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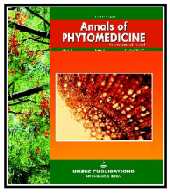

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In vitro α-amylase, α-glucosidase and β-galactosidase inhibitory activity of *Echinochloa colona* (L.) Link

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Abstract

Caryopsis (grains) of *Echinochloa colona* (L) Link was extracted with methanol (MEEC) by maceration and its fractions were prepared by dispersing uniformly in distilled water and subjected to solvent fractionation with toluene (TFEC), n-butyl alcohol (BFEC), and water (AFEC). The methanolic extract and fractions were concentrated under reduced pressure and were evaluated for antidiabetic activity by employing standard *in vitro* techniques (α-amylase, α-glucosidase and β-galactosidase activity were tested by considering the % inhibition of enzymes and compared with standard). *In vitro* antidiabetic studies (among all the fractions and methanol extract), the n-butyl alcohol fraction showed more active α- amylase, α-glucosidase and β-galactosidase inhibitory activity with 71.47 ± 4.53%, 72.89 ± 2.52% and 89.91 ± 0.84% with IC_{50} values of 6.96 mg/ml, 6.81 mg/ml and 7.8 mg/ml, respectively, which are comparable with standard 95.22 ± 0.74 %, 89.26 ± 0.79 % and 98.24 ± 0.90% with IC_{50} values of 5.54 ± 0.63 mg/ml, 5.17 ± 0.020 mg/ml and 10.32 ± 0.21 mg/ml. Results confirm that the n-butyl alcohol fraction of *E. colona* exhibited the highest antidiabetic activity among all the fractions and methanol extract. Further studies are needed for purification, characterization, and structural elucidation of bioactive compounds from n-butyl fractions and also confirm its antidiabetic activity by *in vitro* studies. These substantiate the use of *E. colona* seeds in traditional medicine for the treatment of diabetes.

1. Introduction

World Health Organization (WHO) defined Diabetes mellitus as “a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both”. The effects of diabetes mellitus include long-term damage, dysfunction, and failure of various organs including the kidney, nerves, heart, and gastrointestinal tract (Kaur and Valecha, 2014). The global prevalence of diabetes has reached epidemic proportions with worldwide incidence projected to increase from 424 million in 2017 to 629 million by 2045, Hence, there is always scope for the development of antidiabetic drugs due to their high prevalence and long term complications (Saleem *et al*., 2019).

In recent years, emphasis is on the development of drugs from plants or the treatment of various diseases including diabetes mellitus, the incidence of which is very high all over the world. The reason is that plant drugs could be effective and at the same time have fewer or no side effects (Rother, 2007; Wilson *et al*., 1980). One therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. One of the effective managements of diabetes mellitus, in particular, non-insulin-dependent diabetes mellitus (NIDDM) to decrease postprandial hyperglycemia, is to retard the absorption of glucose by inhibition of carbohydrate hydrolyzing enzymes, such as α-glucosidase and α-amylase, in the digestive organs. α-glucosidase is the key enzyme catalyzing the final step in the digestive process of carbohydrates. Hence, α-glucosidase inhibitors can retard the liberation of d-glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppression of postprandial hyperglycemia. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise (Ali *et al*., 2006).

β-galactosidase is also a member of glycosyl hydrolysis enzyme, which may cause the occurrence in releasing of glucose and galactose by way of cleavage of β-1,4-D-galactoside linkage of lactose. This enzyme activates the final step in the process of carbohydrate digestion, resulting in the breakdown of disaccharides and oligosaccharides into absorbable glucose. Therefore, the inhibition of these hydrolytic enzymes may ameliorate postprandial hyperglycemia by restraining the influx of glucose from the intestinal tract to blood vessels (Li *et al*., 2016).

