ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Antidiabetic and Antitubercular activities of methanol extract of root bark of *Artocarpus integrifolia*

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ABSTRACT

Artocarpus integrifolia is an important medicinal plant belonging to Artocarpus Genus (family Moraceae) is distributed over tropical and sub tropical regions . Various parts of this plant species have been used to cure ailments like inflammation, skin diseases, boils, fever, diarrhea etc. In the present study methanol extract of root bark of this plant has been evaluated for antidiabetic and antitubercular activities. Two compounds homopterocarpin and cycloheterophyllin have been isolated from methanol extract. This is the first time homopterocarpin has been isolated from Artocarpus genus. The α-amylase inhibition was assayed by adapting modified dinitrosalicylic acid method of Bernfeld to assess the antidiabetic activity. Antitubercular activity was evaluated against *Mycobacterium tuberculosis* H37Rv using microplate Alamar Blue(MABA) Assay method. Present study revealed that methanol extract of root bark of *A. integrifolia* was found to have highest inhibiting effect on α-amylase (25.2 % at 100µ g/ml) and also inhibited the growth of *M. tuberculosis* H37Rv (MIC 6.25µg/ml). The compounds isolated viz., homopterocarpin and cycloheterophyllin, from the root bark of this plant, showed low inhibition activity against α- amylase and did not control the H37Rv bacterial growth at low concentrations. This investigation revealed that the methanol extract of root bark of *A. integrifolia* has the potential to be developed further in to natural antidiabetic and antitubercular drug.

Key words: α-Amylase, antidiabetic, antitubercular, *Artocarpus integrifolia,* cycloheterophyllin, homopterocarpin.

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ISSN: 0975-8585

INTRODUCTION

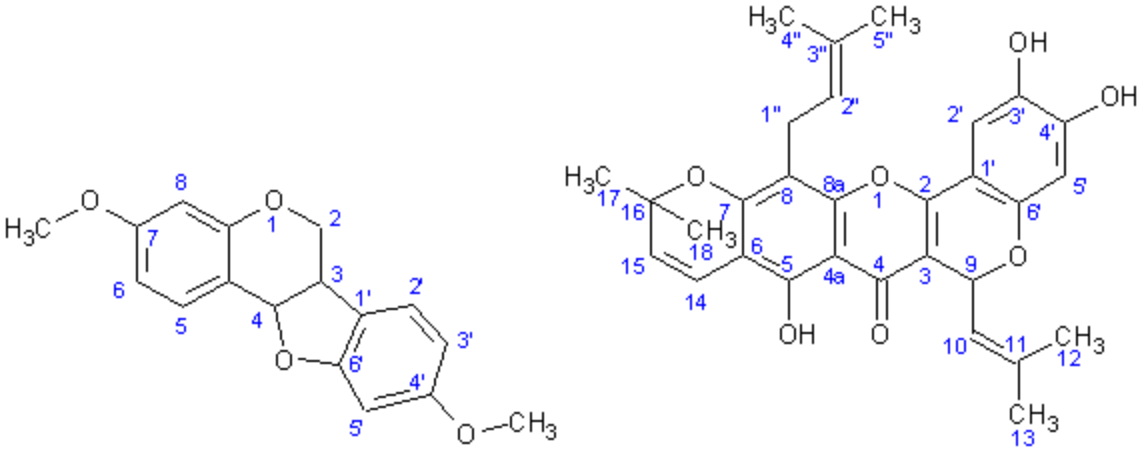
Diabetes mellitus is a chronic disease that occurs when the pancreas does not produce enough insulin, and / or when the body cannot effectively use the insulin it produces. Hyperglycaemia, or high blood sugar is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the systems in the body especially the kidney, nerves and blood vessels [1]. Conventional treatments include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo and disaccharides [2, 3]. Suppression of α-amylase enzyme in the human digestive system would delay the degradation of starch and oligosaccharides to monosaccharides before they can be adsorbed. This would decrease the absorption of glucose and consequently reduce postprandial blood glucose level. The known α-amylase inhibitors used in management of diabetes are acarbose and miglitol, but these drugs are known to be associated with gastrointestinal side effects. Therefore, it becomes necessary to identify α- amylase inhibitors from natural sources having fewer side effects.

Also tuberculosis remains a leading cause of death globally. In 2009 there were an estimated 9.4 million cases of tuberculosis worldwide, with 1.1 million of those occurring in India[4] . Incidence of tuberculosis is greatest among those with conditions impairing immunity, such as human immunodeficiency virus (HIV) infection and diabetes. The consequences of mismanagement of tuberculosis in a patient with diabetes can be severe[5]. During recent years, *M. tuberculosis* has developed resistance against drugs.

Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material.

The plants of Artocarpus species distributed over the tropical and subtropical regions and various parts of the plants of this species have been used as traditional folk medicine by local people against various ailments like inflammation, boils, fever, skin diseases, diarrhea etc. Different species of Artocarpus genus have been evaluated for their pharmacological activities. *A. integrifolia* (syn for *Artocarpus heterophyllus*)[6], which is commonly known as jackfruit contains various chemical components as colouring matters[7] and flavones responsible for various biological activities. Although a lot of pharmacological investigations [8] have been carried out based on the chemical constituents present in it but a lot more can still be explored and utilized in a therapeutic manner. Some of the studies that have been carried out on this plant are antiinflammatory, antioxidant, antifungal, antidiabetic, and antibacterial activities [9-15]. Since antidiabetic and antitubercular activities of the root bark of this plant has not been studied, in the present study we have selected the methanol extract of the root bark and also two pure compounds isolated from the same methanol extract of *A. integrifolia*, homopterocarpin and cycloheterophyllin [Figure 1] to evaluate their antidiabetic activity by α-amylase inhibition assay[16] and antitubercular activity by microplate Alamar Blue Assay[17].

ISSN: 0975-8585



1. Homopterocarpin 2. Cycloheterophyllin

Figure 1: Structures of compounds isolated from methanol extract of *A.integrifolia*.

MATERIALS AND METHODS

General

Melting points were determined in open capillary and are uncorrected. IR spectra were recorded on FT-IR Perkin-Elmer spectrum GX spectrometer. ^{1}HNMR spectra were recorded on Bruker spectrometer operating at 400MHz and mass spectral analysis was carried out with GCMS Shimadzu QP 5050 mass spectrometer.

Plant Material

Root bark of the plant *A. integrifolia* was collected at Anekal Bangalore dist, Karnataka India during September 2009. The plant material was identified by Prof. M. R. Nagaraju, NMKRV College for women, Bangalore.

Extraction and Isolation

The shade dried coarse powder of the root bark (500g) was kept in stoppered flask and were macerated with methanol for 48 hrs with occasional stirring. Then the extract was filtered through whatman filter paper and removal of solvent under reduced pressure gave a dark brown residue (36g). This dark brown mass (30g) was chromatographed on Si gel(300g, 60-120mesh) built in a tall cylindrical column (2.5" dia, 3.5 feet length) using n-hexane. With n-hexane-ethyl acetate mixture as eluent, each fraction was monitored by tlc. The fractions eluted with n-hexane-ethyl acetate (95ml: 5ml) were evaporated to give pale yellow solid(1.1g). This on crystallization with n-hexane afforded colourless needles homopterocarpin (1) (300mg). Fractions eluted with hexane : ethyl acetate (85ml : 15ml) were evaporated to give yellow amorphous solid(0.5g) which on crystallization with n-hexane yielded yellow needles,

cycloheterophyllin (2) (180mg). The structure of isolated compounds was determined by physical and spectral analyses(FT-IR, ^{1}H-NMR, and Mass spectrometry). The spectral data of both these compounds were in good agreement with those in the literature[10,18-21]. The crude extract is expected to have few other prenyl flavones [21], which are to be extracted and studied in future.

Homopterocarpin(1) was obtained as colourless needles mp 60^{0}C ; IR ν_{max}^{KBr}(cm^{-1}) 3475, 2947,1620; ^{1}H NMR (400 MHz, $CDCl_3$) δ5.5(1H,m,2-H), 3.64(1H,t, J=7Hz, 3-H), 4.25(1H, dd,J=7,3Hz,4-H), 7.13(1H,d,8Hz, 5-H), 6.45(2H,m, 6-H,8-H), 7.43(1H,d,J=8Hz,2′-H), 6.64(1H,dd,J= 8,2.5Hz,3′-H),6.48(1H,d,J=2.5Hz,5′-H), 3.77 (3H,s, 7-OCH_3) , 3.79(3H,s,4′-OCH_3); EIMS *m/z* 284 [M]$^+$,285, 283,270, 269,,253,161,148,133.

