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GLUCOSIDASE INHIBITORY ACTIVITY AND *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF ETHYL ACETATE EXTRACTS OF ENDOPHYTES ISOLATED FROM THE ROOT OF *CATHARANTHUS ROSEUS.L*

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ABSTRACT

In this study, the ethyl acetate extracts of EPF and EPA isolated from the root of *Catharanthus roseus* were studied for its alpha glucosidase inhibitory activity and radical scavenging activity. The ethyl acetate extract showed more α-Glucosidase inhibition (83.29 ± 0.02) at 1000 µg/ml than EPF. Further, oral administration of EPA extract prevents the increase in plasma glucose levels significantly 60 minutes after the maltose and sucrose load at 500mg/kg.b.w concentration, which is equal to acarbose. At 500µg/ml and 1000µg/ml concentration, the EPF extract had shown an inhibition percentage for DPPH scavenging assay 47.59 ± 0.37 and 54.50 ± 0.31 respectively, when compared to EPA extract. The inhibition of DPPH radical by the EPF extracts is relatively higher than other extract. It was found that 1000µg/ml of EPA extract scavenged the hydroxyl free radical level to 70.90 ± 0.17 respectively, when compared to the percentage inhibition by EPF. In case of hydrogen peroxide free radical, scavenging assay at 1000µg/ml concentration the EPA extract showed more inhibition (46.84 ± 0.23) when compared to EPF. In case of nitric oxide scavenging assay, the EPA extract inhibited the nitric oxide radical level (48.71 ± 0.29) highly, when compared to EPF, which lowered the H_2O_2 radical level by 19.96 ± 0.23 respectively. This result suggests that EPA present in *C. roseus* play a vital role in the antidiabetic effect and further studies in this actinomycetes will yield novel prototypes for treating diabetes mellitus. The EPA extracts also exhibited the inhibiting effect on rat intestinal α-Glucosidase.

KEYWORDS: Antioxidant property, endophytic fungi, actinomycetes, free radical, α-Glucosidase inhibition, *Catharanthus roseus.*

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INTRODUCTION

Catharanthus roseus L. (Madagascar Periwinkle) is native to Madagascar and belongs to the family Apocynaceae. Synonyms include *Vinca rosea* (the basionym), *Ammocallis rosea*, and *Lochnera rosea*; other English names occasionally used are Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle. In the wild, it is an endangered plant; the main cause of decline is habitat destruction by slash and burn agriculture. It is also however widely cultivated and is naturalised in subtropical and tropical areas of the world. It is an evergreen subshrub or herbaceous plant growing to one meter tall (Huxley 1992). Chattopadhyay (1999) reported that the extracts from the leaves was found to have potential activity as blood sugar lowering agent. α-Glucosidase enzymes in the brush border membrane play important role in converting oligosaccharides into monosaccharides before they can be absorbed. The suppression of the activity of such digestive enzymes would delay the degradation of oligosaccharide, 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; they are designed to specifically delay the digestion of complex carbohydrates, thus significantly reducing postprandial glycemic and insulinemic excursions (Ariane and Jean 2007). Type II Diabetes mellitus is a serious metabolic disorder with rising prevalence in developing countries. Calculation of the excess global mortality revealed that diabetes is likely to be the fifth leading cause of death (Roglic et al., 2005). Primarily, α- glucosidase inhibitors from plants were tested in animal trials for use in humans, but soon turned out to be inefficient (Puls and Keup 1973, Bischoff et al., 1994). Screening of actinomycetes, had also yielded some polypeptide based amylase inhibitors such as tendamistat (Taylor and Barker 1983; Wiegand et al., 1995).