In the present study, *E. colona* (Synonym: *Echinochloa colonum*), belong to the family Poaceae was selected. It is commonly called jungle rice, shama millet, and small barnyard grass. In Telugu, it is called othagaddi and distributed in tropical and subtropics including

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South India and Southeast Asia, and tropical Africa. In India, seeds of EC are used to prepare a food dish khichdi and consumed during festivals, fasting days, the whole plant is used as fodder by grazing animals and it cures ingestion (Borkar *et al*., 2016). Traditionally the grains of the plant (boiled and consumed as rice) were used as antidiabetic by the local tribes of south Rajasthan (India) (Shah *et al*., 2019).

Phytochemical screening of *E. colona* reveals the presence of flavonoids, glycosides, lignin's coumarins, phenols, saponins, and tannins. And quantitative phytochemical analysis revealed that flavonoids 0.210 ± 0.040, phenols 0.004 ± 0.001, saponins 0.039 ± 0.011, and tannins 0.024 ± 0.002. The results suggest that the plant species possess the properties for curing various ailments such as anti-inflammatory and antimicrobial activities (Babu and Savithramma, 2013), wound healing activity (Borkar *et al*., 2015), potential antioxidant (Ajaib and Khan, 2013), hepatoprotective activity (Praneetha *et al*., 2017). And a compound 'tricin' was isolated from the dichloromethane (CH_2Cl_2) fraction of methanol (MeOH) extract of *E. colona* have shown significant phytotoxic effects on the germination and seedling growth of weeds. So, that might be potentially useful as a bioherbicide for weed control in agriculture which should be investigated further in the field for their practical application (Sayani and Chatterjee, 2017). Traditionally, it is used in diabetes, biliousness, constipation, and cooling (Madhava Chetty *et al*., 2008). Hence, the present study is focused on the enzyme inhibitory activity of diabetes. The methanolic extract of caryopsis of *E. colona* and its fractions were tested for α-amylase, α-glucosidase, and β-galactosidase enzyme inhibitory properties by colorimetric method.

2. Materials and Methods

2.1 Materials

α-amylase (porcine pancreas), α-glucosidase (*Saccharomyces cerevisiae*) and β-galactosidase (*E.coli*), P-nitro phenyl β-D-glucopyranoside (PNPG), O-nitro phenyl-β-galactosidase (ONPG), 3, 5-Di-nitro salicylic acid, acarbose, quercetin, sodium potassium tartrate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, disodium hydrogen phosphate, and di-potassium hydrogen phosphate, starch, dimethyl sulfoxide (DMSO), were procured from Gamut Scientifics (SRL), Secunderabad, Telangana, India. All other chemicals and solvents used are of analytical grade.

2.2 Collection of plant material

Caryopsis of *Echinochloa colona* (L) Link were collected from the Kakatiya University Campus, Warangal, Telangana, India. After authentication by Professor V.S. Raju (Taxonomist), Department of Botany, Kakatiya University, Warangal. The voucher specimen (KU/UCPSC/49) of the plant was deposited in the Department of Pharmacognosy, University College of Pharmaceutical Sciences and Warangal, India.

2.3 Preparation of methanolic extract and fractions

Caryopsis (Seeds) of *E. colona* were collected, dried under shade ground into coarse powder and then macerated with methanol in a round bottom flask at room temperature, and then fractionated with toluene, n-butyl alcohol, and aqueous was concentrated under reduced pressure (Rotavapour, Switzerland) to yield green color mass.

2.4 Phytochemical screening

The dried extracts and fractions of *E. colona* were subjected to phytochemical screening for the presence of different classes of organic compounds like alkaloids, carbohydrates, saponins, flavonoids, proteins, and phenolic compounds according to standard methods (Harborne, 1973). Any change of colours or the precipitate formation was used as indicative of positive response to these tests.

