted: 31 October 2014

ABSTRACT

Objectives: The endophytic bacteria *Lactobacillus sp*. has been isolated from the tissues of plant leaves. The bacterial strains were grown in nutrient broth at 37°C for 5 days. To isolate the bioactive compounds, the culture was grown in a broth and the culture broth was centrifuged and the supernatant was collected.

Methods: Solvent-solvent method was applied to extract the bioactive compounds. The antioxidant activity was evaluated with the help of 1,1-diphenyl -2-picrylhydrazyl radical scavenging activity.

Results: The qualitative analysis of extracts indicated presence of carbohydrates, tannins, saponins, alkaloids, glycosides, proteins, amino acids, and saponins. Flavonoids and anthocyanins were showed negative results, the quantitative analysis of the phenolic compounds showed the presence of 0.67 mg/ml. The inhibitory concentration 50 value for antioxidant activity was 35 µg/ml.

Conclusion: The antioxidant activity is due to the presence of different bioactive, and the high levels of phenolic compounds may be responsible for antioxidant activity.

Keywords: *Lactobacillus* sp., Antioxidant activity, Endophytic bacteria, Phenolic compounds.

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 non-infected plants. This effect is at least in part due to the production of phytohormones by endophytes. Endophytic bacteria are plant associated bacteria that colonize and persist in various healthy parts of plants such as fruits, vegetables, stems and roots [5,6]. Endophytic microorganisms enter primarily through root zone; however flowers, stems and cotyledons may also be used as a route of entry [3]. The bacteria may also enter tissues via germinating radicles. Endophytes inside a plant may either become localized at the point of entry or may spread throughout the plant tissues [3].

In view of the increasing prevalence of antibiotic-resistant human and plant pathogens, there is an increasing demand for new antimicrobials from natural sources. Bacterial and fungal endophytes are believed to have a resistance mechanism against pathogenic attack and thus have emerged as a promising source of new antimicrobial compounds. Several antimicrobial metabolites belonging to the class such as alkaloids, flavonoids, phenolic acids, steroids, terpenoids, peptides, tetralones, quinines, etc. have been identified from endophytes.

Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase, and various peroxidases. The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells. Hence, the current study concentrates on the bioactive compounds present in the endophytic bacterial extract and the total phenol compounds, finally their antioxidant activity from *Adhathoda beddomei.*

METHODS

Preparation of endophytic bacterial extract

The bacterial culture was inoculated in 10 ml of nutrient broth for 24 hrs. 2% of inoculums was added into 500 ml beaker containing 300 ml of nutrient-broth and incubated for 5 days. After 5 days, it was centrifuged and the supernatant was collected. The extract was subjected to solvent-extraction, using chloroform in a separating funnel and solvent was evaporated to obtain the endophytic bacterial extract [7].

Bioactive compounds analysis

Bioactive compound analysis was performed by standard methods [8] for detecting the presence of different chemical constituents in the plant extract was employed. The tests for the secondary metabolites *viz*. alkaloids, tannins, saponins, glycosides, flavonoids, antocyanins and betacyanins phenols were carried out.

Determination of total phenolic compounds

The total phenolic compounds are determined by using the Folin– Ciocateau reagent solution. The reaction mixture was

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% $NaHCO_3$. 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 $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 minutes. The absorbance was determined using spectrophotometer at λ_{max}=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-picrylhydrazyl 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 (IC_{50}) value was estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm [10] (with modifications).

% inhibition = ([Absorbance of control–Absorbance of fraction]/ Absorbance of control) × 100.

RESULTS

A preliminary analysis of the bioactive compounds revealed the presence of the carbohydrates, tannins, saponins, alkaloids, glycosides, proteins, aminoacids, 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 by 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 IC_{50} 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, betacyanin 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).

Evaluation of antioxidant activity of the endophytic bacterial extract

Graph 2 shows that with a decrease in concentration of the endophytic bacterial extract, its antioxidant activity increased. In the present study, the IC_{50} value was determined from Graph 2 and was found to be 35 µg/ ml.

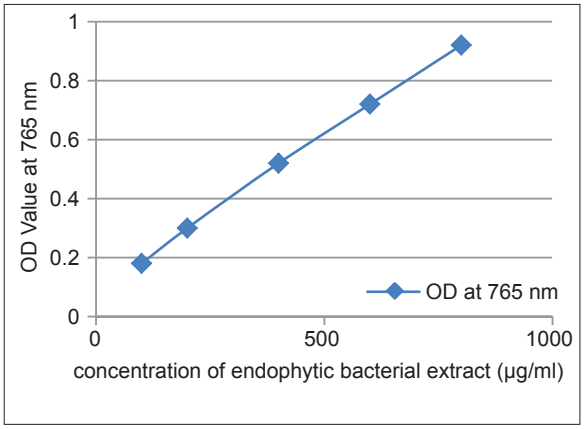
DISCUSSION

An enormous variety of plants have been studied for new sources of natural antioxidants. Phenolic compounds derived from plants were

