Bioactivity guided isolation of antidiabetic and antioxidant compound from *Xylocarpus granatum* J. Koenig bark

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Abstract

The present study was designed to identify antidiabetic and antioxidant constituents from ethanol bark extract of a medicinally important mangrove plant *Xylocarpus granatum* J. Koenig, using in vitro bioactivity-guided fractionation. The repeated fractionation of *X. granatum* ethanol bark extract (XGEB) by silica gel column chromatography yielded a compound with strong antidiabetic and antioxidant potential. The bioactive compounds likely to be present in the XGEB fraction were identified by FT-IR, ¹H & ¹³C NMR and MS analysis and determined as a limonoid derivative Xylococcinsin-I, by comparing spectral data with the literature reports. The isolated compound demonstrated excellent in vitro antidiabetic potential IC₅₀ values of 0.25 and 0.16 mg/ml, respectively for α -amylase and α -glucosidase inhibition study. The antioxidant potential assayed by DPPH, ABTS, superoxide and hydrogen peroxide scavenging studies exhibited that the isolated compound could scavenge these free radicals with IC₅₀ values of 0.041, 0.039, 0.096 and 0.235 mg/ml, respectively. Further, in silico study was performed to find the antidiabetic activity of Xylococcinsin-I by docking it against α -glucosidase enzyme. The study demonstrated that Xylococcinsin-I have satisfactory interactions and binding energies when docked into target which further confirms the possible mode of antidiabetic action of the isolated compound. The bioactivity assays further asserts the antidiabetic and antioxidant efficacy of the isolated compound which strongly suggests that Xylococcinsin-I holds promise in the pharmaceutical industry. The results from this study provide new mechanistic evidence justifying, at least in part, the traditional use of *X. granatum* extract for antidiabetic and antioxidants activities.

Keywords *Xylocarpus granatum* · Antidiabetic · Antioxidant · Bioactivity-guided screening · Docking

Introduction

The mangrove genus *Xylocarpus* that belongs to family *Meliaceae* consists of four mangrove tree species viz. *Xylocarpus granatum*, *X. moluccensis*, *X. mekongensis*, *X. rumphii* growing around littoral of the tropical Indian Ocean and distributed widely in the coastal areas of South-East Asia, Australia and East Africa (Banerjee and Rao 1990). The genus *Xylocarpus* has been reported to possess several secondary metabolites of ethnomedicinal importance such as alkaloids (*N*-methyl flindersine, chelerythrine, dihydrochelerythrine, acetonyl dihydrochelerythrine), flavonoids (catechin, epicatechin, kaempferol, 3-O- β -D-glucoside), monoterpenes, triterpenoids, tetraterpenoids, limonoids (Xylococcinsin A-I, Xylocarpin, Humulin B, Hainangranatums A-J, Sundarbanxylogranins A-E), proanthocyanidins (procyanidin B1, procyanidin B3, procyanidin trimer, procyanidin pentamer, procyanidin hexamer, procyanidin decamer and procyanidin

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undecamer), phenolic acids, steroids (Wangensteen et al. 2009; Pan et al. 2010; Dai et al. 2017).

Xylocarpus granatum J. Koenig is an important mangrove plant used traditionally for treatment of fever, malaria, thrush, cholera, dysentery and diarrhoea in many countries including India (Bandaranayake 2002; Dr Duke's Phytochemical and Ethnobotanical Database 1992). Earlier studies have shown that leaves, fruits and barks of *X. granatum* possess free radical scavenging activities (Uddin et al. 2004; Vadlapudi and Naidu 2009; Das et al. 2016a), antidiabetic (Das et al. 2016b) and antidyslipidaemic activities (Srivastava et al. 2011; Lakshmi et al. 2011). However, *X. granatum* has not been subjected to detailed chemical constitution analysis and the bioactive studies were restricted to its crude extracts. Therefore, it is necessary to investigate the active chemical components of these extracts and identify their biological effects.

Recently, we have comprehensively evaluated the antidiabetic and antioxidant activities of ethanol bark extracts of *X. granatum* in animal model (unpublished data). In the present study, the antidiabetic and antioxidant activities of the ethanol bark extracts of *X. granatum* were investigated by in vitro bioassay guided fractionation technique. This paper describes the isolation and structural identification of bioactive component from ethanol bark extracts of *X. granatum* responsible for antidiabetic and antioxidant activities. Further, in silico analysis of the isolated compound was carried out to derive the possible mode of action for its antidiabetic activity.