Globally, about one million species of endophytic fungi was recorded (Ganley et al., 2004), which can potentially provide a wide variety of structurally unique, bioactive natural products such as alkaloids, benzopyranones, benzoquinones, flavonoids, phenols, steroids, terpenoids, tetralones, xanthones and others (Tan and Zou 2001). The relationship between endophytes and their host plant are thought to be symbiotic, such as that, 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., 2003). Free radicals are highly reactive particles produced by the body either as a by-product during normal biochemical processes, such as enzyme activation. Under normal circumstances, the body is capable of neutralizing these particles and maintains them at a safe minimum level. Excess or abnormal formation of free radicals is potentially dangerous and can lead to oxidation and even irreversible damage of body tissues. 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. Some antioxidant compounds isolated from endophytic fungi and their antioxidant activities have also been reported (Harper et al., 2003; Song et al., 2005). High antioxidant status in plants was reported to hinder the transportation of reactive oxygen species (ROS) across the cell (Neill et al., 2002). Antioxidant compounds in food play an important role as health protecting factors. 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). The antioxidative action, one of the important physiological functions of food, is supposed to protect living organisms from oxidative damages, resulting in the prevention of various diseases (Azuma et al., 1999; Gorinstein et al., 2001). The present study is aimed to analyze the α -Glucosidase

inhibitory and antiradical activities of the ethyl acetate extracts of endophytic actinomycetes and fungi isolated from the root of *C. roseus*.

MATERIALS AND METHODS

Rational and design

Collection of Plant material and Isolation of crude extract

Roots of healthy *Catharanthus roseus* were collected from Loyola College campus, Chennai. The roots were surface sterilized using 3 - 5% sodium hypochlorite solution for 3 minutes followed by 70% ethanol for 30 seconds. Each root was cut and then aseptically transferred to petridishes containing starch casein agar medium supplemented with Nalidixic acid and Actidione for actinomycetes and 2.5% water agar medium supplemented with Streptomycin for fungal isolation. Plates were incubated at 28^0C for a maximum of three weeks. Actinomycetes and fungi growing on the medium were isolated, subcultured and identified. The isolated actinomycetes and fungi were mass-produced by inoculating them in Modified Nutrient Glucose broth (MNGB) and Potato dextrose broth (Himedia, Mumbai). After 10 days of growth, the broth was centrifuged at 8000 rpm for 10 minutes at 4ºC. The supernatant was collected and mixed with an equal volume of ethyl acetate. The organic layer was separated using the separating funnel. The solvent was removed using Rotary evaporator under reduced pressure, and the extract was stored at 4ºC until use.

Chemicals and reagents required for alpha glucosidase inhibition

Maleate buffer (100 mM, pH 6.0), Maltose 40 mM (Substrate), Crude α-glucosidase enzyme, Phosphate buffered saline pH 6.8.

Chemicals and reagents required for free radical scavenging activity

10 mM Sodium Nitroprusside, Griess reagent (1 % sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2 % H_3PO_4), Phosphate buffered saline pH 7.4, 40mM hydrogen peroxide, Phosphate buffer pH 7.4, Fenton reagent (200 µL of 10 mM $FeSO_4 \cdot 7H_2O$, 200 µL of 10 mM EDTA and 200 µL of 10 mM 2-deoxyribose), 10 mM H_2O_2, 2.8 % TCA, 1 % TBA, 0.2 mM DPPH in 99% Ethanol, Ascorbic acid and BHT. All the chemicals and reagents used in this study were purchased from Himedia, Qualigens and SRL, which are analytical grade.

Isolation of α-glucosidase crude enzyme

The crude enzyme solution was prepared according to the method of Kessler et al 1978. After fasting for 24 h, the small intestine between the part immediately below the duodenum and the part immediately above the cecum was removed from the rats. The duodenum was rinsed with ice-cold PBS, and homogenized with maleate buffer (100 mM, pH 6.0). After centrifugation at 8000 rpm for 10 min at 4^0 C, the homogenate was used as the crude enzyme α- glucosidase solution.

