ejpmr, 2018,5(4), 335-339

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

ISOLATION OF OCTADECANEDIOIC ACID FROM *CASSIA FISTULA* LINN AND ITS BIOLOGICAL EVALUATION

Brinda Bolar and *Gangadhar A. Meshram

Organic Research Laboratory, Department of Chemistry, University of Mumbai, Vidyanagri, Kalina, Santacruz (E), Mumbai-400098, India.

***Corresponding Author: Gangadhar A. Meshram**

Organic Research Laboratory, Department of Chemistry, University of Mumbai, Vidyanagri, Kalina, Santacruz (E), Mumbai-400098, India.

Article Received on 07/02/2018 **Article Revised on 28/02/2018** **Article Accepted on 20/03/2018**

ABSTRACT

Cassia fistula L., a semi wild Indian Labernum also known as the golden shower tree is an ornamental tree with beautiful bunches of yellow flower. It is highly reputed for its laxative and purgative properties. The extracts of cassia fistula are widely used as traditional antidiabetic medicines. One of the theraupatic approach for preventing diabetes mellitus-2 is to retard the glucose absorption in the blood via inhibition of glucosidase viz glucoamylase and α-amylase. In the present study Octadecanedioic acid was isolated from the bark of Cassia fistula. The structure was elucidated on the basis of MS, NMR and IR. Octadecanedioic acid exhibited potent inhibitory activity against glucoamylase and α-amylase with IC_{50} value 56μg/mL and 48μg/mL respectively. The antioxidant activity was evaluated by DPPH method. The results indicated that Octadecanedioic acid possess considerable antioxidant activity. The highest radical activity was detected at 81μg/mL. As a result Octadecanedioic acid was isolated from *Cassia fistula* can be used for antioxidant therapy during management of Diabetes Mellitus Type -2.

KEYWORDS: Cassia fistula, Octadecanedioic acid, glucoamylase, α-amylase, antioxidant activity.

1. INTRODUCTION

Diabetes mellitus (DM) is a major health problem all over the world. It is a chronic metabolic disorder which is characterized by abnormal insulin secretion.[1] Insulin is the major hormone regulating the glucose transport in mammals, it stimulates sugar uptake from the circulation into muscles and adipose tissues. Diabetes often referred to as diabetes mellitus describes a group of metabolic diseases in which the person has high blood glucose (blood sugar) either because insulin production is inadequate or because body's cells do not respond to insulin or both. Patients with high blood sugar typically experience polyuria (frequent urination), increasingly thirsty (polydipsia) and hungry (polyphagia). Diabetes is associated with impaired wound healing, exacerbated granulation tissue formation, delayed cellular infiltration, reduced collagen and ineffective neovascularization.[2] The overall lowering of glucose is of pivotal importance in treatment of diabetes, with proven beneficial effects on micro and macrovascular outcomes.[3] Also glucose variability is an independent risk in cardiovascular complications.[4]

Enzymes are macromolecular biological catalysts. Amylase are second largest group of enzyme used in the world. Amylase catalase the hydrolysis of glucosidic linkage in starch and other related oligo- and polysaccharides. These enzymes are widespread among the higher plants, animals and microorganisms. They have been widely utilized in various industrial processes because of their efficiency in starch hydrolysis.[5] One of the most important enzyme is glucoamylase (EC 3.2.1.3 1,4-α-D-glucanglucohydrolase) also known as amyloglucosidase are capable of hydrolyzing glycosidic linkage of α-1,4 type by the successive removal of glucose units from the non-reducing end of the chain, releasing a D-glucose molecule in the β-conformation.[6] Another enzyme is α-Amylase enzyme which are also capable of hydrolyzing the glycosidic linkage.

One of the therapeutic approaches for treating type 2 diabetes mellitus is to decrease the post prandial glucose levels i.e it retards the absorption of glucose through the inhibition of carbohydrates hydrolyzing enzymes present in the small interstinal brush border(glucoamylase and α-amylase). These enzymes are responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption. Inhibitors of these enzymes can delay carbohydrate digestion prolonging overall carbohydrate digestion time, reducing the rate of glucose absorption and hence controlling the post parandial glucose rise in the blood. Acarbose and miglitol are excellent examples of inhibitors used in diabetes control.[7] Acarbose a well known drug used for clinical treatment of diabetes mellitus is a pseudotetrasaccharide produced by Actinoplanes sp. Fermentation, consisting of a polyhroxylated aminocyclohexene derivative (valienamine) linked via its

