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ISOLATION AND CHARACTERISATION OF GALLIC ACID FROM *TERMINALIA BELLERICA* AND ITS EFFECT ON CARBOHYDRATE REGULATORY SYSTEM *IN VITRO*

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ABSTRACT
Acetone Extract of *Terminalia bellerica* fruit rind powder was fractionated by column chromatography. The Chloroform-Ethyl Acetate fraction showed maximum antimicrobial activity. This fraction was further fractionated using silica gel open column chromatography. The fractions of open column chromatography were tested for antimicrobial activity and effect on glucoamylase activity *in vitro*. The bioactivity guided fractionation led to the isolation of a pure compound. It showed significant antimicrobial activity against *E.coli, B. subtilis* and *S. Aureus*, but lower antimicrobial activity against *P. Aeurogenosa* and 30.70 to 42.34 % inhibition of glucoamylase *in vitro* at very low concentrations. The elemental analysis and spectral data reveals that it is Gallic acid. It is found to be effective inhibitor of glucoamylase therefore may be hypoglycaemic agent showing good agreement with the earlier literature.
KEY WORDS: *Terminalia bellerica*, Gallic Acid, Antibacterial, glucoamylase inhibitor, Hypoglycaemic.

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INTRODUCTION

The concept of ethnopharmacology has evolved from the requirements for studies in light of modern science on the drugs used in the traditional medicines. In 1981 Bruhn and Holmsted defined ethnopharmacology as the interdisciplinary scientific exploration of biological active agents traditionally observed by man. The main component of ethnopharmacology may be defined as pharmacology of drugs used in ethnomedicine[1]. The herbal products can be isolated and identified as potential for medicines and a valuable research leads to new molecules with novel structural features which could be exploited for making drugs of better therapeutic index[2]. The large number of herbal products has been reported for the care of diabetes mellitus literature[3]. Plant drugs are considered to be less toxic and free from side effects than synthetic drugs[4]

Triphala is a popular herbal formula from India that consists of equal parts of three myrobalans taken without seed: Amalaki (*Embilica officinalis*), Bibhitaki (*Terminalia bellerica*) and Haritaki (*Terminalia chebula*) with potential anticancer properties and also effective in colon cleansing. Bibhitaki is a word derived from Sanskrit and it means something that keeps you away from all kinds of diseases. *Terminalia bellerica* is used in Indian traditional system of medicine to treat various diseases including diabetes. Chloroform extract of *Terminalia chebula* seed powder showed significant anti diabetic and renoprotective effects[5]. Effect of continuous administration of dried 75% methanolic extract of fruits of *Terminalia bellerica* suspended in water was studied in alloxan induced hyperglycemia. Its fruit possesses antidiabetic and antioxidant activities[6]. In mammalian tissue and in yeast glycogen is debranched by a two component enzyme system-Amylo (1-6) Glucosidase/ Oligo 1-4- Glucotransferase. The first and necessary step is the action of the transferase which repositions the outer glucose units of the side chains leading to the exposure of single α-Glucose unit linked 1-6 to the rest of the molecule. The 1-6 bonds are then hydrolysed by glucoamylase which is specific for the glucose unit. The overall action of the system is to redistribute the 1-4 linked glucose unit and to release glucose unit involved in the 1, 6 bond as the sole low molecular weight

product. 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 from *Terminalia bellerica* and study the effect of isolated compound on carbohydrate regulatory enzymes *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 the seeds removed. The fruit rind was powdered in electrical mill and the powder was stored in the sealed containers till used.

Extraction and isolation of Gallic Acid

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 and antimicrobial studies were carried out. The extracts were also tested to study their effect on glucoamylase activity *in vitro*. The fraction obtained in acetone (A4) was further separated onto silica gel column. All the fractions collected were tested for bioassay and effect on glucoamylase activity *in vitro*. And further bioactivity guided fractionation yielded the pure compound 1 which was further characterized as Gallic acid.

Antimicrobial study

The extracts were bioassayed 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[7]. 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. 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. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute.

RESULTS AND DISCUSSION

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, hepatoprotective, hypoglycaemic and hypotensive properties[8]. Some studies have also been done on hepatoprotective and renoprotective activity. The percentage yields of the various extracts obtained from soxhlet extraction are recorded (**Table 1)**. Amongst the soxhlet extractions the fractions obtained with various solvents were tested for antimicrobial activity. The acetone fraction showed maximum antimicrobial activity; therefore it was further fractionated by column chromatography. The Petroleum ether and chloroform fractions showed no antimicrobial activity. The ethyl acetate fraction and the acetone fraction showed maximum antimicrobial activity. The methanol fraction showed moderate antimicrobial activity. All the fractions showed increase in the glucoamylase activity (**Table 2 and Table 3**). The percentage yields of the fractions obtained by column chromatography of A4 are reported (**Table 4**). The antimicrobial study and its effect on glucoamylase activity was studied. It is observed that fractions B1 and B2 have remarkable antimicrobial activity, whereas fraction B3 has moderate and fraction B4 good antimicrobial capacity. Fraction B1 was found mild initiator of glucoamylase. The fractions B6 and B7 were observed to increase the activity of glucoamylase. But fractions B3, B4 and B5 surprisingly showed significant inhibition of glucoamylase activity. Amongst the fractions B3, B4 and B5; B4 showed maximum extent of inhibition of the glucoamylase (**Table 5 and Table 6**). As fraction B4 observed potent antimicrobial, it was further fractionated by column chromatography. The percentage yields of the sub-fractions obtained by fractionation of B4 are recorded (**Table 7**). Sub fractions C1 and C2 did not showed antimicrobial activity but C3, C5, C6 and C7 are moderate whereas sub fractions C4 is excellent antimicrobial activity. The fractions C1, C2, C3, C6 and C7 showed remarkable effect on glucoamylase activity (**Table 8 and Table 9**). Fraction C4 on further purification yielded pure compound which was found antimicrobial and inhibitory effect on glucoamylase *in vitro*. The elemental Analysis, IR, ^{1}H-NMR, ^{13}C-NMR, GC-MS revealed the compound, structurally to be gallic acid. Gallic acid shows significant antibacterial activity against *Bacillus subtilis, Staphylococcus aureus* and *Eischerichia coli.* The inhibition of glucoamylase by gallic acid at different concentrations is recorded (**Table 10**). In the present study we have observed that the inhibitory effect of gallic acid is more prominent at low concentration (below 100 µg). Thus, may be useful as hypoglycaemic agent.