In vitro α-glucosidase inhibitory assay

In order to investigate the inhibitory effect of the extract of endophytic fungi (EPF) and actinomycete (EPA) from *C.roseus*, an *in vitro* α- glucosidase inhibition test was performed. α-Glucosidase from yeast was used extensively as a screening material for α-glucosidase inhibitors, but the results do not always agree with those obtained in mammals.(Mariko et al., 2006) Therefore, the rat small intestine homogenate was used as α -glucosidase solution. The inhibitory effect was measured by the modified method from Dahlqvist (1964) proposed by Oku et al., 1982. The assay mixture consisted of 100 mM maleate buffer (pH 6.0), substrate solution (100 µl), and the extract (50-1000 µg/ ml). Acarbose was used as reference drug as α-glucosidase inhibitor. The mixture was preincubated for 5 min at 37°C, and the reaction was initiated by adding the crude α -glucosidase solution (50 µl) after incubation for 10 min at 37º C, the glucose released in the reaction mixture was determined by the GOD-POD method. Absorbance was read at 505 nm. The rate of carbohydrate

decomposition was calculated as a percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of prevention was calculated by the following formula:

Amount of glucose released in control – Amt. of glucose in test / Control ×100

In vivo α-glucosidase inhibitory assay

The efficacy of the ethyl acetate extract of endophytic actinomycetes was checked *in vivo* at 500 mg/ Kg by the method of Abesundara et al., (2004). Briefly, the animals were fasted for 12 h. Then zero hour blood samples were taken from the orbital sinus. Then, the animals were treated with extract or vehicle. Ten minutes after the treatment with the extracts, maltose/ sucrose solution (2g/ Kg) was given to the animals. 30, 60 and 120 minutes after the administration of maltose/sucrose solution, plasma glucose levels were estimated using GOD-POD method. Acarbose (3 mg/ Kg) was used as positive control. The percentage of inhibition was calculated with the following formula: % inhibition: (Control – Test) / Control x100

DPPH radical scavenging assay

DPPH (1, 1 -diphenyl-2-picryl hydrazyl) radical scavenging activity of EPF and EPA were determined with the method proposed by Sanchez et al., (1998). The extracts and standard reference compounds were dissolved with 99% ethanol at various concentrations. 1 ml of various concentrations (50-1000 µg/ml) of the extracts and standard reference compounds were mixed with 1 ml of 0.2 mM DPPH separately and made up using 99% ethanol to 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.

Hydroxyl radical scavenging assay

The ability of the EPF and EPA extracts to scavenge the hydroxyl radical generated by the Fenton reaction was measured according to the modified method of Chung et al., (1997). The Fenton reaction mixture containing 200 µL of 10 mM $FeSO_4 \cdot 7H_2O$, 200 µl of 10 mM EDTA and 200 µl of 10 mM 2-deoxyribose was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) containing 200 µl of ethyl acetate extracts of EPF and EPA. Then, 200 µl of 10 mM H_2O_2 was added to the mixture and incubated for 4 hrs at 37 °C. After incubation, 1ml of 2.8 % TCA and 1ml of 1 % TBA were added and placed in a boiling water bath for 10 min. The resultant mixture was then allowed to cool to room temperature and centrifuged at 8000 rpm. The absorbance was recorded at 532 nm.

Hydrogen peroxide radical scavenging assay

The ability of the EPF and EPA to scavenge hydrogen peroxide was determined according to the method of Ruch et al., (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The ethyl acetate extracts of endophytic fungus and actinomycetes, (50-1000 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide using a UV-VIS spectrophotometer.

Nitric oxide radical scavenging assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by the Griess reaction (Green et al., 1982). The reaction mixture (3 ml) containing 10 mM sodium nitroprusside in phosphate-buffered saline and EPF and EPA and the reference compounds at different concentrations (50, 100, 200, 400, 500 and 1000 µg/ml) were incubated at 25 °C for 150 min. A 0.5 ml aliquot of the incubated sample was removed at 30 min intervals and 0.5 ml Griess reagent (1 % sulfanilamide, 0.1% naphthylethylene diamine

dihydrochloride in 2 % H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546 nm. All the tests were performed in triplicates. Butylated hydroxytoluene and Ascorbic acid were used as a positive control. Percentage inhibition of the nitric oxide generated was measured by the following formula:

Radical scavenging (%): (OD control – OD test sample) / OD control × 100

Based on the results obtained in the alpha glucosidase and antioxidant activity, the extracts of endophytic fungus and actinomycetes were subjected to HPLC in order to analyze the various components present in the mixture.