2.5 α-amylase enzyme inhibitory assay

This assay was carried out by using a modified procedure of McCue and Shetty (McCue and Shetty, 2004). The stock solution of extracts was prepared by dissolving up to 10 mg of each extract in 1 ml of dimethyl sulfoxide (DMSO). A total of 250 µl of extracts (1.25-10 mg/ml) was placed in a tube and 250 µl of 0.02 M sodium phosphate buffer (pH6.9) containing α-amylase solution (0.5 mg/ml) was added. This solution was pre-incubated at 25°C for 10 min, after which 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at particular time intervals and then further incubated at 25°C for 10 min. The reaction was terminated by adding 500 µl of Di-nitro salicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 ml distilled water and the absorbance was measured at 540 nm using (ELICO-SL-159-UV-Visible Spectrophotometer). A control was prepared using the same procedure replacing the extract with distilled water.

$$\%\text{Inhibition} = \frac{(\text{Absorbance (control)} - \text{Absorbance (extract)})}{\text{Absorbance (control)}} \times 100$$

2.6 α-glucosidase enzyme inhibitory assay

The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis with some modifications (Apostolidis *et al*., 2007). Stock solutions of extracts were prepared by dissolving up to 10 mg of each extract in 1ml of DMSO. A total of 50 µl of extracts (1.25-10 mg/ml) and 100 µl of yeast α-glucosidase solution in phosphate buffer (pH 6.9) were incubated at 25°C for 10 min, followed by the addition of 50 µl of 5 Mmol/l, P-nitropheny l-A-D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9). The reaction mixture was then incubated at 25°C for 5 min and their action was terminated by adding 3 ml of 100 Mm sodium carbonate solution into the mixture and absorbance of liberated p-nitrophenol was observed at 405 nm using UV-Visible Spectrophotometer. Acarbose was used as a positive control (standard) and the inhibitory activity of α-amylase and α-glucosidase was calculated by using the following formula:

$$\%\text{Inhibition} = \frac{(\text{Absorbance (control)} - \text{Absorbance (extract)})}{\text{Absorbance (control)}} \times 100$$

2.7 β-galactosidase inhibitory assay

The β-galactosidase solution was adjusted to 0.2 U/ml by 0.1 M phosphate buffer (pH 7.3). Two hundred microliters of enzyme solution. 100 µl of different concentrations of BFEC, TFEC, AFEC, and 2.4 ml of 0.1 M phosphate buffer (pH 7.3) were mixed and incubated at 37°C for 15 min. After pre-incubation, 100 µl of 30 mM O-nitro phenyl-β-D-galactoside (ONPG) solution in 0.1M phosphate buffer (pH 7.3) was added and incubated at 37°C for 30

min. The reaction was terminated by 2 ml of 0.2 M sodium carbonate solution. The absorbance of the mixture solution was measured by a Spectrophotometer at 410 nm. All the assays were performed in triplicates.

$$\%\text{Inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

The IC_{50} values defined as the concentration of the extract that inhibited 50% of the enzyme activity were determined from plots of % inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values. (Zeng *et al.*, 2016].

Table 1: α-amylase inhibitory activity and IC_{50} values by *E. colona* extract, fractions and acarbose

Test sample	Concentration (mg/ml)	% inhibition	IC_{50}
MEEC	1.25	09.52 ± 0.63	8.10 ± 0.84
	2.5	19.64 ± 1.23	
	5	26.23 ± 2.12	
	10	70.56 ± 3.54	
BFEC	1.25	09.82 ± 0.74	6.96 ± 0.54
	2.5	18.86 ± 1.29	
	5	36.34 ± 2.92	
	10	71.47 ± 4.53	
TFEC	1.25	08.79 ± 0.55	7.46 ± 0.54
	2.5	17.58 ± 0.96	
	5	34.35 ± 1.96	
	10	68.34 ± 3.46	
AFEC	1.25	05.44 ± 0.52	8.99 ± 0.25
	2.5	10.44 ± 1.08	
	5	20.42 ± 2.37	
	10	62.97 ± 4.64	
Acarbose	1.25	16.42 ± 0.34	5.04 ± 0.63
	2.5	29.66 ± 1.24	
	5	53.87 ± 0.98	
	10	95.22 ± 0.74	

Data expressed as mean ± SD(n=3).