nitrogen atom to a 6-deoxyglucose,which is itself α-1,4-linked to a maltose moiety. It is a competitive inhibitor of α-amylase and glucoamylase and the mechanism of inhibition seems to be due to the unsaturated cyclohexene ring and the glycosidic nitrogen linkage that mimics the transition state for the cleavage enzymatic of glucosidic linkages.[8,9] Compounds such as (-)-Epicatechin from pterocarpus marsupium Roxb., Quercetin from Baubinia purpurea L., β-Sitosterol from cassia fistula L., are excellent inhibitors for glucoamylase and α-amylase which results in control of blood glucose level in the body.[10]

Diabetes management includes controlling hyperglycemia, oxidative stress and hyperlipidemia.[11] Oxidative stress produced by cellular respiration and environmental factors is the main cause of diabetes mellitus complications. Superoxide anion, hydrogen peroxide and radical hydroxyl are the most dangerous radicals known.[12] It has been proposed that Chronic and acute overproduction of reactive oxygen species (ROS) under pathophysiologic conditions could contribute to the initiation and development of Cardiovascular diseases.[13] Therefore increased attention is now been paid on the antioxidant activity of natural products originating from traditionally used medicinal plants or dietary sources.[14,15]

Anticancer drugs are frequently associated with toxic and other side-effects.[16-17] Saturated fatty acid is found in high concentration in food items. It has been reported to inhibit human breast cancer cell proliferation *in vitro*[18-20] and breast tumorigenesis *in vivo.*[21-22] Fatty acids has also been shown to induce apoptosis of breast cancer cells and to arrest breast cancer cell-cycle.[23] Dietary fatty acids has been associated with a decrease in incidence of mammary tumor in carcinogenesis models.[24] Cassia fistula L. which is also known as Amaltas is known for its disease killing property. It is a highly valuable medicinal plant in Ayurveda has anti-dysentry, anti-microbial, anti-glycaemic,anti-diarrheal, anti-obesity, anti-pyretic and hepatoprotective properties.[16] The whole plant is used to treat diarrhoea. The seeds, flowers and fruits are used to treat fever, abdominal pain, skin diseases and leprosy.[17] The root of the plant is prescribed as a tonic, astringent, febrifuge and strong purgative.[18,19,20,21,22,23,24] Phytochemical investigation of this plant revealed the presence of long-chain hydrocarbons, triglycerides, sterols, chromones, flavonoid, anthraquinones, sugar, diterpenoid and triterpenoids from its leaves, flowers, seeds, pods and fruit.[25]

In the present study we have isolated long chain hydrocarbons from the bark of *Cassia fistula*. The isolated compound was further subjected to glucoamylase, α-amylase and antioxidant assay.

2. MATERAILS AND METHODS

Plant Extraction

The bark of the plant was collected from Campus of University of Mumbai, Kalina, Santacruz, Mumbai-98. The plant material was identified and authenticated at Blatter Herbarium, St.Xavier's College, Mumbai-400001. The voucher specimen No.R4144 was reserved for further reference. It was shade dried for a week at 25°C and was ground to a coarse powder in a grinder. The powdered bark (50g) was extracted in various solvents like dichloromethane, pet-ether, ethyl acetate and butanol. The dichloromethane extract was subjected to isolation of compound.

Isolation of compound

The bark material was sonicated with dichloromethane for 48 hrs. The extract obtained was evaporated to dryness. The extract was kept at 4°C. The extract obtained was subjected to column chromatography in Chloroform: Ethyl acetate system in the ratio of 95:5, 90:10, 85:15 and so on. In Chloroform: Ethyl acetate (50:50) system a Brownish white powdered compound was obtained. The compound obtained was further subjected to column chromatography in Pet Ether: Chloroform system in the ratio of 95:5, 90:10, 85:15 and so on. In 100% Chloroform system white powdered compound was obtained which gave single spot in TLC. This compound was further subjected to spectroscopic analysis.

Glucoamylase assay

0.5 ml of the reaction mixture containing 0.1 ml modulator, 0.3 ml of 100 mM acetate buffer (pH 4.5) and 0.1 ml of glucoamylase were incubated at 37°C for 30 min. Then, add 0.5 ml of starch solution (5mg/ml prepared in 100 mM acetate buffer (pH 4.5) and incubated further at 37°C for 30 min. The reaction was terminated by keeping the test tubes in boiling water bath for 1-2 min, cooled under running tap water; add 2 ml of DNS (3,5-dinitrosalicylic acid) and the test-tubes were kept in boiling water bath for 15minutes. The test-tubes were cooled and diluted with 7 ml of distilled water.