HPLC fingerprinting

High Performance Liquid Chromatography (HPLC) was applied to assess the quality and quantity of the EPA and EPF extracts from *C. roseus*. HPLC analysis was performed on a Kromasil C (18) column (250 mm × 4.6 mm ID, 5µm) with methanol and water (percentage) as mobile phase. The column temperature was set up and the flow - rate was 1 (ml/min^{-1}). The reference solution of chemical standards and sample were injected into HPLC system, separately. The experiment was carried out by Shimadzu LCsolution Analysis Report Company.

RESULTS

The aim of the study was to show the different inhibitory effect of rat intestinal alpha glucosidase with different concentrations and to study the free radical scavenging activities by ethyl acetate extracts of endophytic fungi (EPF) and actinomycetes (EPA) isolated from the root of *C. roseus.*

Alpha glucosidase

On analyzing the inhibitory effect of rat intestinal alpha glucosidase by the extracts at different concentrations, the ethyl acetate extract of endophytic actinomycetes (EPA) isolated from the root of *C. roseus*, had shown a maximum inhibitory effect, than the other extracts (Table 1). Whereas, the crude ethyl acetate extract of endophytic fungi (EPF) showed a less activity (32.42 ± 0.03) even at 1000µg/ml concentration. Based on the results obtained from the *in vitro* study the ethyl acetate extract of actinomycetes was found to show a high activity. Hence, *in vivo* studies were carried out using endophytic actinomycetes on lowering maltose and sucrose absorption in the intestine. At 30 minutes after maltose load, the normal control animals had shown an increase in plasma glucose level; where as the EPA treated as well as the acarbose treated animals had not shown any significant rise in plasma glucose level. Chemical analysis revealed that the major constituents of the extracts are alkaloid compounds. Our results revealed that the ethyl acetate extract of endophytic actinomycetes were able to inhibit alpha glucosidase inhibitory activities *in vitro* in a dose dependent manner. At 60 min after sucrose load, the control animals had shown an increase in plasma glucose level 118.83 ± 0.06 whereas the EPA treated as well as the acarbose treated animals had not shown any rise in plasma glucose level (Table 2,3).

Antioxidant property

The free radical scavenging activity of ethyl acetate extracts of endophytic fungi and actinomycetes was observed in the presence of DPPH, Hydroxyl, Hydrogen peroxide and Nitric oxide respectively. In DPPH free radical scavenging activity, the extract of endophytic fungi was found to show a high percentage of inhibition (54.50 ± 0.31) at 1000µg/ml, whereas the ethyl acetate extract of endophytic actinomycetes showed a least activity 17.89 ± 0.47 (Table 4). In hydroxyl free radical scavenging activity, endophytic actinomycetes showed high activity 70.90 ± 0.17 at 1000µg/ml whereas when compared to endophytic fungi it showed only 47.66 ± 0.34 (Table 5). In the case

of hydrogen peroxide free radical scavenging activity, both endophytic fungi and actinomycetes showed moderate inhibition percentage, 42.86 ± 0.27 and 46.84 ± 0.23 at 1000µg/ml. For nitric oxide scavenging activity, endophytic actinomycetes showed high activity 48.71 ± 0.29 whereas endophytic fungi showed a least activity 19.96 ± 0.23. BHT and Ascorbic acid were used as the positive control, which showed 72.76 ± 0.17 and 63.00 ± 0.25 at 500 µg/ml respectively (Table 6,7). HPLC analysis shows that the extract of EPF is a mixture of compounds as it shows many peaks (Fig 1a) whereas the extract of EPA has a major compound at 2.850Mv (Fig 1b) respectively. Purification of the extracts from these actinomycetes is under progress. The EPA has a potential ability to inhibit the alpha glucosidase enzyme thereby causing partial digestion and keeping the blood glucose level normal and scavenge the free radicals hydrogen peroxide more when compared to the other free radicals.