Cycloheterophyllin(2) was obtained as yellow needles mp 218^{0}C; IR ν_{max}^{KBr}(cm^{-1}) 3423,1653,1627,1595; ^{1}H NMR(400MHz, $CDCl_3$) δ6.23(1H, d, J=8Hz,9-H),5.47(1H, d, J=8Hz ,10-H),1.70(1H, s,12-H),1.97(1H,s,13-H),6.73(1H, d ,J=8Hz,14-H),5.62(1H, d, J=8Hz,15-H),1.45(1H ,s,17-H),1.46(1H, s,18-H,), 7.27(1H, s,2′-H),6.5(1H,s,5′-H),3.5 (1H, dd, J=8.0,2.0 Hz ,1″-H), 5.26(1H,m,2″-H),1.87(1H,s,4″-H),1.97(1H,s,5″-H),13.25(1H,s,5-OH); EIMS *m/z* 502[M]$^+$,487, 459, 448, 447, 431 ,403, 236, 205, 189, 153, 69,55,43.

Assay for α- amylase inhibition

The α-amylase inhibition was assayed by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as the decrease in the units of maltose liberated. A modified dinitrosalicylic acid (DNS) method of Bernfeld was followed and activity of α-amylase (Himedia Mumbai) was assayed with different concentrations of sample along with different volumes of solvent, with control. The test tubes were added with 1ml of sodium phosphate buffer (50mM pH 7.0-7.3), different volumes of solvent, different concentrations of sample, 0.5ml of starch (in buffer) and 0.1ml of enzyme (from 1mg/ml sample in buffer). The blank tube was added with 1ml of DNS before adding enzyme. The tubes were incubated at 37^{0}C for 10 min followed by addition of 1ml of DNS. The tubes were incubated in boiling water bath for 10 min, cooled and read for absorbance at 540nm against blank. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the following formula.

$Activity = [(C_m \times E_z) / (Mw \times I_t)] \times dilution\ factor$

C_m = *Conc. of maltose liberated.*

E_z = *ml. of enzyme used*

Mw = Mol wt. of maltose.

I_t =*incubation time (min)*

The antidiabetic activity was investigated through the inhibition of α-amylase, an enzyme that assisted the digestion of starch and so reduced the glucose absorption. The α-amylase activity was calculated and α-amylase inhibition was expressed as a percentage of inhibition and calculated by the following equations:

ISSN: 0975-8585

% activity = activity in presence of compound / control activity × 100
%inhibition = 100 - % activity

Antitubercular activity using Alamar Blue Dye

Antitubercular activity was evaluated against *M. tuberculosis* H37Rv using well known microplate Alamar Blue Assay method. This methodology is non toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 20µlitre of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation, the 96 wells plate received 100 µ liter of the Middle brook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2µg/ml. Plates were covered and sealed with parafilm and incubated at 37^0C for 5 days. After this time, 25 µlitre of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

RESULTS

Amylase inhibition assay

Three different concentrations of methanol extract, homopterocarpin and cycloheterophyllin of *A.integrifolia* were tested to detect amylase activity and the inhibition of amylase activity. At 100µg/ml and 50µg/ml concentrations of methanolic extract had the highest α-amylase inhibition of 25.2% and 18.4% respectively, than compared to homopterocarpin and cycloheterophyllin. At the concentrations of 25µg/ml all the three samples exhibited very low inhibition of α-amylase enzyme. [Table 1].

This study revealed that the methanolic extract of the root bark of the plant was more sensitive as it displayed significant amylase inhibition at 100 and 50µg/ml. whereas homopterocarpin and cycloheterophyllin showed very low enzyme inhibition (less than 7%). The comparative activities of samples are shown in [Graph 1].

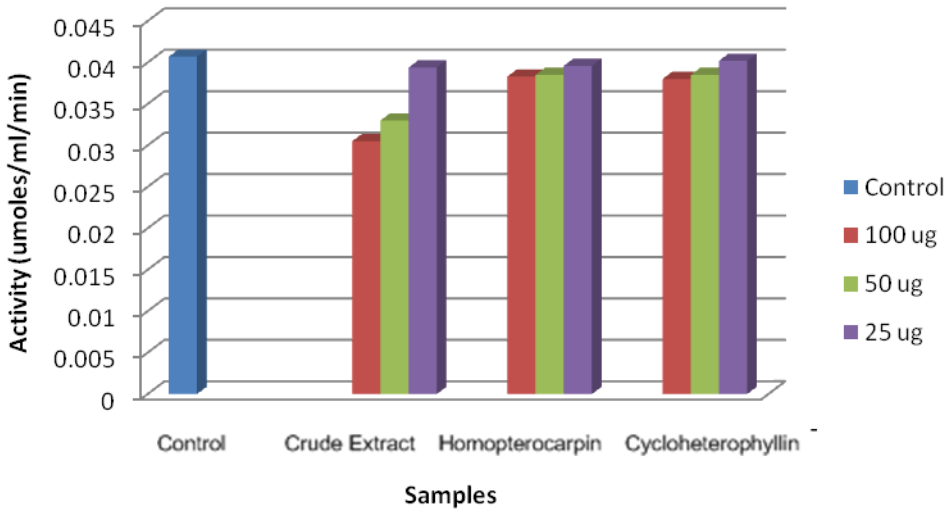
Table 1: Inhibition activity of different concentrations of methanol extract and pure Compounds of *A.integrifolia* on α-amylase.