Spectral Data

UV Spectrum: λmax – 220 nm and 270nm.

IR Spectrum: 3496 cm^{-1},1667 cm^{-1}, 1318 cm^{-1}, 3280 cm^{-1},1610 cm^{-1}, 1541cm^{-1} , 1425cm^{-1}

^{1}H NMR: δ 7.077, δ 3.325, δ 2.006, δ 1.238

^{13}C NMR Spectrum: 169.028 (C-1), 144.970 (C-4 and C-5), 138.193 (C-5), 120.564 (C-2), 108.945 (C-3 and C-7)

CONCLUSION

Pure Gallic Acid was isolated from the fruit rind of *Terminalia bellerica*. It is found effective inhibitor of glucoamylase therefore may be a hypoglycaemic agent. The structure was established on the basis of chromatographic and spectroscopic evidences. The data obtained from elemental analysis, IR, ^{1}NMR, ^{13}C NMR, GCMS confirms the compound isolated is Gallic Acid.

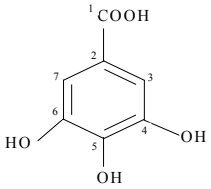

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Table 1: Extraction of various fractions by soxhlet

Solvent	Fraction	% yield
Pet. Ether	A1	1.013
Chloroform	A2	0.343
Ethyl Acetate	A3	7.587
Acetone	A4	6.532
Methanol	A5	11.358

Table 2: Bioassay of A1 to A5 Extracts

Fraction	*Bacillus subtilis*	*Staphylococcus Aureus*	*Eischerichia coli*	*Pseudomonas aeurogenosa*
A1	-	-	-	-
A2	-	-	-	-
A3	14	16	14	17
A4	14	14	20	17
A5	14	17	16	16

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

Table 3: Effect of A1 to A5 Extracts On Glucoamylase Activity *In Vitro*

Fraction	Activity (U)	%Increase
Control	6.66	-
A1	9.48	42.34
A2	8.71	30.78
A3	14.35	113.96
A4	19.48	192.49
A5	20.76	211.71

Table 4: Fractionation of A4 by silica gel column chromatography

Fraction	Solvent	% yield
B1	Pet. Ether	1.44
B2	Pet. Ether +Chloroform (50:50)	2.455
B3	Chloroform	5.11
B4	Chloroform+ Ethyl Acetate(50:50)	15.73
B5	Ethyl Acetate	27.46
B6	Ethyl Acetate +Methanol (50:50)	14.727
B7	Methanol	18.00

Table 5: Bioassay of fractions B1 to B7

Fractions	*Bacillus subtilis*	*Staphylococcus Aureus*	*Escherichia coli*	*Pseudomonas aeurogenosa*
B1	-	-	-	-
B2	-	-	-	-
B3	6	4	5	6
B4	16	19	21	24
B5	14	14	17	16
B6	14	15	13	13
B7	14	14	20	17

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

Table 6: Effect of B1 to B7 fractions on Glucoamylase *in vitro*

Fraction	Activity (U)
Control	6.66
B1	7.43
B2	6.66
B3	5.38
B4	1.53
B5	3.33
B6	9.74
B7	9.74

Table 7: Sub-fractionation of B4 by silica gel column chromatography

Fraction	Solvent	% yield
C1	Pet. Ether	0.892
C2	Pet. Ether +Chloroform(50:50)	13.53
C3	Chloroform	8.678
C4	Chloroform+Ethyl Acetate(50:50)	8.857
C5	Ethyl Acetate	5.571
C6	Ethyl Acetate +Methanol(50:50)	21.85
C7	Methanol	28.28

Table 8: Bioassay of fractions C1 to C7

Fraction	*Bacillus subtilis*	*Staphylococcus Aureus*	*Escherichia coli*	*Pseudomonas aeurogenosa*
C1	-	-	-	-
C2	-	-	-	-
C3	6	4	5	6
C4	10	8	7	6
C5	4	6	8	4
C6	2	4	6	7
C7	6	7	5	4

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

Table 9: Effect of sub-fractions C1 to C7 on glucoamylase *in vitro*

Fraction	Activity (U)	%Inhibition/Increase
Control	6.66	-
C1	11.02	65.46
C2	8.71	30.78
C3	8.20	23.12
C4	5.89	11.56
C5	7.40	11.11
C6	8.46	27.02
C7	9.74	46.24

Table10: Effect of different concentrations of gallic acid on glucoamylase activity

Amount (µg)	Activity (U)	% Inhibition
Control	6.66	-
2	3.84	**42.34**
4	4.61	**30.70**
6	5.64	15.31
8	5.12	23.12
10	6.15	07.65
20	6.15	07.65
40	5.64	15.31
60	5.12	23.12
80	5.13	23.14
100	6.15	07.65

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