Table 1

In vitro α -glucosidase inhibition using ethyl acetate extracts of EPF and EPA of C. roseus

Concentration (µg/ml)	% of inhibition of Alpha-glucosidase		
	EPF	EPA	Acarbose
50	0.78 ± 0.04	5.75 ± 0.05	74.53 ± 0.01
100	3.23 ± 0.28	16.84 ± 0.06	81.23 ± 0.02
200	14.57 ± 0.02	34.76 ± 0.02	87.98 ± 0.01
400	18.64 ± 0.04	45.00 ± 0.00	91.78 ± 0.02
500	28.60 ± 0.02	55.54 ± 0.02	94.08 ± 0.04
1000	32.42 ± 0.03*	83.29 ± 0.02*	96.71 ± 0.03

All values represent (mean ± SE) taken from two experiments each with 3 replicates per treatment was tabulated The values (mean ± SE) for four animals; * values indicates that the values significantly differ from corresponding reference values.

Table 2

In vivo lowering of maltose level in the blood using ethyl acetate extracts of endophytic actinomycete of C. roseus

Tested material	'0' hour	30 minutes	60 minutes	120 minutes
Control	86.97 ± 0.07	96.17 ± 0.04	109.28 ± 0.04*	109.67 ± 0.06
Treated	94.72 ± 0.03	93.04 ± 0.03	93.04 ± 0.03	108.47 ± 0.04*
Acarbose	97.14 ± 0.07	97.46 ± 0.05	94.95 ± 0.04*	96.30 ± 0.01

The values (mean ± SE) for four animals; 109.28 ± 0.04 indicates that the values significantly differ from corresponding zero hour values.

Table 3

In vivo lowering of sucrose level in the blood using ethyl acetate extracts of endophytic actinomycetes of C. roseus

Tested material	'0' hour	30 minutes	60 minutes	120 minutes
Control	90.78 ± 0.04	104.58 ± 0.05	118.83 ± 0.06*	100.33 ± 0.06
Treated	94.01 ± 0.02	96.01 ± 0.06	105.18 ± 0.03	106.15 ± 0.04*
Acarbose	95.46 ± 0.06	94.78 ± 0.05	101.64 ± 0.07*	99.45 ± 0.07

The values (mean ± SE) for four animals; * values indicates that the values significantly differ from corresponding zero hour values.

Table 4
DPPH radical scavenging activity of EPF and EPA extracts

Concentration (µg/ml)	% Inhibition of DPPH free radical			
	EPF	EPA	BHT	Ascorbic acid
50	17.19 ± 0.24	02.14 ± 0.15	68.10 ± 0.39	72.24 ± 0.18
100	25.10 ± 0.10	06.05 ± 0.21	73.23 ± 0.19	77.90 ± 0.35
200	19.62 ± 0.25	09.25 ± 0.24	77.79 ± 0.16	83.78 ± 0.20
400	37.63 ± 0.35	12.99 ± 0.15	84.86 ± 0.13	89.13 ± 0.36
500	47.59 ± 0.37*	14.74 ± 0.32*	90.00 ± 0.46	92.59 ± 0.31
1000	54.50 ± 0.31*	17.89 ± 0.47*	93.92 ± 0.45	94.64 ± 0.41

*Each value represents Mean ± SE of triplicates, in two experiments. The values (mean ± SE) for four animals; * values indicates that the values significantly differ from corresponding BHT and Ascorbic acid values.*

Table 5
Hydroxyl radical scavenging activity

Concentration (µg/ml)	% Inhibition of Hydroxyl free radical			
	EPF	EPA	BHT	Ascorbic acid
50	0.83 ± 0.05	34.18 ± 0.33	86.38 ± 0.34	87.84 ± 0.14
100	0.36 ± 0.04	42.57 ± 0.11	88.82 ± 0.14	88.76 ± 0.33
200	03.84 ± 0.42	47.10 ± 0.31	90.73 ± 0.36	88.58 ± 0.41
400	21.38 ± 0.32	50.07 ± 0.38	92.28 ± 0.15	92.79 ± 0.25
500	30.58 ± 0.57*	60.95 ± 0.29*	97.79 ± 0.48	96.05 ± 0.14
1000	47.66 ± 0.34*	70.90 ± 0.17*	98.74 ± 0.19	98.94 ± 0.18