Experimental

Collection of plant materials and preparation of the extracts

Barks of *Xylocarpus granatum* (family-Meliaceae) were collected from Mahanadi delta mangrove forest area of Odisha coast (India). The mangrove plant *X. granatum* was collected from Kansaridia area of Mahanadi delta region of Odisha located in between 20°18'–20°32'N latitude and 86°41'–86°48'E longitude during winter. The specimen was authenticated by Prasanna Kumar Nayak, Herbarium keeper, Integrated Coastal Zone Management Project (ICZMP), Forest Department, Govt. of Odisha, India. The plant specimen was identified at Department of Natural Products, Institute of Minerals and Materials Technology, Bhubaneswar (RRL-B), Odisha, India and voucher specimen (VS No. RRL-B 12567) was deposited. The barks were dried for 15 days under shade condition. The samples were pulverized and extracted with ethanol by soxhlation. After extraction, the extracts were concentrated under reduced pressure in rotary evaporator, and the samples were stored at 4 °C till further use.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Butylated hydroxy toluene (BHT), Ascorbic acid and α -glucosidase, α -amylase, H_2O_2 were purchased from SRL India, Ltd. Acarbose was purchased from Sigma Aldrich India. All the chemicals and reagents used in the study were of analytical grade.

Chromatographic fractionation of ethanol bark extract

The *X. granatum* ethanol bark extract (XGEB) obtained from powder (10 g) was subjected to column chromatography (4 × 100 cm, silica gel 60–120 mesh) as the stationary phase. The column was eluted with gradient of petroleum ether–chloroform (100:0–0:100) followed by gradient of chloroform–methanol (100:0–75:25) solvents. A total of 120 fractions of 100 ml each were collected. Each fractions were then screened for their in vitro antidiabetic (by α -amylase inhibition and α -glucosidase inhibition assay) and in vitro antioxidant activities (by DPPH, ABTS, superoxide, hydrogen peroxide scavenging). All these fractions were then pooled to afford 12 major fractions (A–L). Amongst all the fractions, the fraction 'G' eluted from chloroform: methanol fraction (95:5) indicated highest in vitro antidiabetic and antioxidant activities and yielded 150 mg of purified compound. The inactive and less active fraction were discarded. Finally, the active fraction elute was selected for the structural characterization by FTIR, NMR (1H & ^{13}C) and MS analysis.

Characterization of bioactive fraction and structure elucidation

The isolated compound was characterized by FT-IR, 1H , ^{13}C NMR and MS analysis. The IR spectra were recorded with a Shimadzu FTIR spectrometer with the compound prepared as KBr pellets. 1H and ^{13}C NMR spectra were recorded with a Bruker AVANCE II 400 NMR Spectrometer using tetramethyl silane (TMS) as an internal standard. Mass spectral data were obtained with a WATERS, Q-TOF MICROMASS (LC–MS) mass spectrometer.

Evaluation of antidiabetic properties

The antidiabetic activities of the different fractions and the isolated compound were evaluated on the basis of their potential to inhibit carbohydrate metabolizing enzymes such as α -amylase (Ali et al. 2006) and α -glucosidase (Apostolidis

et al. 2007). The experiments were carried out in triplicate and data were expressed as mean $\pm$ standard deviation (SD).

Evaluation of antioxidant properties

The antioxidant activities of the different fractions and the isolated compound were determined by DPPH (Maisuthisakul et al. 2007), ABTS (Thaipong et al. 2006), super oxide (Das et al. 2016a) and H_2O_2 (Rath et al. 2011) scavenging assays. The experiments were carried out in triplicate and data were expressed as mean $\pm$ standard deviation (SD).

Data retrieval and molecular docking

To understand the binding mechanism of the isolated compound, Xylococcin-I with α -glucosidase, we retrieved the solved crystal structure of *Bacillus cereus* Oligo-1,6-Glucosidase (PDBID: from Protein Data Bank (www.pdb.org) and the 3D structure of Xylococcin-I (ChemSpider ID:10272292) from ChemSpider database (<http://www.chemspider.com>) (Watanabe et al. 1997). The binding pocket analysis of '1,6-glucosidase' was performed in Metapocket-2 meta-server. The best binding cavity was considered for docking purpose. The molecular docking of Xylococcin-I was performed with 1,6-glucosidase using AutoDock 4.2 (Morris et al. 2009). Three individual docking calculations were performed with three different grid boxes [(50 $\times$ 50 $\times$ 50) (60 $\times$ 60 $\times$ 60) and (70 $\times$ 70 $\times$ 70)] to find out the potential binding conformation. The list binding energy scored conformation was considered as the best conformation. The detailed procedure of molecular docking (using AutoDock) was adopted from a recent study (Maharana et al. 2014). The molecular visualizations and interaction analysis was performed using Discovery studio visualizer (Accelleris Inc., San Diego, CA).

