Isolation, Characterization and *In vitro* Glucoamylase activity of Phytochemical from the barks of *Terminalia benghalensis* (Banyan Tree)

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***Abstract* - Diabetes is a significant health issue that has gradually grown worldwide. The disorder is associated with substantial morbidiy, death and long term complications. It is a major contributing factor to cardiovascular disease. Therefore, there is a need to develop successful natural antidiabetic agents that can be used on a long term basis without any side effects. In the present work, column chromatography was used to separate the phytoconstituents of the bark powder extract of Terminalia benghalensis (Banyan tree). The phytoconstituents were characterized by IR, 1H-NMR, 13C-NMR and Mass spectra. The antibacterial activity of Cyanidin was also studied. The in vitro antidiabetic activity was evaluated using glucoamylase inhibition assay. The phytochemical screening of the bark powder extract yielded an anthocyanidin. The percentage inhibitory action against α-glucosidase enzyme was evaluated. The results suggest that cyanidin marginally inhibits glucoamylase enzyme.**

Index Terms*— *Cyanidin, glucoamylase, Terminalia benghalensis

I. INTRODUCTION

Diabetes mellitus has become an alarming global problem in recent years. Post prandial hyperglycemia plays an important role in the development of Diabetes Mellitus type II and the resulting complications. One therapeutic approach to treat postprandial hyperglycemia is to retard the cleavage of glucose form disaccharide via inhibition of α – glucosidase in the digestive organs[1]. α – glucosidase (EC 3.2.1.20) is a glucosidase that acts on 1,4 – α bonds, breaking down starch and disaccharides into glucose. This enzyme is ubiquitous in plants, micro-organisms and animal tissues, although the substrate specificity of α-glucosidase differs greatly depending on the source[2]. The knowledge of medicinal plants is a starting point for the modern science. Otherwise, researchers looking for naturally occurring medicine would have the mundane and dangerous task of wandering the planet ad randomly ingesting plants. Plants produce a large number of bioactive compounds. Fruits and vegetables contain a high amount of phytochemicals which may defend against free radical damage[3]. Separating and analyzing the active components of extracts from medicinal plants offer approaches to studying their pharmacological, pharmacokinetic and toxicological processes. Ficus benghalensis (Banyan tree) is a large tree with aerial roots. It grows wild in lower Himalayas and is found all over India. Different [arts of the tree have been found to possess medicinal properties, leaves are good for ulcers, aerial roots are useful in treating gonorrhoea, seeds and fruits are used as cooling agent and tonic as well[4]. A water extract of bark of Ficus benghalensis plant has been shown to possess a hypoglycaemic effect by a group of workers[5]. The water extract of the bark has been reported to possess hypercholesteraemic and hypolipidemic effects[6]. In the present study we have attempted the isolation of a single components from bark powder of Ficus Benghalensis up to homogeneity by silica gel column chromatography, solvent extraction method and preparative TLC method. The compounds isolated were structurally characterized by spectral analysis and the effects of isolated compounds in varying concentrations on glucoamylase were studied in-vitro.

II. MATERIALS AND METHODS

A. Chemicals

Porcine pancreatic α-amylase (EC 3.2.1.1) was procured from SRL Ltd. and A. Niger Glucoamylase (EC 3.2.1.3) were procured from SRL Ltd., Mumbai. All other chemicals were used of analytical grade. Glass double distilled water was used to carry out enzyme assays.