The absorbance was recorded at 530 nm using spectrophotometer and liberated glucose was estimated. The % inhibition (I) was calculated as,

$$\% I = \{(Ac - As)/Ac\} \times 100$$

Where, Ac and As are the absorbance of the control and the sample respectively.

α-Amylase assay

0.5 ml of the reaction mixture containing 0.1 ml modulator, 0.3 ml of 20 mM phosphate buffer(pH 4.5) and 0.1 ml of α-Amylase were incubated at 37°C for 30 minutes. Then added 0.5 ml of starch solution (10 mg/ml prepared in 20 mM phosphate buffer pH 7.0) and incubated further at 37°C for 30 minutes. The reaction was then terminated by keeping the test tubes in boiling water bath for 1-2 minutes, cooled under running tap water. 1 ml of DNS (3,5-dinitrosalicylic acid) was added

and the test-tubes were kept in boiling water bath for 15 minutes. The test tubes were cooled and diluted with 7 ml distilled water. The absorbance was recorded at 530 nm using spectrophotometer and liberated glucose was estimated. Acarbose was used as a standard for both the assays. The maximum inhibition was determined from plots of % inhibition versus modulator and calculated as shown

$$\% \ I = \{(Ac - As)/Ac\} \times 100$$

Where, Ac and As are the absorbance of the control and the sample respectively.IC_{50} values of Acarbose and Octadecanedioic acid was determined from the plots of percentage inhibition versus concentration (μg/ml).

DPPH Radical scavenging assay

1 ml of various concentrations of the extracts in methanol was added to a 1 ml of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed standing for 30 minutes. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Inhibition of free radical DPPH in % inhibition was calculated in the following way:

$$\% \ I = \{(Ac - As)/Ac\} \times 100$$

Where Ac is the absorbance of the control reaction (containing all reagents except the test compound) and As is the absorbance of the test compound. Ascorbic acid is used as control and methanol as blank.

Results are presented as mean ± standard error of the mean.

Spectral Characterization

IR, ^{1}H, ^{13}C-NMR and EI-MS were carried out to identify the compound. Infrared was recorded using Perkin Elmer,^{1}H and ^{13}C NMR spectra were recorded using $CDCl_3$ as a solvent on broker Avance II 300 MHz NMR spectrometer respectively and EI-Mass spectrum was obtained using High resolution on Perkin Elmer EI-Mass spectrometer, in the Department of Chemistry, University of Mumbai, Kalina, Santacruz (E),Mumbai-400098

Spectral Data

Melting Point: 124-127°C

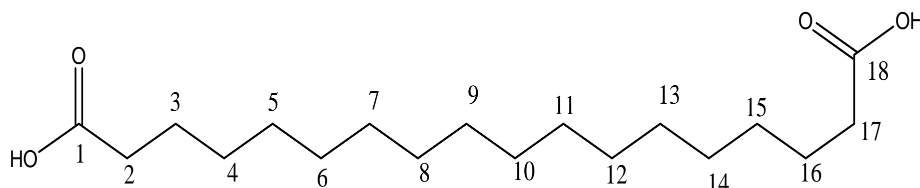
Nature: White amorphous powder.

FT-IR: 3368.90 cm^{-1},2915.58 cm^{-1}, 2847.93 cm^{-1},1734.73 cm^{-1},1471.91 cm^{-1},1462.79 cm^{-1},1414 cm^{-1},1046 cm^{-1},1170.87cm^{-1} 1101.24 cm^{-1}.

^{1}H NMR: δ1.257 (s), δ1.654 (t), δ2.365 (t), δ10.935 (s).

^{13}C NMR: δ180.449, δ34.142, δ29.734, δ24.712.

EI-MS: 314(M+), 296, 278, 250, 237, 209, 196, 168, 154, 140, 126, 112, 98, 84, 70, 55.



3. RESULTS AND DISCUSSION

The compound was white amorphous in nature with the melting point of 124 to 127 °C. The IR spectra of the compound gave absorption band at 3368.90 cm^{-1} which is a characteristic of –OH stretching. Absorption at 2915.58 cm^{-1} and 2847.93 cm^{-1} is due to aliphatic C-H stretching. Absorption frequency at 1734 cm^{-1} is due to –CHO stretching.