Statistical analysis

Statistical evaluation was performed with SPSS for Windows, version 20 (IBM Corporation) software using one-way ANOVA with post hoc Bonferroni test. A level of $p < 0.05$ was accepted as statistically significant. The experiments were carried out in triplicate and data were expressed as mean $\pm$ standard deviation (SD).

Results and discussion

Isolation and structure elucidation of isolated compound

The ethanol bark extract of *X. granatum* was subjected to bioassay-guided fractionation on silica gel column

chromatography using petroleum ether, chloroform and methanol gradient elution system. The fraction eluted with chloroform: methanol (95:5) exhibited highest in vitro anti-diabetic and antioxidant activities amongst all the fractions was collected and selected for isolation and characterization. The in vitro bioactive compounds thus isolated were characterized and identified by FT-IR, NMR (1H & ^{13}C) and MS analysis.

The FT-IR spectra of the isolated compound obtained from XGEB exhibited characteristic absorption bands at 3499/cm, 2918/cm, 2858/cm, 1731/cm, 1523/cm, 1458/cm, 1218/cm, 1123/cm and 1036/cm (Fig. 1). It was found that the spectra consist of strong and broad band in the range of 3500–3000/cm indicating the presence of free and hydrogen bonded hydroxyl (–OH) group. Sharp and strong peak at 2918/cm and 2858/cm indicated the presence of aliphatic –C–H stretching. The signals at 1731/cm was due to the characteristic absorption of –C=O. The band at 1458/cm corresponded to the bending vibration of CH_2 . The stretching bands at 1116/cm and 1059/cm evident for the presence of C–O linkage in XGEB. The FT-IR spectra indicate the presence of functional groups such as hydroxyl, carbonyl, methyl side chain and etheral linkage. Therefore, it was further investigated with other sophisticated analytical techniques to confirm the presence of the functional groups and to elucidate the structure.

The 1H -NMR spectrum revealed the presence of δ 0.5(6H) correspond to methylene proton in the structure, δ 1.4(18H) corresponds to methyl protons, δ 1.9(3H) indicate the presence of sp^3 C–H, δ 2.6(3H) corresponds to the protons adjacent to oxygen of ester and lactone ring, δ 3.2(8H) corresponds to proton adjacent to carbonyl and methyl protons in the methyl ester group, δ 3.8(3H) for the protons in acetyl group, δ 5.2(2H) for protons in hydroxyl group and δ 7(3H) for protons in furan ring (Fig. 2). The ^{13}C NMR data shows δ at 13.84, 22.10, 28.72, 29.05, 31.31, 38.93, 39.14, 39.35, 39.56, 39.77, 39.97, 40.18,

Fig. 1 FTIR spectrum of isolated compound from ethanol bark extracts of *X. granatum*

Fig. 2 Structure elucidation by ^1H NMR for isolated compound

39.95, 40.18, 78.31, 78.64 and 78.97. This indicates the presence of 17 different groups of carbon (Fig. 3).

The mass spectra data shows fragmented ion peak at 102, 262, 309, 367, 387, 389, 403, 437, 599, 663 and 685 (Fig. 4). The important neutral molecule losses include water, acetic acid, 2-methyl butanoic acid, furan, ethyl acetate, methoxy and hydroxyl groups. The base peak was found at 387. $[\text{M} + \text{K}]^+$ at m/z 685.18741, $[\text{M} + \text{Na}]^+$ 669 and the M^+ peak at 646.298950 was used to establish its molecular formula as $\text{C}_{34}\text{H}_{46}\text{O}_{12}$.

The ^1H and ^{13}C NMR and MS spectroscopic data of the isolated compound was compared with reported data (Alvi et al. 1991) and the structure was identified

as Xylocensin-I, a limonoid derivative with molecular weight of 646. The structure of the isolated compound is depicted in Fig. 5.

Antidiabetic activities

Diabetes mellitus characterized by prolonged hyperglycaemic condition are reported to be controlled effectively by inhibiting carbohydrate metabolizing enzymes like α -amylase, α -glucosidase, sucrase in the digestive tract (Shim et al. 2003). Inhibitors of these enzymes prolong carbohydrate digestion causing a reduction in the rate of glucose absorption leading to blunting of postprandial

Fig. 3 Structure elucidation by C^{13} NMR spectra of isolated compound

hyperglycemia (Chiasson and Rabasa-Lhoret 2004). Phytochemicals from different plants including mangrove plants have been reported for their hypoglycemic property which may be attributed to inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β cell regeneration, and enhancing the insulin releasing activity (Tiware and Rao 2002; Das et al. 2016b).