*The values (mean ± SE) for four animals; * values indicates that the values significantly differ from corresponding BHT and Ascorbic acid values.*

Table 6
Hydrogen peroxide radical scavenging activity

Concentration (µg/ml)	% Inhibition of Hydrogen peroxide free radical			
	EPF	EPA	BHT	Ascorbic acid
50	02.88 ± 0.17	11.77 ± 0.16	46.07 ± 0.28	47.10 ± 0.38
100	17.03 ± 0.16	18.85 ± 0.11	56.57 ± 0.15	56.22 ± 0.18
200	19.93 ± 0.28	25.18 ± 0.26	59.17 ± 0.35	60.98 ± 0.15
400	27.87 ± 0.17	36.15 ± 0.21	65.46 ± 0.14	67.18 ± 0.19
500	31.96 ± 0.35	42.86 ± 0.27*	72.81 ± 0.44	72.76 ± 0.17
1000	42.74 ± 0.14*	46.84 ± 0.23*	83.97 ± 0.15	85.40 ± 0.13

*The values (mean ± SE) for four animals; * values indicates that the values significantly differ from corresponding BHT and Ascorbic acid values.*

Table 7
Nitric oxide radical scavenging activity

Concentration (µg/ml)	% Inhibition of Nitric oxide free radical			
	EPF	EPA	BHT	Ascorbic acid
50	1.93 ± 0.20	04.96 ± 0.13	22.12 ± 0.34	35.32 ± 0.16
100	03.8 ± 0.18	21.85 ± 0.26	29.81 ± 0.22	42.91 ± 0.03
200	05.55 ± 0.04	26.82 ± 0.19	45.38 ± 0.25	44.73 ± 0.12
400	07.17 ± 0.10	35.19 ± 0.28	55.46 ± 0.16	50.71 ± 0.04
500	11.45 ± 0.16	49.65 ± 0.21*	63.20 ± 0.38	63.00 ± 0.25
1000	19.96 ± 0.23	48.71 ± 0.29*	68.11 ± 0.36	73.38 ± 0.21

The values (mean ± SE) for four animals; * values indicates that the values significantly differ from corresponding BHT and Ascorbic acid values.

