Endophytes of *Datura metel* L. for their antioxidant and antidiabetic potential

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Abstract- *Endophytes living asymptomatically within the plant tissues have been found in many plant species. Endophytes also constitute a valuable source of secondary metabolites for the discovery of new potential therapeutic drugs. In this study, endophytic fungi (both extra and intracellular) and actinomycete were obtained from the stem and root of Datura metel L. and evaluated for their antioxidant and antidiabetic potential. The ethyl acetate extracts of the endophytic isolates were screened for their antioxidant potential by DPPH activity and antidiabetic activity by alpha glucosidase inhibitory assay. In DPPH assay, it was noted that endophytic actinomycete recorded the highest reduction percentage of 51.97 when compared to that of fungi which showed only a percentage of 41.11. Endophytic actinomycete recorded highest antidiabetic potential of about 77.58% which is almost equal to the control. This endophytic actinomycete can be used as potential novel source of natural antioxidants as well as alpha glucosidase inhibitor.*

Keywords- Endophytes, actinomycete, antioxidant, DPPH and antidiabetic.

I. INTRODUCTION

Datura metel L. is a shrub, 0.5 to 2 meters in height; branches coarse, erect, smooth or slightly hairy. Leaves ovate to oblong-ovate, 9 to 18 cm long, with in-equilateral at base, pointed, irregularly and shallowly lobed at margins. Flowers axillary, solitary, white or nearly purple, and are very large. Calyx cleft at the apex, and divided into linear teeth, green, ca. 6 cm long: Corolla ca. 15cm long and ca. 8 cm in diameter; The fruits are rounded, nodding green, stout spines ca. 3.5 cm in diameter. Seeds are numerous, nearly smooth, and pale brown.

The term endophyte was first used by De Bary in 1886 to describe microbes that reside inside plants. Globally, there are at least one million species of endophytic fungi in all plants (Ganley *et al*., 2004), which can potentially provide a wide variety of structurally unique, bioactive natural products such as alkaloids, benzopyranones, benozoquiones, flavonoids, phenols, steroids, terpenoids, tetralones, xanthones and others (Tan & Zou, 2001). The relationship between endophytes and their host plant is thought to be symbiotic, such as endophytes obtain nutrients and protection from the host but contribute to effective host defense against pathogens, herbivores or abiotic stress (Redman *et al*., 2002; Arnold *et al*., 2001).

Actinomycetes are Gram-positive, filamentous bacteria, normally occur as spores resistant to desiccation and starvation, but in the presence of sufficient moisture and nutrients, the spores can germinate and form vegetative mycelium (Williams *et al*., 1989). The actinomycetes are well-known saprophytic bacteria and are quantitatively and qualitatively important in the rhizosphere, where they may influence plant growth and protect plant roots against invasion of root pathogenic fungi (Cao *et al*., 2004).

An antioxidant acts as a free radical scavenger and neutralizes these reactive particles by binding to their free electrons. By destroying free radicals, antioxidants help to detoxify and protect the vital body tissues and organs. Antioxidants come in the form of enzymes, hormones, vitamins, minerals and phytochemicals. Extensive research indicates that antioxidants inhibit and control free radical damage. The screening of plant extracts and natural products for antioxidant and antimicrobial activity has revealed the potential of higher plants as a source of new agents to serve the processing of natural products (Rios *et al*., 1998).

α-Glucosidase enzymes in the intestinal lumen and in the brush border membrane play an important role in carbohydrate digestion by degrading starch and oligosaccharides to monosaccharides before they are being absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation (Puls *et al*., 1997). α-Glucosidase inhibitors (AGIs) offer an alternative as they are designed to specifically delay the digestion of complex carbohydrates, thus significantly reducing postprandial glycemic and insulinemic excursions (Ariane & Jean, 2007).

II. MATERIALS AND METHODS

2.1 Isolation and identification of endophytes

Stems and roots of healthy plants of *Datura metel* L. were collected and sterilized to remove microorganisms and soil particles. Surface sterilized stem and root samples were split into pieces and they were aseptically transferred to petri dishes. For the isolation of actinomycete, starch casein agar (SCA) is used and for the isolation of fungi, water agar is used. Actinomycete and fungus were isolated, subcultured in potato dextrose agar and identified. *Fusarium* sp. is an endophytic fungus (Fig 1) isolated from the stem and *Stenotrophomonas* sp. is an actinomycete (Fig 2) isolated from the roots of *Datura* were recorded.

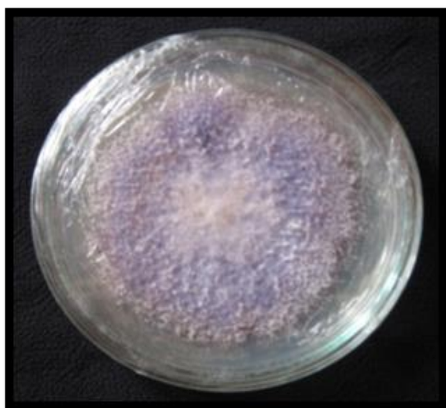

Fig 1 Isolation and culture of endophytic fungus after 8 days of incubation

Fig 2 Isolation of actinomycete from the roots of *Datura*

2.2 Cultivation and sampling

The endophytic fungus and actinomycete were grown in 1 litre flasks containing 500 ml of potato dextrose broth medium. The test endophytes were inoculated and incubated for 3 weeks. Endophytic fungal culture was kept under dark condition. (Fig 3)

Fig 3 Mass production of endophytic fungus on potato dextrose broth

The flask which contains actinomycete culture was kept under rotary shaker. After incubation, the culture filtrate was extracted and filtered through three layers of cheese cloth to remove mycelia and actinomycete. Then the culture filtrate was extracted with equal volumes of solvent ethyl acetate. The organic phase was collected and the solvent was then removed by evaporation under reduced pressure at 45°C using rotary vacuum evaporator. The dry solid residue was redissolved in ethyl acetate and the crude extract was evaluated for their antioxidant and antidiabetic properties.