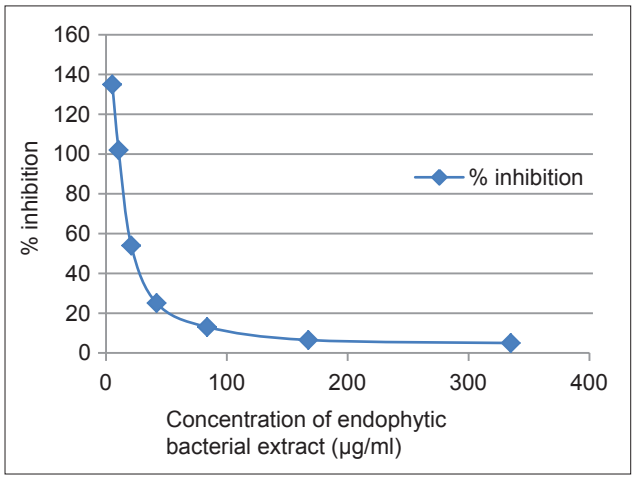
Table 1: Preliminary analysis of bioactive compounds in the endophytic bacterial extract

Test	Observation	Result
Test for carbohydrate	Appearance of reddish ring	+
Test for tannins	Appearance of greenish black colour	+
Test for saponins	Formation of bubbles	+
Test for flavonoids	No observation	-
Test for alkaloids	Appearance of white precipitate	+
Test for anthocyanin and betacyanin	No color change	-
Test for glycosides	Appearance of yellow color	+
Test for proteins and amino acids	Formation of blue color	+
Test for steroids and phytosterols	Reddish brown ring	+

+: Positive, -: Negative



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



Graph 2: Percentage inhibition by endophytic bacterial extract

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. *Chaetomuium* 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 ethylaacetate 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 new pharmacological agents.

REFERENCES

1. Sturz AV, Christie BR, Nowak J. Bacterial endophytes: Potential role in developing sustainable system of crop production. Crit Rev Plant Sci 2000;19:1-30.
2. Wellington B, Marcela TB. Delivery methods for introducing endophytic bacteria into maize. BioControl 2004;49:315-22.
3. Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW. Bacterial endophytes in agricultural crops. Can J Microbiol 1997;43(10):895- 914.
4. Buchenauer H. Biological control of soil-borne disease by rhizobacteria. J Plant Dis Protec 1998;105(4):329-48.
5. Mclnroy JA, Kloepper JW. Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil 1995;173(2):337-42.
6. Sturz AV, Christie BR, Matheson BG, Nowak J. Biodiversity of endophytic bacteria potential role which colonize red clover nodules, root, stem and foliage and their influence on host growth. Biol Fertil Soil 1997;25:13-9.
7. Carmen A, Brandon R. Isolation and identification of endophytic bacteria from roots of lemon grass (*Cymbopogon citratus*). Eur J Agric Sci 2013;10:37-40.
8. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. Lloydia 1978;41:234-46.
9. Milan SS. Total phenolics content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extract. Kragujevac J Sci 2011;33:63-72.
10. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. Gen Pharmacol 1999;32:661-7.
11. Yuhernita Y, Juniarti J. Secondary metabolites analysis of methanol extract of Surian (Toona sureni (Bl.) Merr) leaf as antioxidant potential. Makara Sains 2011;15(1):48-52.
12. Baghiani A, Boumerfeg S, Belkhiri F, Khennouf S, Charef N, Harzallah D, *et al*. Antioxidant and radical scavenging properties of *Carthamus caeruleus* L. extracts grow wild in Algeria flora. Comun Sci 2011;1(2):128-36.
13. Khennouf S, Iratni N, Baghiani A, Harzallah D, Arrar L. Antioxidant and antibacterial activities of extracts from *Artemisia herba alba* Asso leaves and some phenolic compounds. J Med Plant Res 2010;4(13):1273-80.
14. Fernandes MR, Costa-Silva TA, Pfenning LH, Costa-Neto CM, Heinrich TA, Alencar SM, *et al*. Biological activities of the fermentation extract of the endophytic fungus *Alternaria alternata* isolated from *Coffea arabica* L. Braz J Pharm Sci 2009;45(4):677- 85.
15. Scholz E, Rimpler H. Proanthocyanidins from *Krameria triandra* root. Planta Med 1989;55(4):379-84.
16. Conde E, Moure A, Domínguez H, Parajó JC. Fractionation of antioxidants from autohydrolysis of barley husks. J Agric Food Chem 2008;56:10651-9.
17. Strobel GA. Endophytes as sources of bioactive products. Microbes Infect 2003;5(6):535-44.