Sample	OD at 540nm	Conc. of maltose Liberated(g)	Activity of α-amylase (moles/ml/ min)	Activity	Inhibition
Control	1.74	147	0.0407	100	0
Crude extract(100 g)	1.27	110	0.0305	74.8	25.2
Crude extract (50 g)	1.5	120	0.033	81.6	18.4
Crude extract(25 g)	1.64	142	0.0394	96.5	3.5
Homopterocarpin(100 g)	1.6	138	0.0383	93.8	6.2

ISSN: 0975-8585

Homopterocarpin(50 g)	1.62	140	0.0385	95.2	4.8
Homopterocarpin(25 g)	1.65	143	0.0396	97.2	2.8
Cycloheterophyllin(100 g)	1.59	137	0.038	93.1	6.9
Cycleheterophyllin(50 g)	1.62	140	0.0385	95.2	4.8
Cycloheterophyllin(25 g)	1.73	145	0.0402	98.6	1.4

Graph 1: Comparative activities of different concentrations of methanol extract and pure compounds of *A.integrifolia*.



Anti-TB activity using Alamar Blue Dye

The anti-TB activity of samples was determined using MABA as the analytical method. The samples were dissolved in DMSO, and final drug concentrations tested were 100 to 0.2µg/ml. The methanol extract was active against H37Rv at MIC 6.25 µg/ml, and cycloheterophyllin inhibited the bacterial growth at MIC 25 µg/ml, where as homopterocarpin was resistant upto 100µg/ml. [Table 2].

Table 2: Antitubercular activity of the methanol extract and pure compounds of *A.integrifolia*.

Compounds tested	MIC(µgm/ml) H37Rv
Methanol extract	6.25
Homopterocarpin	Resistant upto 100
Cycloheterophyllin	25

DISCUSSION

Two known compounds viz, homopterocarpin, a pterocarpan and cycloheterophyllin, a prenylated flavone were isolated from the methanolic extraxt of *A. integrifolia*. The spectral data of both these compounds were in good agreement with those in the literature. This

pterocarpan was first isolated and characterized as heartwood constituents of the pterocarpus species. [17-20] Surprisingly to the best of our knowledge, homopterocarpin has never been found in the Artocarpus genus before. Since crude extract is expected to have few other prenyl flavones, they have to be separated by column chromatography and subjected to the biological studies.

Findings of the present study revealed that methanol extract of *A. integrifolia*, found to have highest inhibitory effect on α-amylase (25.2% at 100µg/ml) and also inhibited the growth of *M. tuberculosis* H37Rv (MIC 6.25µg/ml) Where as the pure compounds homopterocarpin and cycloheterophyllin showed low inhibition activity(less than 7%) against α-amylase and did not control the H37Rv bacterial growth at lower concentrations. The activity shown by the methanol extract may be a synergistic action of several compounds.

CONCLUSION

The bio-activity study of the methanol extract of *A. integrifolia* exhibited interesting anti-diabetic and anti-tuberculosis activities, even at very low concentration (less than 1mg) which led to the isolation of two known compounds homopterocarpin and cycloheterophyllin. This is the first time homopterocarpin has been isolated from Artocarpus genus. This investigation reveals that the methanol extract of root bark of *A. integrifolia* has the potential to be developed further into a natural antidiabetic and antituberculosis drug. Further investigation of the methanol extract is necessary to isolate and analyze the constituents present in the plant, responsible for their action on α-amylase and *M. tuberculosis* H37Rv.

ACKNOWLEDGEMENTS

We thank RSS Trust, Principal, staff members of Dept of Chemistry NMKRV College for women with PG and Research centre, Jayanagar Bangalore, India, for providing necessary facilities.We thank Dr. N.R.Krishnawsamy for his help in structure elucidation. We acknowledge Dr.K.G.Bhatt, Maratha Mandal's Dental college, Hospital and Research Centre Belgaum, India for the evaluation of antituberculosis activity. We also thank NMR centre and Dept of organic chemistry, Indian Institute of Science, Bangalore, India for helping us in recording NMR, IR and Mass spectra.

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