The ^{1}H NMR spectrum of the compound revealed that the compound has long aliphatic chain. A singlet at δ1.257 represents a long aliphatic chain. There are triplets observed in the region of δ1.654 and δ2.365 are due to presence of $-CH_2$ neighboring groups. A peak at δ10.935 is a singlet due to presence of –COOH group.

The ^{13}C spectrum of the compound revealed that the compound has a –COOH group in the region of δ180.449. The compound showed $-CH_2$ groups in the region of δ34.142, δ29.734 and δ24.712.

The EI-MS of the compound showed a molecular ion at m/z 314 with weak intensity. The fragments have m/z (relative intensity) 296 (12), 278 (14), 250 (10), 237 (11), 209 (7), 196 (5), 168 (9), 154 (12), 140 (14), 126 (18), 112 (38), 98 (100) (base peak), 84 (62), 70 (40), 55 (66). These data were compared with the data reported in the literature.

The isolated compound was further studied for its effect on the carbohydrate metabolizing enzymes, α-amylase and glucoamylase in the range of 2-10 μg/ml. Acarbose is used as reference glucosidase inhibitor.

Table 1: Effect of Octadecanedioic acid on Glucoamylase.

Concentration (µg/ml)	% Inhibition (Octadecanedioic acid)	IC_{50} (µg/ml)	Concentration (µg/ml)	%Inhibition (Acarbose)	IC_{50} (µg/ml)
20	28	56	20	38	57.5
40	32		40	45	
60	63		60	51	
80	68		80	56	
100	72		100	64	

Table 2: Effect of Octadecanedioic acid on α-Amylase.

Concentration (µg/ml)	% Inhibition (Octadecanedioic acid)	IC_{50} (µg/ml)	Concentration (µg/ml)	%Inhibition (Acarbose)	IC_{50} (µg/ml)
20	36	48	20	33	50
40	48		40	42	
60	56		60	61	
80	63		80	66	
100	72		100	74	

The percentage inhibition at 20, 40, 60, 80, 100 µg/ml concentrations of Octadecanedioic acid showed concentration dependent increase in percentage inhibition. As the highest concentration of 100 µg/ml showed maximum inhibition of 72% whereas Acarbose showed inhibition of 74% in the maximum concentration of 100 µg/ml. Biological evaluation with glucoamylase showed IC_{50} values of 56 µg/ml and 57.5 µg/ml for Octdecanedioic acid and Acarbose. α- Amylase showed maximum inhibition of 72% by Octadecanedioic acid at the concentration of 100 µg/ml and Acarbose showed maximum inhibition of 74% at the concentration of 100 µg/ml. The IC_{50} values of 48 µg/ml for Octdecandioic acid and of 50µg/ml for Acarbose showed excellent inhibitory action of isolated compound over standard Acarbose.

Antioxidant activity of Octadecanedioic acid was tested against DPPH radical at varying concentrations of 20,40,60,80,100µg/ml. Results obtained are shown in the Table 3. The activity of the standard (Ascorbic acid) was much higher than Octadecanedioic acid.

Table 3: DPPH Radical scavenging activity of Octadecanedioic acid and Ascorbic acid.

Sample	Radical Scavenging Activity
Octadecanedioic acid	81
Ascorbic acid	5.30

4. CONCLUSION

The aim of the study was to analyse the activities of Octadecanedioic acid with respect to hypoglycemic conditions and anticancer activity. Our present study, confirms antioxidant as well as antidiabetic of Octadecanedioic acid. The above compound isolated from *Cassia fistula* reportedly shows good antimicrobial activity.