B. General

Melting point was determined in an open capillary tube using Buchi M-560 melting point instrument. IR spectra was obtained on Perkin-Elmer Frontier 91579 FT-IR spectrophotometer using ATR attachment. All 1H and 13C NMR (300 and 75 MHz respectively) spectra were recorded on Bruker Avance Spectrometer with 1% TMS as internal standard. Chemical shifts are expressed as δ. Abbreviations used in the splitting pattern were as follows: s for singlet, d for doublet, t for triplet and dd for doublet of doublet. Mass spectra was obtained on ThermoFinnigan Discovery- Max GC-MS. Elemental analysis was performed on Elemental Analyzer model 'EURO EA 3000'. UV-Double Beam Spectrophotometer on which absorbance was recorded by using 'Shimadzu UV 2400'model

C. Extraction

Ficus benghalensis (bark) were collected fresh from Kalina campus; University of Mumbai brought to the laboratory and washed with distilled water. The bark kept for drying under sunlight. The dried barks were ground in electrical mill and the bark powder was kept separately in amber coloured glass bottle. A suspension of bark powder in alcohol was stirred manually for half an hour and the resultant mixture was kept overnight in refrigerator at 8˚C temperature, after 24 hr. the excess of solvent was removed by vacuum distillation and centrifuged for 5 minutes at 6000 rpm. The process was repeated for seven days. The centrifugates were collected together and evaporated to dryness at 80˚C in an oven. From the solid extract, fractionation is carried out with different solvents (petroleum ether, chloroform, ethyl acetate and methanol). Methanol extract was then subjected for the isolation of active component by silica gel column chromatography using various solvents in the order of increasing polarities. Finally, the fraction obtained from methanol was further fractionized by preparative TLC using a Chloroform: Acetic acid: Methanol (45:30:25.) solvent system. The spot-on TLC having 0.31 Rf was scraped out, and extracted with alcohol. The residue (Compound 1) thus obtained was recrystallized in methanol and referred as Compound (1) and was subjected to spectroscopic analysis, GC-MS, IR, 13C-NMR, 1H-NMR, DEPT-135, Elemental analysis to elucidate the structure. Compound (1) was studied for its effect on the enzymes, glucoamylase and α-amylase, in varying concentrations (10-100µg/mL).

D. Glucoamylase inhibition assay

The glucoamylase inhibition was determined as per the method described by Miller[6] with slight modifications. The assay mixture containing 300µL of 100mM acetate buffer (pH 4.5), 100µL of glucoamylase and 100µL of modulator in the concentration range 20-100µg/mL were incubated for 30 min at 37^{o}C followed by addition of 500µL of starch solution (5mg/mL prepared in 100mM phosphate buffer pH 4.5). After 30 min, the reaction was terminated by keeping the test-tubes in boiling water bath for 1-2minutes and cooled under running tap water. 2mL of 3,5-dinitrosalicylic acid (DNS) color reagent was added, placed in boiling water bath for 15 min., cooled to room temperature and diluted to 7mL with distilled water. The absorbance was measured at 540nm using UV Spectrophotometer. Acarbose was used as positive control. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute. The maximum inhibition was determined from plots of percent inhibition versus modulator and calculated as below,

% Activity = (enzyme activity of test / enzyme activity of control) X 100,

% Inhibition = (100 - % activity)

III. RESULTS AND DISCUSSION

Characterization of compound-1

UV-visible (nm) : 217, 273 .

IR spectrum (cm^{-1}): 3423, 1661, 1631, 1508, 1382, 1301, 1254, 1225, 1115, 1089, 1039, 1009, 974, 884

1H NMR (ppm): 9.085 (1H, s), 8.166-8.119 (2H, m), 7.562 (1H, s, -OH), 7.029- 6.980 (3H, m), 6.517 (1H, s, -OH), 6.258 (1H, s, -OH), 5.635 (2H, s, -OH).

13C NMR (ppm) : 163.1, 157.2, 156.6, 155.7, 149.3, 147.7, 147.4, 133.3, 127.6, 121.9, 118.2, 117.3, 114.0, 102.9, 92.32.

Mass : m/z 325 (M+)

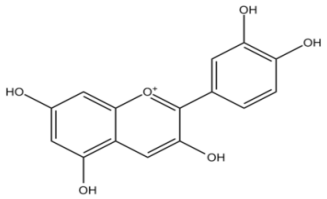
Elem. Analysis : Carbon – 52.509%, Hydrogen – 4.551%, Oxygen - 42.94%

Compound-1 (shiny red color) shows melting point 250-254°C. From HPLC study it is confirmed that isolated compound is single and pure with 96.84% purity.

