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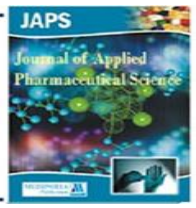

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Effect of Epigallocatechin gallate isolated from Terminalia Bellerica fruit rind on glucoamylase activity in vitro

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ABSTRACT

Chloroform-Ethyl Acetate fraction of Terminalia bellerica fruit rind powder showed maximum antimicrobial activity. This fraction on further purification by column chromatography gave a single spot compound, which after characterisation was found to be Epigallocatechin gallate. Epigallo catechin gallate showed significant antimicrobial activity against *E.coli, B. subtilis* and *S. Aureus*. Epigallocatechin gallate inhibits 23.13 to 46.24 % activity of glucoamylase at very low concentrations, therefore may be used as hypoglycaemic agent showing good agreement with the earlier literature.

Key words: Terminalia bellerica, Epigallocatechin gallate, Antibacterial, glucoamylase inhibitor, Hypoglycaemic.

INTRODUCTION

Most of the drugs used today are obtained from natural sources or semi-synthetic derivatives of natural products as mentioned and used in the traditional system of medicine. Hence it is a logical approach for drug discovery to screen traditional natural products instead of randomly synthesized chemical compound. The herbal products can be isolated and identified as potential for medicines. A valuable research leads to new molecules with novel structural features which could be exploited for making drugs of better therapeutic index. The large number of herbal products has been reported for the cure of diabetes mellitus. Plant drugs are considered to be less toxic and free from side effects than synthetic drugs. *Terminalia bellerica* known as belleric myrobalan is a large deciduous tree, common to the plants on lower hills of South East Asia. This plant exhibits several pharmacological effects including antibacterial, antimalarial, antifungal, anti HIV, antioxidant and antimutagenic effects (Aquil et al., 2004, Bajpai et al., 2005, Padam et al., 1996, Valsaraj et al., 1997). Methanolic extract (75%) of Terminalia bellerica reduced the serum glucose level both in normal and alloxan induced diabetic rats (Sabu et al., 2002). Terminalia bellerica exhibits BP lowering effect possibly through Ca^{++} antagonist mechanism and thus provides a sound mechanistic background for its medicinal use in hypertension (Khan et al., 2008). It's fruit possesses antidiabetic and antioxidant activities (Sabu et al. ,2009). In Type-2 diabetes, the body does not produce enough insulin or properly use it. The cause of diabetes is a mystery although both genetic and environmental factors such as obesity and lack of excercise appear to play a role. Currently available therapies for diabetes include insulin and various oral anti-diabetic agents such as Sulphonyl Ureas, biguanides, α glucosidase inhibitors and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation.

Many of these oral antidiabetic agents have a number of serious adverse effects; thus managing diabetes without any side effects is still a challenge (Saxena et al., 2004). Therefore the search for more effective and safer hypoglycaemic agents has continued to be an important area of investigation. The glucosidase transferase debranching system may be regarded as an integral part of the overall phosphorylase pathway for the degradation of glycogen. In the present study attempt has been made to isolate bioactive component Epigallocatechin gallate from Terminalia bellerica and the effect of on glucoamylase in vitro.

MATERIALS AND METHODS

Plant Material

Terminalia bellerica dry fruits were purchased from the local market and washed with distilled water. The fruits were dried and only the fruit rind was powdered in electrical mill and the powder was stored in the sealed containers till used.

Extraction and isolation of Epigallocatechin gallate

500g of fruit rind powder was subjected to Soxhlet Extraction with different solvents from petroleum ether, Chloroform, Ethyl Acetate, Acetone and Methanol. The extracts obtained were dried removing excess of solvent by distillation. Antimicrobial studies were carried out. Each extract was tested to study the effect on glucoamylase activity in vitro. The Ethyl Acetate (A3) fraction was further purified onto silica gel column. All the fractions were tested for bioassay and the effect on glucoamylase activity in vitro. The Ethyl Acetate fraction showed maximum antimicrobial activity therefore it was further fractionated by column chromatography. Maximum antimicrobial activity was observed with ethyl acetate and acetone fraction. Also all the fractions were found good activator of glucoamylase in vitro. The ethyl acetate fraction, A3 on repetitive chromatographic fractionation yielded chloroform-ethyl acetate fraction (Q4) which showed maximum antimicrobial activity and also was found potent inhibitor of glucoamylase in vitro. Therefore fraction Q4 was subjected to purification up to homogeneity by column chromatography. The sub-fractions of Q4 were tested for antimicrobial activity and effect on glucoamylase in vitro. Sub-fraction R5 on further purification yielded pure compound which was found bioactive and also good glucoamylase inhibitor in vitro. Thus the bioactivity guided fractionation yielded the pure compound-2 which was further characterized by spectroscopic techniques as Epigallocatechin gallate.