2.3 DPPH radical scavenging assay

The free radical scavenging activities of extracts were measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). This was performed in accordance with the method proposed by Sanchez *et al*. (1998). The extracts and standard reference compounds were prepared with 99% ethanol at various concentrations (50-1000 µg/ml). 1 ml of various concentrations (50-1000 µg/mL) of the extracts and standard reference compounds were dissolved in 1 ml of 0.2 mM DPPH separately and made up using 99% ethanol in 10 ml test tube to achieve a final volume of 3 ml. The mixture was vortexed and incubated for 90 min at room temperature. The optical density was measured at 517 nm.

$$\text{Radical scavenging (\%)} = \frac{\text{OD control} - \text{OD test sample}}{\text{OD control}} \times 100$$

2.4 Alpha Glucosidase inhibitory assay

In vitro α-glucosidase inhibition test was performed to investigate the inhibitory effect of the ethyl acetate extract of endophytic fungi and actinomycete isolated from *Datura metel* L. Rat small intestine homogenate was used as α-glucosidase (maltose α-glucosidase) solution because it would better reflect the *in vivo* state. The inhibitory effect was measured using the method slightly modified from Dahlqvist (1964).

Assay Procedure

The assay mixture consisted of 100 µl of maleate buffer (100 mM, pH 6.0), 40 mM sugar substrate solution (100 µl) and the ethyl acetate extract of endophytic fungi and actinomycete (200-1000 µg/ml). The mixture was preincubated for 5 min at 37°C and the reaction was initiated by adding the crude α-glucosidase solution (50 µl) to it. The solution was incubated for 10 min at 37°C. The glucose released in the reaction mixture was determined using the glucose kit (Merckotest, GOD-POD Method) and the absorbance was read at 505 nm. Acarbose was used as reference drug to compare the α-glucosidase inhibitory effect of the extract. The rate of carbohydrate decomposition was calculated as the percentage ratio of the amount of glucose released when the carbohydrate was completely digested.
Absorbance of the sample

$$\text{Glucose released (mg/dL)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times 100$$

The inhibitory rate of the extract was calculated by the following formula

$$\%\ \text{inhibition} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

III. RESULTS AND DISCUSSION

In DPPH free radical scavenging activity, the extract of endophytic actinomycete was found to show high percentage of inhibition of DPPH (51.97%) at 1000 µg/ml whereas endophytic fungi showed comparatively lesser inhibition of DPPH (41.11%) at 1000 µg/ml. (Table 1).

Table 1: Antioxidant activity of ethyl acetate extracts of EPF and EPA isolated from *D. metel* L. by DPPH free radical scavenging assay

Concentration (µg/ml)	% Inhibition of DPPH free radical			
	EPF	EPA	BHT	Ascorbic acid
50	4.93	12.5	68.88	72.60
100	10.85	28.28	73.58	78.60
200	23.02	29.27	78.11	84.11
400	25.0	32.87	85.08	89.78
500	38.42	49.34	90.92	93.19
1000	41.11	51.97	94.81	95.46

EPF - endophytic fungi EPA - endophytic actinomycete.

BHT and ascorbic acid were used as the positive control. Even though both endophytic fungi and actinomycetes showed activity, actinomycetes found to show maximum activity in scavenging the free radical. The effect of free radical scavenging activity of our crude endophytic fungus extract on DPPH radicals is due to their hydrogen donation ability. The results showed that the crude extract is a moderate free radical scavenger which may limit the occurrence of free radical damage in human body.

As shown in Table 2, incubation of the ethyl acetate extract at different concentrations with intestinal α-glucosidase enzyme caused an increased activity with 77.58% inhibition when incubated with crude ethyl acetate extract of endophytic actinomycete at 1000 µg/ml. Crude ethyl acetate extract of endophytic fungi showed only moderate inhibition percentage of 58.62% at 1000 µg/ml concentration. However, the inhibitory effect of the crude ethyl acetate extract of endophytic actinomycete was comparable to that of the acarbose, which is well known α-glucosidase inhibitor.

Table 2: *In vitro* α-glucosidase inhibition activity using ethyl acetate extracts of EPF and EPA isolated from *D. metel* L.

Concentration (µg/ml)	% Inhibition of α-glucosidase		
	EPF	EPA	Acarbose (Reference)
200	1.72	18.96	74.56
400	33.62	27.58	81.28
600	42.24	45.68	88.01
800	56.89	72.41	92.68
1000	58.62	77.58	96.78

EPF - endophytic fungi EPA - endophytic actinomycete.

IV. CONCLUSION

The results indicated that *Stenotrophomonas* sp. isolated from *Datura metel* L. was found to be an effective α-glucosidase inhibitor rather than *F. oxysporum*. Hence higher inhibitory activities of crude ethyl acetate extracts of *Stenotrophomonas* sp. against α-glucosidase confirmed its potential use in treatment of obesity and diabetes. In vitro screening of the crude extracts for antioxidant activity showed that the *Stenotrophomonas* sp. effectively scavenged the free radicals when compared with that of *Fusarium oxysporum*.

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