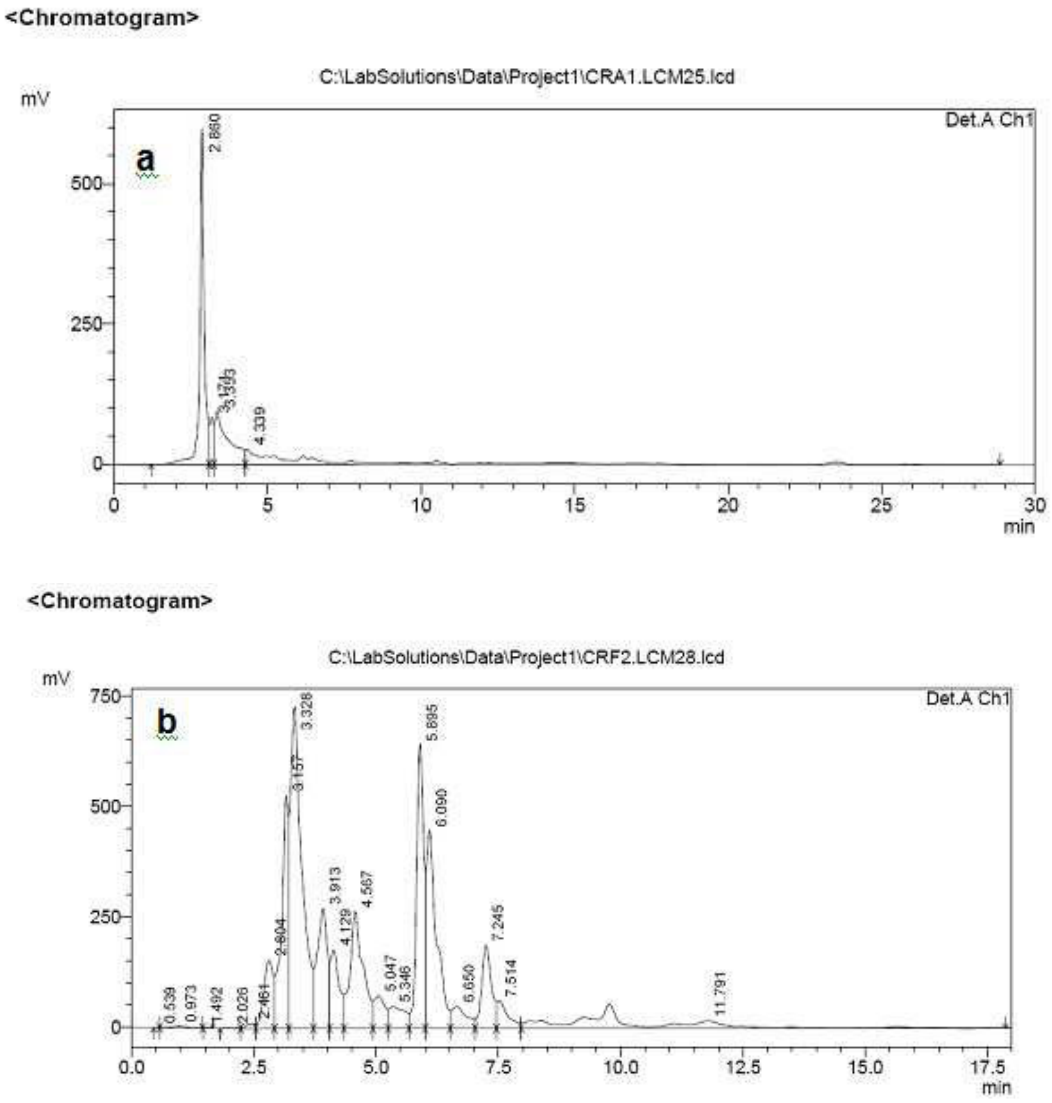

Figure 1
HPLC profile of ethyl acetate extract of a) EPA b) EPF isolated from the root of C. roseus

DISCUSSION

Alphaglucosidase

Agents with alpha glucosidase inhibitory activity have been useful for the control of postprandial hyperglycemia in patients with frank diabetes. These drugs reversibly inhibit the digestion of disaccharides, and thus the conversion of disaccharides into monosaccharides, eliciting attenuated postprandial blood glucose levels. There are many natural products with alpha glucosidase inhibitory activity. Many reports have shown a significant reduction by treating with plant extracts (Chidambaram and Subash 2007). In the present study, the *α-glucosidase* inhibitory effect of the crude ethyl acetate extract of EPA was comparable to that of the acarbose, which is the standard alpha glucosidase inhibitor. The enzyme inhibitors impede digestion through their action of digestive enzymes (amylase and glucosidase) play a key role in the digestion of plant starch and portions. In *in vitro* study, CKD-711 an amino oligosacharide showed similar effects to acarbose on porcine intestinal maltase and sucrase, IC_{50s} of 2.5 and 0.5 MU g/ml, respectively, whereas it had about 2 fold lower (Kwon et al., 2002). Living organisms use enzyme inhibitors as a major tool to regulate glycolytic activities of alpha amylase (Young et al., 2003). In rat fed on starch and sucrose meals, the dose of CKD-711 which reduced the postprandial blood glucose increment by 50 percent in comparison to control rats (ED_{50}) were 3.07 and 1.15 mg/kg, respectively, and acarbose had ED_{50} of 1.94 and 1.15 mg/kg, respectively (Kwon et al., 2002). Our results showed strong inhibition of alpha glucosidase activity. Higher inhibitory activities of crude ethyl acetate extracts of EPA against alpha glucosidase confirmed, suggest its potential in prevention and therapy of obesity and diabetes. In most of the cases, the mechanism of inhibition occurs through the direct blockage of the active center at several sub sites of the enzyme (Payan, 2004).