Antimicrobial study

The extracts were tested for bioassay against four microorganisms *Staphylococcus aureus* (NCTC 3750), *Pseudomonas aeurogenosa* (Fisch's Immuno type-4), and *Bacillus Subtilis* (ATCC9373) and *Escherichia coli* (ATCC10148) by agar cup diffusion method (Barns et al., 2005). All bioassays were carried out in triplicate and average values were taken.

Glucoamylase activity

1mL of the reaction mixture containing 0.5mL of starch solution (5mg/mL prepared in 100 mM acetate buffer pH 4.5) and a suitable amount of glucoamylase enzyme (0.1mL) and 0.4mL of buffer (100 mM) were incubated at 37^{o}C for 30 minutes. After half an hour the reaction was terminated by keeping the test tubes in boiling water bath for 1-2 minutes, cooled under running tap water and the liberated glucose was estimated by DNS method (Miller G., 1959). A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute.

RESULTS AND DISCUSSION

The sub-fractions R1 to R8 were studied extensively for antimicrobial studies and effect on glucoamylase activity in vitro. The results of Bioassay are shown in Table 1. The effect of the sub-fractions on glucoamylase activity are shown in Table 2.

Table 1: Bioassayof fractions R1 to R8

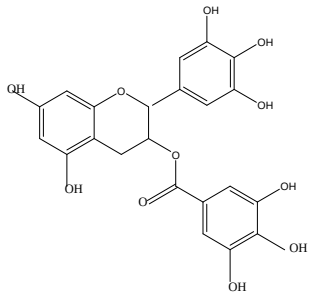
Fraction	*Bacillus subtilis*	*Staphylococcus Aureus*	*Escherichia coli*	*Pseudomonas aeurogenosa*
R1	-	-	-	-
R2	-	-	-	-
R3	8	7	6	8
R4	7	4	4	5
R5	19	18	16	12
R6	14	15	7	15
R7	14	16	16	13
R8	16	14	8	12

The values indicate mean zone of inhibition in mm excluding control (8mm)

Table 2: Effect of sub-fractions R1 to R8 on glucoamylase activity in vitro.

Fraction	Activity (U)	%Inhibition/Increase
Control	6.66	-
R1	6.64	0.3
R2	6.67	0.15
R3	5.64	15.31
R4	5.63	15.46
R5	5.62	15.61
R6	9.74	46.24
R7	5.64	15.31
R8	12.82	92.49

The fraction R5 showed maximum antimicrobial activity. This on further purification yielded pure compound which was found antimicrobial and glucoamylase inhibitor in vitro. The elemental Analysis and spectral data revealed the compound structurally to be Epigallocatechin gallate.



Spectral Data.

IR: 3300-3400cm^{-1}, 3286 cm^{-1}, 3063 cm^{-1}, 1702 cm^{-1}, 1610 cm^{-1}, 1307 cm^{-1} , 1025 cm^{-1}.

^{1}H NMR : δ 4.82, δ 4.94, δ 5.03, δ 7.08 , δ 2.587,

^{13}C NMR : ppm 25.4 (C-4), 68.5 (C-3), 77.2 (C-2), 94.5 (C-6), 95.2 (C-8), 98.08 (C-10), 105.5 (C-2$'$ & C-6$'$), 108.9 (C-2$''$ & C-6$''$), 120.147 (C-1$''$), 129.43 (C-1$'$), 132.4 (C-5$'$), 138.4 (C-4$'$), 144.88 (C-3$'$), 145.2 (C-5), 155.82 (C-9), 156.41 (C-7), 166.29 (C-7$''$).

The inhibition of glucoamylase by various concentrations of epigallocatechin gallate is shown in Table 3. Epigallocatechin gallate shows significant inhibition of glucoamylase activity in vitro at very low concentrations i.e. from 2µg to 100µg. The maximum inhibition of the enzyme is observed at 2µg and 4µg concentration of the compound.

Table 3: Effect of Epigallocatechin gallate on glucoamylase activity in vitro.