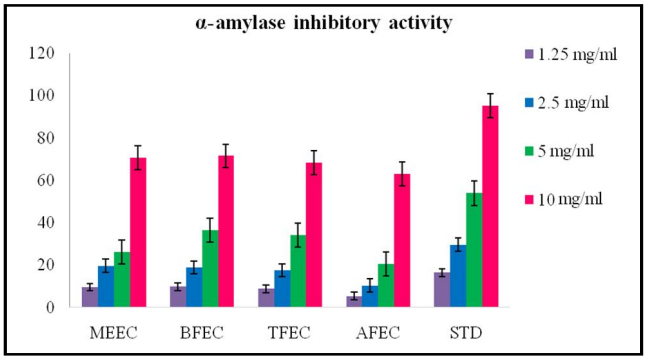

Figure 1: α-amylase inhibition of *E. colona* methanolic extract, fractions and acarbose. Data expressed as mean ± SD(n = 3).

3. Results

The phytochemistry screening of the methanolic extracts and fractions revealed the presence of carbohydrates, steroids, saponins, flavonoids, and phenolic compounds and the absence of alkaloids. Methanolic extract and its fractions of *E. colona* seeds inhibited the catalysis of α-amylase at 1.25, 2.5, 5, 10 mg/ml concentration and the percentage inhibition was dose-dependent. The percentage inhibition of methanolic extract of *E. colona* (MEEC) ranging from 9.5 to 70.56% with IC_{50} value 8.10 mg/ml, n-butyl alcohol fraction (BFEC) ranging from 9.8 to 71.47% with IC_{50} value 6.96 mg/ml, toluene fraction (TFEC) shown percentage inhibition ranging from 8.7 to 68.34% with IC_{50} value 7.46 mg/ml and aqueous fraction (AFEC) has shown percentage inhibition ranging from 5.4 to 62.97% with IC_{50} value 8.99 mg/ml. Among all the test samples, *E.colona* has shown remarkable α-amylase enzyme inhibition, *i.e.*, 71.47% at 10 mg/ml concentration, and it was well comparable with the standard drug acarbose (95.2% inhibition at 10 mg/ml concentration with IC_{50} value 5.04 mg/ml).

Table 2: α-glucosidase inhibitory activity and IC_{50} values by *E. colona* extract, fractions and acarbose

Test sample	Concentration (mg/ml)	% inhibition	IC_{50}
MEEC	1.25	09.74 ± 0.62	6.89 ± 0.32
	2.5	21.19 ± 0.72	
	5	35.24 ± 1.34	
	10	69.87 ± 2.26	
BFEC	1.25	09.98 ± 0.68	6.81 ± 0.25
	2.5	19.17 ± 0.84	
	5	37.17 ± 1.55	
	10	72.89 ± 2.52	
TFEC	1.25	09.82 ± 0.47	6.91 ± 0.36
	2.5	18.86 ± 0.96	
	5	36.62 ± 1.06	
	10	71.86 ± 2.73	
AFEC	1.25	08.95 ± 0.72	7.41 ± 0.45
	2.5	17.34 ± 1.32	
	5	34.15 ± 2.50	
	10	67.15 ± 3.68	
Acorbose	1.25	13.08 ± 0.56	5.17 ± 0.20
	2.5	25.95 ± 1.23	
	5	54.67 ± 0.51	
	10	89.26 ± 0.79	

Data expressed as mean ± SD(n=3).

