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Article

Independent and synergistic activity of the flavonoids of *Gracilaria corticata* as promising antidiabetic agents

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The therapeutic approaches for Type 2 Diabetes Mellitus rely most on the usage of oral hypoglycaemic drugs. These drugs have adverse side effects and hence alternative medicines are continuously explored. The present study intends to investigate the antidiabetic potential of the flavonoids present in *Gracilaria corticata*. The flavonoids were isolated (FEGC) and their inhibitory activity on the carbohydrate hydrolysing enzymes such as α-amylase and α-glucosidase was analysed. The flavonoids were found to inhibit α-amylase and α-glucosidase with an IC_{50} value of 302 µg and 75 µg respectively. The synergistic effect of FEGC and luteolin was also investigated and the results show that both FEGC and luteolin inhibited synergistically at half their IC_{50} values. The observations of this study reveal that the flavonoids of *G. corticata* have potential antidiabetic activity and can act independently or synergistically in the management of Type 2 Diabetes Mellitus

Keywords: α-amylase. α-glucosidase. Antidiabetic. *Gracilaria corticata*. Luteolin. Synergistic.

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder which manifests itself as hyerglycemia. The prevalence of DM is increasing at an alarming rate and it is estimated that more than 550 million people would be afflicted by it by the year 2030. Type 2 DM is the most common form of DM and its incidence is triggered by genetic, environmental and lifestyle factors (Olokoba, Obateru, Olokoba, 2012). Insulin resistance coupled by insufficient insulin secretion by the β-islet cells of the pancreas cause Type 2 DM (Kahn, 2008). The short and long term complications of diabetes and its relatively late diagnosis account for the morbidity and mortality seen in diabetes (Malviya *et al*., 2010). DM is prevalent more in certain ethnic groups and in low and middle income countries (Azevedo, Alla, 2008, Global Burden of Diabetes, 2011).

The treatment for DM involves hypoglycaemic drugs, diet changes and lifestyle modifications. The spectrum of hypoglycaemic drugs includes insulin, insulin secretagogues, insulin sensitizers and enzyme inhibitors. Chronic consumption of these drugs results in undesirable side effects and economic burden (Ahmad, Crandall, 2010, Siddiqui *et al*., 2013). Hence complementary and alternative medicine is sought after in many countries around the world. Medicinal plants, plant phytoconstituents and sea weeds are the most common forms of alternative medicine used and researched worldwide (Malviya, Jain, Malviya, 2010).

Marine organisms are promising resources of several compounds with medicinal values. Algae, also referred to as sea weeds, are large group of marine organisms, unicellular or multicellular, capable of carrying out photosynthesis (Pulz, Gross, 2004). Algae form a component of traditional medicine, Chinese medicine in particular (Yamamoto *et al*., 1984). Sea weeds are rich sources of bioactive molecules like flavonoids, terpenoids, vitamins etc and hence serve as excellent food and medicine. Algae belonging to all three categories namely, Red, Brown and Green algae are consumed as food and found to possess pharmacological activities (Kaliaperumal, Kalimuthu, 1997).

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Gracilaria corticata is a red seaweed with immense industrial and biotechnological potential, owing to the presence of phycocolloids (de Almeida *et al*., 2011). *G. corticata* exhibits antioxidant, antidiabetic, antimicrobial, cardioprotective, gastroprotective and hepatoprotective activities. The pharmacological activities of *G. corticata* are manifested by its phytoconstituents such as flavonoids, quinines, phenolics, glycosides etc (Balakrishnan, Jenifer, Esakkilingam, 2013). *G.corticata* are rich in flavonoids and the present study aims at comparing the antidiabetic potential of the flavonoids present in *G.corticata* and luteolin. The inhibition of α-amylase and α-glucosidase by luteolin and *G. corticata* extract was studied to evaluate their hypoglycaemic activity.

METHODS

Chemicals

Acarbose, α-glucosidase and p-nitrophenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich, St. Louis, USA and α-amylase was purchased from Himedia. All the other chemicals used were of analytical grade.

Collection of *Gracilaria corticata* seaweed

Seaweeds of *Gracilaria corticata* were collected in the month of June from south sea coast of Mandapam, Rameshwaram, Tamil Nadu, India.