Bark, fruits and leaves of *X. granatum* have been reported earlier for their potential to treat diabetes (Srivastava et al. 2011; Das et al. 2016a). However, scientific data are lacking on the bioactive compounds from bark extracts of this plant responsible for their antidiabetic property. Therefore, *X. granatum* ethanol bark extracts were investigated for α -amylase and α -glucosidase inhibitory effects and bioassay-guided fractionation was carried out in search of α -amylase and α -glucosidase inhibitors. The antidiabetic activities of the crude and silica gel eluted different column fractions of XGEB were determined using α -amylase and α -glucosidase inhibition assays and the results were presented in Table 1. The results suggested that the isolated compound Xylocensin-I could inhibit both α -amylase and α -glucosidase enzymes with IC_{50} values being 0.28 and 0.16 mg/ml, respectively. Under the similar condition, the crude bark

extract of *X. granatum* and standard drug Acarbose inhibited strongly α -amylase enzyme with IC_{50} values of 0.36 and 0.15 mg/ml, respectively. Similarly, crude bark extract of *X. granatum* and Acarbose displayed α -glucosidase inhibition activities with IC_{50} values being 0.17 and 0.11 mg/ml, respectively. These findings suggest the possible mode for hypoglycaemic effects of the Xylocensin-I isolated from ethanol bark extract of *X. granatum*.

Antioxidant activities

In the present study, antioxidant activity of the crude ethanol bark extract and column-isolated fractions of *X. granatum* were evaluated in a series of standard antioxidant assays such as; (1) DPPH, (2) ABTS, (3) superoxide and (4) hydrogen peroxide scavenging assays and the results are summarized in Table 2. Xylocensin-I exhibited strong DPPH, ABTS, superoxide and hydrogen peroxide scavenging potential as revealed by the present study with IC_{50} values being 0.041, 0.039, 0.096 and 0.235 mg/ml, respectively (Table 2). The crude ethanol bark extract and standard antioxidant compound BHT exhibited strong activity against DPPH assay with IC_{50} values of 0.043 and 0.047 mg/ml, respectively. The

Fig. 4 MS spectra of bioactive fraction from ethanol bark extracts of *X. granatum*

Fig. 5 Structure of the isolated compound from ethanol bark extracts of *X. granatum*

ABTS and hydrogen peroxide scavenging assays displayed effective antioxidant potentials of crude ethanol bark extract and BHT. Similarly, *X. granatum* crude ethanol bark extract and Ascorbic acid also exhibited superoxide scavenging

Table 1 In vitro antidiabetic activities (expressed as IC₅₀ in mg/ml) of crude and isolated compound from ethanol bark extract of *X. granatum*

Compounds	α -Amylase inhibition	α -Glucosidase inhibition
Crude (XGEB)	0.36 ± 0.01 ^a	0.17 ^a
Xylocensin-I	0.28 ± 0.05 ^b	0.16 ± 0.02 ^a
Acarbose	0.15 ± 0.01 ^c	0.11 ± 0.04 ^a

Data are expressed as mean ± SD ($n=3$). Means in the same column followed by the different superscripts are significantly different ($p < 0.05$)

activity with IC₅₀ values of 0.1 and 0.083 mg/ml, respectively. The crude extract of *X. granatum* showed stronger H₂O₂ scavenging activity than Xylocensin-I at the same concentrations. It is possible that antioxidant activity of the crude bark extract did not come from any single compound and it emerged from the synergistic action of different phytochemicals present in the crude extract. The present study was in agreement with the previous reports that have shown the leaves and barks of *X. granatum* exhibited strong radical scavenging activity (Uddin et al. 2004; Vadlapudi and Naidu 2009; Das et al. 2016a).