Amount (µg)	Activity (U)	% Inhibition
Control	6.66	-
2	3.84	42.34
4	3.83	42.34
6	3.58	46.24
8	5.12	23.12
10	5.13	23.12
20	6.15	07.65

Terminalia bellerica fruit is found beneficial as an excellent laxative. The ripe fruit is used as an astringent. It has been proved beneficial in the treatment of diarrhoea and also possesses antioxidant, antispasmodic, bronchodilator, hypercholestrolemic, antibacterial, cardioprotective, hepato-protective, hypoglycaemic and hypotensive properties. Methanolic extract has an immunomodulatory property that can differently alter both macrophage phagocytic activity and proliferation of splenic lymphocytes. Furthermore, the *T. bellerica* extract has immunosuppressant effects at low concentrations while stimulatory activity is observed at high concentrations. These results suggest a potential therapeutic application of this plant in the treatment of disease associated with the functions of phagocytes and lymphocyte (Aurosorn et al., 2008). Crude extracts of Terminalia bellerica exhibit antisecretory and antonociceptive effects (Khan et al., 2010). Phylanthus emblica and Terminalia bellerica extracts appear to be an effective against hepatocellular carcinoma and lung cancer cells and less toxic against normal cells (Khosit et al., 2008). Most of the plant extracts exhibited hypoglycemic, hypolipidemic, and antioxidant effects in animals as well as in humans, which may be helpful in approaches to treating diabetes and associated complications (Mankil et al., 2006).

Many medicinal plants constitute a rich source of bioactive chemicals that are largely free from adverse effects and have excellent pharmacological actions. They could lead to the development of new classes of possibly safer antidiabetic agents. Therefore, much effort should be focused on assessing natural products and herbal plants for the discovery of potentially useful α-glucosidase inhibitors or other treatment approaches to diabetes. In our study we have observed that Eepigallocatechin gallate, the compound isolated first time from Terminalia bellerica is glucoamylase inhibitor. This inhibitory effect (42.34 to 46.24% inhibition) is more prominent at low concentration (below 10 µg) which at higher concentrations (above 10 µg) shows decrease in % inhibition and near 100 µg shows activation of glucoamylase.

CONCLUSION

Thus, we conclude that Epigallocatechin gallate at very low concentration being promising inhibitor of glucoamylase, may be useful agent to control the level of blood glucose in diabetic conditions.

REFERENCES

Aquil F, Ahmad I, Antibacterial properties of traditionally used Indian medicinal plants, Methods Find Exptl. Clinical Pharmacology, 2004; 29: 79-92.

Aurasorn S, Pattana S, Kornkanok I. Effects of Terminalia bellerica Roxb. Methanolic extract on mouse immune response in vitro, Maejo International Journal of Science and Technology, 2008; 2(02): 400-407.

Bajpai M, Pande A, Tewari S, Prakash D. Phenolic contents and antioxidant properties of some food and medicinal plants, International journal Food science Nutrition, 2005; 56: 287-291.

Barns A, Victor Lorian. Procedure for testing antimicrobial properties in agar media, Antibiotics in laboratory medicine, 5th edition (2005); 1-23.

Khan A, Gilani A. Pharmocodyanamic evaluation of Terminalia bellerica for its antihypertensive effect, Journal of Food and Drug Analysis, 2008; 16(3): 6-14.

Khan A, Gilani A. Antisecretory and Analgesic activities of Terminalia bellerica, African Journal of Biotechnology, 2010; 9(18): 2717-2719.

Khosit P, Sriharut C, Chatri N, Noppamas S, Chariya H. Synergistic growth inhibitory effects of Phyllanthus emblica and terminalia bellerica extracts with conventional cytotoxic agents : Doxorubicin and cisplatin against human hepatocellular carcinoma and lung cancer cells, World Journal of Gastroentorology, 2008; 14 (10) : 1491-1497.

Mankil J, Moonsoo P, Hyun C, Yoon-Ho K et al, Anti-diabetic agents from medicinal plants, Current Medicinal Chemistry, 2006;13:1203-1218.

Miller G. Use of Dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 1959; 31: 427-431.

Padam S, Grover I, Singh M. Antimutagenic effects of polyphenols isolated from Terminalia bellerica, myrobalan in Salmonella typhimurium, Indian Journal of Experimental Biology; 1996; 34: 98-102.

Sabu M, Kuttan R. Antidiabetic activity of medicinal plants and its relationship with their anti-oxidant property, Journal of Ethnopharmocology, 2002; 81: 155-160.

Sabu M, Kuttan R. Antidiabetic & Antioxidant activity of T bellerica Roxb, Indian journal of experimental biology, 2009; 47(4) : 270-5.

Saxena A, Kishore V. Role of selected medicinal Indian plants on management of Type 2 diabetes: A review, Journal of Alternative Complementary Medicine, 2004; 10: 369-378.

Valsaraj K, Pushpagandhan P, Smitt U, Adsersen A, Christensen S, Sittie A, Nyman U, Nielsen C, Olsen C. New anti HIV-1 antimalarial and antifungal compounds from Terminalia bellerica, Journal of Natural Products, 1997; 60: 739-742.