REFERENCE

1. J.W. Baynes. Role of oxidative stress in development of complication of diabetes. Diabetes, 1991; 40(4): 405-412.
2. S.A. Eming. T.krieg and J.M. Davidson. J, Inflammation in wound repair: Molecular and cellular mechanisms. Invest, Dermatol, 2007; 127: 514-525.
3. P.C. Yu, Z. Bosnyak and A. Ceriello. The importance of glycated haemoglobin (HbA(1c)) and post parandial glucose (PPG) control on cardiovascular outcomes in patients with type-2 diabetes. Diabetes Res. Clin. Prac, 2010; 89: 1-9.
4. A Ceriello. Hyperglycaemia and the vessel wall: The pathophysiological aspect on the atherosclerotic burden in patients with diabetes. Eur. J. Cardiovasc. Prev. Rehabil, 2010; 17(1): S15-S19.
5. J Bryjak. Glucoamylase, α-amylase and β-amylase immobilization on acrylic carriers. Biochemicals engineering journal, 2003; 16: 347-355.
6. T Pasin and V Benassi. Purification and functional properties of a novel glucoamylase activated by manganese and lead produced by Aspergillus japonicas. Int. J. biological macromolecules, 2017; 102: 779-788.
7. P Michelle and P Monteri. Pouteria torta epicarp as a useful source of α-amylase inhibitor in the control of type 2 diabetes. Food and Chemical Toxicology, 2017; 1-8.
8. Yoon SH and Robyt JF. Study of the inhibition of four alpha amylase by acarbose and its 4IV-α-maltohexaosyl and 4IV-α-maltododecaosyl analogues, Carbohydr. Res., 2003; 338: 1969-1980.
9. Gyemant G, Kandra L, Nagy V and Somsak L. Inhibition of human salivary α-Amylase by glucopyranosylidene-spiro-thiohydantoi. Biochem Biophys Res Commun, 2003; 312: 334-339.

10. R.M. Perez, M.A. Zavala and S. Perez. Antidiabetic effect of compounds isolated from plants. Phytomedicine, 1998; 5(1): 55-75.
11. S.Poovitha and M Siva Sai. Protein extract from the fruit pulp of Momordica dioica shows antidiabetic, anti-lipidemic and antioxidant activity in diabetic rats. Journal of Functional Foods, 2017; 33, 181-187.
12. E. Alberto and J. Cornejo. Antidiabetic effect, antioxidant activity and toxicity of 3’,4’-Di-Oacetyl-cis-khellactone in streptozotocin- induced diabetic rats. Bioorganic & Medicinal Chemistry Letters, 2016; 26: 4086-4091.
13. N.R. Madamanch and A. Vendrov. Oxidative stress and vascular disease. Arteriosclerosis, Thrombosis and Vascular Biology, 2005; 25: 29-38.
14. H.T. Liu and Y.F. Wang. Characterization of in vivo antioxidant constituents and dual-standard quality assessment of Danhong injection. Biomedical Chromatography, 2013; 27(5): 655-663.
15. Z.Y. Ma, S.R. Zheng and H.X. Han. The bioactive components of Coreopsis tinctoria (Asteraceae) capitula: antioxidant activity in vitro and profile in rat plasma. Journal of Functional Foods, 2016; 20: 575-586.
16. S.Kaur and M.Kumar. Modulatory effects of Cassia fistula fruits against free radicals and genotoxicity of mutagens. Food and Chemical Toxicology, 2016; 98: 220-231.
17. P.K. Pandey and D.Singh. Cassia fistula seeds trypsin inhibitors as antibiosis agent in Helicoverpa armigera pest management. Biocatalysis and Agricultural Biotechnology, 2016; 6, 202-208.
18. R.K. Gupta. Medicinal and Aromatic plants. CBS Publishers and distributors, 1st edition, 2010; 116-117.
19. C.P. Khare. Indian medicinal plants. Springer, 2007; 128.
20. A.K. Gupta, N. Tandon and M. Sharma. Quality standards of Indian Medicinal plants, Medicinal Plants Unit, Published by Indian Council of Medicinal Research, 2008; 2: 47-53.
21. K.M. Nadkarni. Indian Materia Medica. Bombay Popular Prakashan, 2009; 1: 285-286.
22. R.N. Chopra, S.L. Nayar and I.C. Chopra. Glossary for Indian Medicinal Plants. National Institute of Science Communication and Information Resources, 2006; 54.
23. Anonymous. The wealth of India, First Supplement Series (raw Materials). National Institute of Science Communication and Information Resources, CSIR, 2007; 3: 340-342.
24. S.S. Agarwal and M. Paridhavi. Clinically useful herbal drugs. Ahuja publishing House, 2005; 281-282.
25. P.Dias and K. Balasubramanian. Insulin mimetic impact of Catechin isolated from Cassia fistula on glucose oxidation and molecular mechanism of glucose uptake on Streptozotocin-induced diabetic wistar rats. Phytomedicine, 2010; 17: 28-36.