Antioxidant property

DPPH radical scavenging activity

The DPPH radical scavenging activity is a standard procedure applied to the evaluation of antiradical activity. DPPH radical scavenging method is a standard procedure applied for the evaluation of antiradical activity. DPPH gives a strong absorption at 517 nm. If the electron pairs off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolourization is stoichiometric in respect to the number of electrons taken up (Ilhami et al., 2006). The DPPH free radicals, which are stable in ethanol shows maximum a proton donating substance such as antioxidant. The effect of free radical scavenging activity of our crude EPF extract on DPPH radicals is thought to be due to their hydrogen donation ability of endophytic fungi of *C. roseus*. The results showed that the crude extract of endophytic fungi is a moderate free radical scavenger that it reacts with free radicals, which may limit the occurrence of free radical damage in human body. According to Prochazkova et al., (2001), many stress situations caused an increase in the total antioxidant activity. Absorbance decreases as a result of the colour change from purple to light yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH free radical (Nazif, 2002). The strongest antioxidant fungal strain, AcapF3, was found in *Agrostis capillaris*, which also showed strong antioxidant activity using the DPPH method by Jung et al., (2004).

Hydroxyl radical scavenging activity

Oxygen free radicals, especially hydroxyl radicals, can accelerate the peroxidation of the polyunsaturated fatty acids in the cell membrane, generating malonaldehyde which is easy to react with phospholipid and hence the characteristics of the cell membrane can be changed (Zeng et al., 2007). This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contribute to

ageing, carcinogenesis, mutagenesis, cytotoxicity and several other diseases. Among the oxygen radicals specifically, the OH radical is the most reactive and severely damages the adjacent biomolecules such as, all proteins, DNA, Poly Unsaturated Fatty Acids (PUFA) and almost any biological molecule. Free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH) can react with many substances like lipids, proteins, *etc*. They are very unstable and react rapidly with other groups or substances in the animal body, leading to cell or tissue injuries (Jae et al., 2005).Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living cells body against various diseases. The ability of the extract to quench hydroxyl radicals seems to directly relate to the prevention of propagation of the process of lipid peroxidation, and the extract seems to be a good scavenger of active radical species, thus reducing the rate of chain reaction.

Hydrogen Peroxide radical scavenging activity

Hydrogen peroxide itself is not very reactive, as it can sometimes be toxic to cell because it may give rise to OH radical in the cells. Addition of hydrogen peroxide to cells in culture can lead to transition metal ion dependent OH radicals mediated DNA damage (Wu-Yang et al., 2007). Superoxide anion is a reduced form of molecular oxygen and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen (Ani et al., 2006). Hydrogen peroxide scavenging activity by ethyl acetate extract of EPA showed a more activity compared to EPF. Hence, EPA acts as a moderate scavenger. Scavenging of hydrogen peroxide by our crude endophytic extract may be attributed to their phenolic nature, which can donate electrons to H_2O_2, thus neutralizing it to water (Wettasinghe and Shahidi 2000). Thus removing hydrogen peroxide is very important for life being away from damage.

Nitric oxide radical scavenging activity

Nitric oxide is a diffusible free radical, which plays many roles as an effector molecule in diverse biological systems including neuronal signaling, and regulation of cell mediated toxicity. On the other hand, nitric oxide (NO), which has an enormous range of beneficial functions in organisms, including regulation of vascular tone, ventilation, hormone secretion, inflammation, immunity and neurotransmission, is also suspected to be cytotoxic or cytostatic to host cells, and to act as a toxic radical (Takako Yokozawa et al., 2001). Suppression of NO released may be partially attributed to direct NO scavenging, as the extract decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. In the present study, nitric oxide was scavenged more by endophytic actinomycetes when compared to endophytic fungi which showed least activity. Therefore, ethyl acetate extracts of EPA was found to inhibit the activity of alpha glucosidase as well as to scavenge the free radicals such as DPPH, Hydrogen peroxide and Nitric oxide. However, the component responsible for the activities of various mechanisms seems to be unclear. Therefore, it is suggested that further work be performed on the isolation and identification of active molecule from EPA.

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