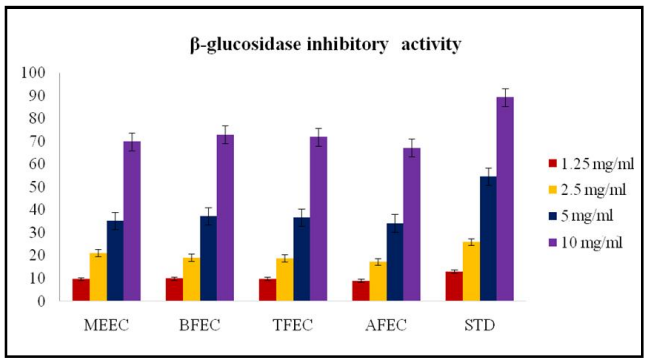

Figure 2: α-glucosidase inhibition of *E. colona* methanolic extract, fractions and acarbose. Data expressed as mean ± SD(n = 3).

Table 3: β-galactosidase inhibitory activity and IC_{50} values by *E. colona* extract, fractions and quercetin

Test sample	Concentration (mg/ml)	% inhibition	IC_{50}
MEEC	1.25	06.25 ± 0.53	11.62 ± 0.75
	2.5	10.24 ± 0.74	
	5	22.43 ± 1.45	
	10	42.64 ± 1.65	
	20	82.45 ± 1.85	
BFEC	1.25	06.74 ± 0.50	11.20 ± 0.90
	2.5	12.33 ± 0.28	
	5	24.35 ± 2.49	
	10	47.49 ± 1.58	
	20	89.91 ± 0.84	
TFEC	1.25	06.41 ± 0.64	11.32 ± 0.47
	2.5	11.63 ± 0.84	
	5	22.86 ± 1.93	
	10	40.28 ± 1.97	
	20	87.10 ± 2.79	
AFEC	1.25	0 6.60 ± 0.40	12.31 ± 0.11
	2.5	11.88 ± 0.57	
	5	22.61 ± 0.64	
	10	41.40 ± 0.90	
	20	83.48 ± 1.09	
Quercetin	1.25	06.87 ± 0.32	10.32 ± 0.21
	2.5	12.42 ± 0.57	
	5	27.29 ± 0.83	
	10	49.43 ± 1.24	
	20	98.24 ± 0.86	

Data expressed as mean ± SD, (n=3).

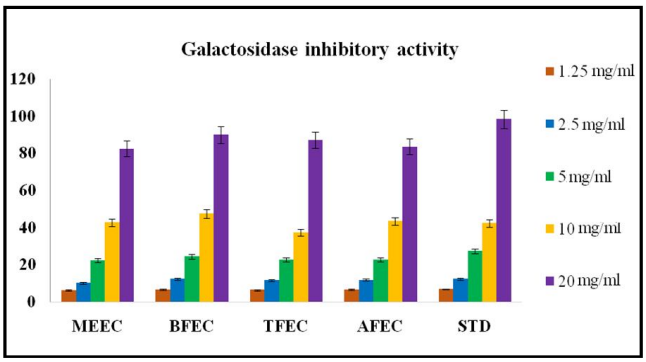

Figure 3: β-galactosidase inhibition of *E. colona* methanolic extract, fractions and acarbose. Data expressed as mean ± SD(n = 3).

Methanolic extract and its fractions of *E. colona* have inhibited the catalysis of α-glucosidase at 1.25, 2.5, 5, 10 mg/ml concentrations and the percentage inhibition was dose-dependent. The percentage inhibition of methanolic extract of *E. colona* ranging from 9.74 to 69.87 % with IC_{50} value 6.89 mg/ml, n-butyl alcohol fraction (BFEC) ranging from 9.98 to72.89 % with IC_{50} value 6.81 mg/ml, toluene fraction (TFEC) shown percentage inhibition ranging from 9.82 to 71.86 % with IC_{50} value 6.91 mg/ml and aqueous fraction (AFEC) has shown percentage inhibition ranging from 8.94 to 67.155% with IC_{50} value 7.41 mg/ml. Among all the test samples, *E. colona* has shown remarkable α-glucosidase enzyme inhibition, *i.e.*, 72.89 % at 10 mg/ml concentration, and it was well comparable with the standard drug acarbose (89.26 % percentage inhibition at 10 mg/ml concentration with IC_{50} value 5.17 mg/ml).