Based on in vitro antidiabetic and antioxidant results of the present work, the ethanol bark extract and isolated

Table 2 In vitro antioxidant activities (expressed as IC₅₀ in mg/ml) of crude and isolated compound from ethanol bark extract of *X. granatum*

Compounds	DPPH scavenging	ABTS scavenging	Superoxide scavenging	H ₂ O ₂ scavenging
Crude (XGEB)	0.043 ± 0.007 ^a	0.041 ± 0.009 ^a	0.1 ± 0.033 ^a	0.212 ± 0.013 ^a
Xylocensin-I	0.041 ± 0.001 ^a	0.039 ± 0.008 ^a	0.096 ± 0.018 ^a	0.235 ± 0.056 ^a
BHT	0.047 ± 0.004 ^a	0.076 ± 0.007 ^b	–	0.356 ± 0.018 ^b
Ascorbic acid	–	–	0.083 ± 0.013 ^a	–

Data are expressed as mean ± SD (*n* = 3). Means in the same column followed by the different superscripts are significantly different (*p* < 0.05)

Table 3 Molecular docking results of iE-DAP and zNOD1-LRR at different active sites

Grid box	Binding energy (kcal/mol)	Ligand efficiency	No. of H-bonds
50 × 50 × 50	− 7.71	− 0.17	2
60 × 60 × 60	− 7.57	− 0.16	1
70 × 70 × 70	− 8.60	− 0.18	1

compound Xylocensin-I from the chloroform: methanol (95:5) fraction of *X. granatum* were believed to capable of inhibiting the carbohydrate metabolizing enzymes along with counteracting free radicals. Earlier studies have reported the antimalarial and anti-ulcerogenic activities of Xylocensin-I isolated from *X. granatum* and *X. moluccensis* (Lakshmi et al. 2012, 2015). However, no studies have reported antidiabetic or free-radical scavenging activities of Xylocensin-I. The results of the present work have suggested that the antidiabetic and antioxidant properties exerted by bark extracts of *X. granatum* may be partly due to the presence of limonoid derivative Xylocensin-I.

Molecular docking of Xylocensin-I on α -glucosidase enzyme

To understand the binding mechanism of Xylocensin-I with α -glucosidase enzyme molecular docking was performed. In this analysis 3D structure of Xylocensin-I, obtained from Chemspider database was docked with α -glucosidase enzyme (retrieved crystal structure of *Bacillus cereus* protein, Oligo-1,6-glucosidase, PCBD: from Protein data bank), using AutoDock 4.2. The molecular visualization of interaction was analysed using Discovery studio visualizer. The molecular docking of Xylocensin-I was performed with α -1,6-glucosidase enzyme revealed that, out of three docking calculations with three different grid boxes [(50 × 50 × 50) (60 × 60 × 60) and (70 × 70 × 70)], complex-3 (with 70 × 70 × 70 grid box) was found to be more appropriate with binding energy of − 8.60 kcal/mol (Table 3). The interaction visualized in Fig. 6, indicates that the residues Ser222

and Lys293 (dotted line) were involved in H-bonding. In addition, the residues Gly141, Ala143, Leu162, Asp285, Lys293, Asp329 (pink coloured residues) were involved in electrostatic interaction and the Ala142, Phe163, Phe203, His224, Phe227, Met228, Pro257, Phe281, Ser228, Gly292, Trp294 (green coloured residues) showed the hydrophobic interaction.

The mechanism of the action of the isolated bioactive compound was an important parameter to be investigated. The docking study of Xylocensin-I was performed to gain an insight on the interaction energy using the α -glucosidase. It is well established that drug showing interaction energy more than − 5 kcal/mol for any ligand is considered to have good affinity (Umamaheswari et al. 2011). From the present bioinformatics study, it was observed that Xylocensin-I showed strong binding affinity with the protein α -glucosidase as evident by its low binding energy (− 8.60 kcal/mol). This docking study demonstrated that Xylocensin-I may elicit its antidiabetic activity by inhibiting the α -glucosidase enzyme.

Conclusion

To the best of our knowledge, for the first time a comprehensive study was carried out on the antidiabetic and antioxidant capacities of the bark extracts of *X. granatum*. A bioassay-guided fractionation and purification of ethanol bark extract resulted in the identification of the limonoid compound, Xylocensin-I in the bark of *X. granatum* which afford an essential basis for the use of this plant in the treatment of diabetes and other oxidative stress-related complications. The study reports strong α -amylase and α -glucosidase inhibitory effects along with radical scavenging potentials of Xylocensin-I. The docking studies further confirmed the antidiabetic potentials of the bioactive compound Xylocensin-I. These findings hold great perception in the development of alternative antidiabetic agents. Further studies of Xylocensin-I in suitable animal model along with its toxicity study are necessary for promoting this molecule as antidiabetic new lead compound.

Fig. 6 Docking study. **a** Binding mode of active ligand (Xylococcin-I) in α -glucosidase enzyme binding pocket; **b** α -Glucosidase (1OUK)—Xylococcin-I docking structure

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Author's contribution HNT and LS had conceptualized and designed the work. SKD have conducted all the experiments. DS helped in the in vitro experiments. SKS helped in data analysis. The bioinformatics study has been carried out by SKP. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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