The absorption peak at λ_{max} 273 nm reflects a high probability of occurrence of n→π* transition due to –

C=O group whereas absorption peak at λ_{max} 217 nm indicates presence of $\pi \rightarrow \pi^*$ transition due to enol group. The broad band around at 3423 cm^{-1} confirmed the presence of O-H group While band around at 3423 cm^{-1} due to –C-H stretching of aromatic nature. The band around at 1176 cm^{-1} also confirms the presence of ester linkage. 1H-spectrum of compound-1 shows five aromatic protons at δ 5.685 (s), δ 6.258 (s), δ 6.517 (s), δ 7.562 (s), and δ 8.135 (s), five phenolic protons at δ 8.119 (s), δ 7.029 (s), δ 6.980 (s) and δ 8.166 (s) suggests the presence of eleven protons in the structure. The five phenolic protons were confirmed by D_2O exchange. ^{13}C-spectrum of a compound exhibited eleven peaks at δ 96, 104, 116, 122, 128, 135, 157, 160, 162, 167 and 176 suggest eleven types of carbon atoms. The percentage composition of Carbon, Hydrogen and Oxygen was found 52.509 %, 4.551 % and 42.94 % respectively, similar to the actual percentage composition of Cyanidin which contains 52.6 %, 4.6 % and 42.9 % of Carbon, Hydrogen and Oxygen. In mass spectrum, molecular ion peak was obtained at 325 m/z.

Thus, from the interpretation of the above spectral data compound-1 has assigned the structure –



The isolated compound was further studied for its effect on the carbohydrate metabolizing enzyme.

Sr. no.	Concentration (ppm)	Activity (U) Control 9.044U
1	2	**5.741**
2	4	6.408
3	6	7.908
4	8	8.575
5	10	8.658
6	20	7.325
7	40	7.575
8	60	8.742
9	80	9.825
10	100	9.992
11	200	9.075
12	400	11.659
13	600	14.326
14	800	15.993
15	1000	17.327

Table I – Effect of varying concentrations on the activity of Glucoamylase activity.

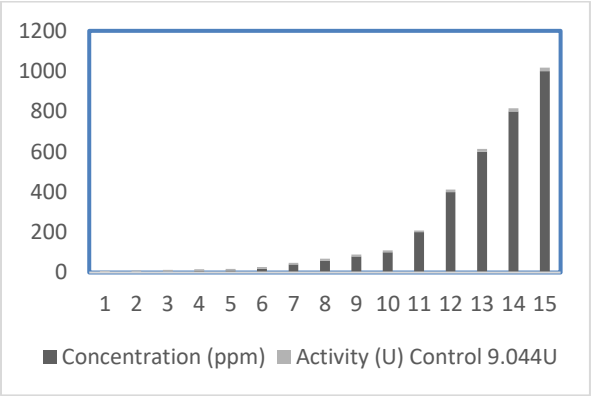


Fig.a. Effect of Compound-1 on glucoamylase activity

The effect of varying concentrations of Cyanidin, 2ppm - 1000ppm, on glucoamylase activity was studied in vitro. It is observed from Table I and Fig. a that lowers concentrations of Cyanidin inhibits glucoamylase activity to the maximum extent whereas at higher concentrations Cyanidin shows minimum inhibition of activity. It showed maximum inhibition (36.52%) at 2ppm concentration of Cyanidine

IV. CONCLUSION

The present study was undertaken to evaluate the effect of Cyanidin on glucoamylase activity in vitro. To the best of our knowledge, first time report on the isolation of cyanidin from Ficus benghalensis bark extract and it's biological evaluation. Cyanidin from Ficus benghalensis bark extract showed strong inhibitory activity against glucoamylase. Thus, Ficus benghalensis may be a good natural source of glucosidase inhibitor used to control post prandial blood glucose and its complications.

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