Preparation of extracts

G. corticata seaweeds were cleaned and the necrotic parts were removed. The seaweeds were washed with tap water to remove any associated debris and shade dried at room temperature (28 ± 2 ºC) for 5-8 days or until they are brittle easily by hand. After complete drying, the seaweed material (1.0 kg) was ground to a fine powder using an electrical blender. Forty gram of powdered seaweeds was extracted with 200 mL of water by cold maceration until the extract was clear. The extract was evaporated to dryness under reduced pressure using a rotary vacuum evaporator and the resulting extract was stored at 4 °C for future use. The prepared extract of *Gracilaria corticata* seaweed was named as Aqueous Extract of *Gracilaria corticata* (AEGC).

Extraction of total flavonoids

The total flavonoids present in *G. corticata* were extracted with the assistance of microwaves. The extraction was carried out in a domestic microwave oven. The AEGC irradiated for 20 min in the oven at regular intervals to maintain the temperature below 80 ˚C (1 min on and 2 min off). The extract (FEGC) obtained was cooled, filtered, evaporated using a rotary evaporator and stored at 4 ˚C for further study.

Estimation of flavonoid content

The total flavonoid content of FEGC was estimated by aluminium chloride method. Briefly, 1 mL of the extract (100 mg/mL) was diluted to 5 mL and 300 µL of sodium nitrite (5% w/v) was added and incubated for 5 minutes. This was followed by the addition of aluminium chloride (10% w/v). After 6 minutes, 2 mL of sodium hydroxide (1 M) was added and the resulting mixture was diluted to 10 mL with d.water. After an incubation period of 15 minutes, the color developed was read at 510 nm. The amount of flavonoid present in the extract was calibrated from a quercetin standard curve. The experiment was done in triplicate.

Assay of alpha amylase inhibition

In vitro amylase inhibition was studied by the method of Bernfeld (1955). In brief, 100 µL of the extract was allowed to react with 200 µL of α-amylase enzyme and 100 µL of phosphate buffer (2 mM, pH 6.9). Hundred microlitres of 1% starch solution was added after 20 min incubation. 200 µL of the buffer served as the control. About 500 µL of dinitrosalicylic acid reagent was added to both control and test and incubated at 60 ˚C for 5 min. Acarbose was used as the standard inhibitor. The absorbance was recorded at 540 nm and the experiment was done in triplicate.

Assay of alpha glucosidase inhibition

In vitro α-glucosidase inhibition was performed by pre-incubation of equal volumes of extract, sodium phosphate buffer (1 mM, pH 6.9) and α-glucosidase enzyme for 5 min and then addition of 0.1 mL of p-nitrophenyl-α-D-glucopyranoside, followed by incubation at 25 ˚C for 10 min. Acarbose was used as the standard inhibitor. The absorbance was recorded

at 405 nm and the percentage of inhibition was calculated.

Calculation of percentage of inhibition

The percentage inhibition of the enzymes was calculated using the formula

$$\text{Inhibition}(\%) = \frac{100 \text{ x } (\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}}$$

Determination of Combination Index (CI)

$$CI = \frac{C_{a,x}}{IC_{a,x}} + \frac{C_{b,x}}{IC_{b,x}}$$

$C_{a,x}$ is IC_{50} of luteolin
$C_{b,x}$ is IC_{50} of FEGC
$IC_{a,x}$ is the concentration of luteolin in combination required to achieve 50% inhibition
$IC_{b,x}$ is the concentration of FEGC in combination required to achieve 50% inhibition

Statistical analysis

All the observed values are expressed as mean of six experiments ± standard deviation. The IC50 values were calculated by regression analysis.

RESULTS AND DISCUSSION

One common therapeutic approach for treating DM is preventing the post-prandial increase of glucose in blood. This can be achieved by interfering with glucose absorption and thus with carbohydrate hydrolysing enzymes. α-amylase and α-glucosidase convert oligo- and di-saccharides into monosaccharides for absorption. Inhibition of these hydrolysing enzymes delays the absorption process and thus indirectly prevents the increase in the postprandial levels of glucose. This study was conducted primarily to analyse the antidiabetic potentials of flavonoids present in AEGC (FEGC) and luteolin. The additive effect of FEGC and luteolin was also investigated.