Methanolic extract and its fractions of *E. colona* have inhibited the catalysis of β-galactosidase at 1.25, 2.5, 5, 10, 20 mg/ml concentration and the percentage inhibition was dose-dependent. The percentage inhibition of methanolic extract of *E. colona* (MEEC) ranging from 6.25 to 82.45 %. With IC_{50} value 8.32 mg/ml, n-butyl alcohol fraction (BFEC) ranging from 6.74 to 89.91 % with IC_{50} value 7.81 mg/ml, toluene fraction of (TFEC) shown percentage inhibition ranging from 6.41 to 87.10 % with IC_{50} value 9.40 mg/ml and aqueous fraction (AFEC) has shown percentage inhibition ranging from 6.60 to 83.48 % with IC_{50} value 10.31 mg/ml. Among all the test samples, *E. colona* has shown remarkable α-glucosidase enzyme inhibition, *i.e.*, 89.91% at 10 mg/ml concentration, and it was well comparable with the standard drug quercetin (98.24% percentage inhibition at 10 mg/ml concentration with IC_{50} value of 10.32 mg/ml).

The present study suggests that one of the mechanisms by which *E. colona* exhibited its anti-hyperglycemic potential is through the inhibition of α-amylase, α-glucosidase, and β-galactosidase enzymes. N-butyl alcohol fraction (BFEC) showed the most active α-amylase, α-glucosidase, and β-galactosidase inhibitory activities with IC_{50} values of 6.96 mg/ml, 6.81 mg/ml, and 11.20 mg/ml, respectively. Aqueous fraction (AFEC) showed mild inhibitory effect against α-amylase, α-glucosidase, and β-galactosidase when compared with that standard reference drugs acarbose, quercetin with IC_{50} values of 5.04 mg/ml, 5.17 mg/ml, and 10.32 mg/ml, respectively.

4. Discussion

Management of the blood glucose level is a critical strategy in the control of diabetes complications. Inhibitors of saccharide hydrolyzing enzymes (α-amylase, α-glucosidase, and β-galactosidase) have been useful as oral hypoglycemic agents for the control of hyperglycemia, especially in patients with type 2 diabetes mellitus. α-amylase is one of the main enzymes in the human body which is responsible for the breakdown of starch into more simple sugars. α-amylase hydrolyzes complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α-glucosidase to monosaccharides and are absorbed through the small intestines into the hepatic portal vein; therefore, increases postprandial glucose levels. The enzyme inhibitory activity of caryopsis methanolic extract and its fractions of *E. colona* might be due to the presence of several phytochemicals such as flavonoids, steroids/triterpenoids, and their glycosides, saponins, and phenolic compounds. The order of α-amylase, α-glucosidase, and β-galactosidase inhibition activity with *E. colona* extract and its fractions, when compared with standard, were found to be: BFEC>TFEC>MEEC>AFEC, BFEC>MEEC>TFEC>AFEC and BFEC>TFEC>MEEC>AFEC, respectively. The present study suggests that one of the mechanisms by which *E. colona* exhibited its antihyperglycemic potential is through the inhibition of α-amylase, α-glucosidase, and β-galactosidase enzymes.

5. Conclusion

Among the methanolic extract and its fractions of *E. colona*, n-butyl alcohol fraction exhibited maximum α-amylase, α-glucosidase and β-galactosidase inhibition with IC_{50} values of 6.96 mg/ml, 6.81 mg/ml, and 11.20 mg/ml. The results of the study elaborated scientific support regarding the use of three fraction extracts, *i.e.*, *E. colona* to treat diabetes through a mechanism based on its α-amylase, α-glucosidase and β-galactosidase enzyme inhibiting activity. However, further studies are recommended to validate these effects.

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Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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