The flavonoid extraction was performed using microwaves. Microwave assisted extraction of flavonoids has been reported for several plants. Alghazeer *et al.* (2017) has reported such extraction for brown algae. The flavonoid content in FEGC was found to be 18 mg/g of extract.

The α-amylase inhibitory potential of luteolin and FEGC were studied and the concentration range under study was 12.5 to 400 µg. The results indicate that inhibition of the enzyme was better with luteolin and FEGC also showed significant inhibition. Around 50% inhibition of enzyme was shown by luteolin, FEGC and acarbose at 25 µg concentration. Maximum inhibition of the enzyme was found at 200 µg. The IC50 of luteolin was 34.5 µM and that of FEGC was 302 µg.

Similarly, the α-glucosidase inhibition study revealed that the inhibitory potential was dose dependent and maximum inhibition was observed at 200 µg concentration for all the three components studied. The IC50 of luteolin was 2.4 µM and that of FEGC was 75 µg.

The percentage inhibition of enzymes at the IC50 concentrations was also studied. It was observed that luteolin exhibited 60% and 45% inhibitory activity for amylase and glucosidase respectively and FEGC exhibited 58% and 52% inhibitory activity for amylase and glucosidase respectively (Table I). These results were almost compatible with those reported by Kim, Kwon and Son (2000) and Tadera *et al*. (2006).

The flavonoids inhibit amylase activity by forming complexes with starch, thereby suppressing the digestion of starch molecules (Takahama, Hirota, 2018). Few studies suggest that the inhibitory activity of flavonoids is related to the structure of flavonoids. The hydroxyl groups, the substitution on B ring, the double bonds and the linkage of B ring were reported to enhance the inhibitory potential (Tadera *et al*., 2006; Proenca *et al*., 2017).

The synergistic effect of FEGC and luteolin at four different doses were studied, i.e., 0.5, 1, 2 and 4 times their IC50 concentrations. The combination index was calculated and it was found that luteolin and FEGC functioned synergistically in inhibiting α-amylase at concentrations half their IC50 values. However, such a synergistic effect was not observed with α-glucosidase and luteolin and FEGC exhibited additive effect at 0.5 x IC50 concentrations (Table II).

The observations however imply that the combination of luteolin and FEGC can be investigated *in vivo* for their antidiabetic potential. Omojokun *et al*. (2014) have reported on the combination of quercetin and rutin in experimental diabetic rats.

This study shows that the flavonoids of *G. corticata* are potent antidiabetic agents and can be used to treat or manage type 2 DM. *G. corticata* has been reported to

possess a wide spectrum of pharmacological properties (de Almeida *et al*., 2011). Nevertheless, the potential of the flavonoids of *G.corticata* are yet to be explored.

TABLE I - Inhibitory activity of FEGC and luteolin against α-amylase and α-glucosidase

Flavonoid	α-amylase		α-glucosidase	
	Inhibition (%)	IC_{50}	Inhibition (%)	IC_{50}
FEGC	58	302[a]	52	75[a]
Luteolin	60	34.5[b]	45	2.4[b]

a – value expressed as mg
b – value expressed as μM
The values mentioned are average of three independent experiments.

TABLE II - Activity of FEGC and luteolin as a combination against α-amylase and α-glucosidase

Flavonoid	Luteolin		FEGC	
	$C_{a,x}$	$IC_{a,x}$	$C_{b,x}$	$IC_{b,x}$
α-amylase	9.8	44.5	302	1510
α-glucosidase	0.686	0.709	75	73

$C_{a,x}$ is IC_{50} of luteolin
$C_{b,x}$ is IC_{50} of FEGC
$IC_{a,x}$ is the concentration of luteolin in combination required to achieve 50% inhibition
$IC_{b,x}$ is the concentration of FEGC in combination required to achieve 50% inhibition.

CONCLUSION

The management of Type 2 diabetes using the antidiabetic potential of the flavonoids present in *G. corticata* was investigated. The flavonoids were observed to possess potent antidiabetic activity and were found to act synergistically with luteolin, a flavonoid that has manifested itself as an important antidiabetic agent. The *in vivo* antidiabetic effect of the flavonoids of *G. corticata* must be evaluated.

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