WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 6, Issue 1, 696-704. Research Article ISSN 2277– 7105

ISOLATION AND BIOLOGICAL EVALUATION OF (24*R*) STIGMAST-5-EN-3-OL FROM *CASSIA FISTULA* BARK.

Brinda Bolar, Jennifer George and Gangadhar A. Meshram*

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

Article Received on
03 Nov. 2016,

Revised on 24 Nov. 2016,
Accepted on 15 Dec. 2016

DOI: 10.20959/wjpr20171-7576

***Corresponding Author**
Dr. Gangadhar A. Meshram
Organic Research Laboratory, Department of Chemistry, University of Mumbai, Vidyanagri, Kalina, Santacruz (E), Mumbai-400098, India.

ABSTRACT

Cassia fistula is known as the Golden rain tree which belongs to Fabaceae family. In Ayurvedic medicine it is well known as a disease killer. The extracts of *cassia fistula* are widely used as traditional antidiabetic medicines. One of the therapeutic 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 (24*R*) Stigmast-5-en-3-ol was isolated from the bark of *cassia fistula*. The structure was elucidated on the basis of MS, NMR and IR. (24*R*)Stigmast-5-en-3-ol exhibited potent inhibitory activity against glucoamylase and α-amylase with IC_{50}value 5.8 µg/mL and 7.198 µg/mL respectively. The antioxidant activity was evaluated by DPPH method. The results indicated that (24*R*) Stigmast-5-en-3-ol possess considerable antioxidant activity. The highest radical activity was detected at 71.42 µg/mL. As a result (24*R*) Stigmast-5-en-3-ol isolated from cassia fistula can be used for antioxidant therapy during management of Diabetes Mellitus Type-2 as well.

KEYWORDS: *Cassia fistula*, (24*R*) Stigmast-5-en-3-ol, glucoamylase, α-amylase, antioxidant activity.

1. INTRODUCTION

India has one of the most diverse cultural traditions associated with the use medicinal plants. Many medicinal plants used for several years are present in Ayurveda and have interesting multilevel activities.[1] The phytochemical evaluation of plants which have been used in olden age for medicinal purposes have often resulted in isolation of compounds with excellent biological activities. The chemical profile of a single plant may change over time as it reacts

to changing environmental conditions which result in change in its biochemical pathway.

The Secondary metabolites and pigments present in the plant have many therapeutic action in humans, which can be refined to produce drugs. These secondary metabolites include alkaloids, phenols, terpenoids, glycosides and others. Such plants having therapeutic properties are called as Herbs.[2]

Cassia fistula linn is an important medicinal plant known as the golden shower tree.[3,4,5] The whole plant possess medicinal properties useful in treatment of skin diseases, inflammatory disease, anorexia, rheumatism and jaundice. It is also used in the treatment of liver troubles, tuberculosis glands and in the treatment of haematemesis, pruritus, leucoderm and diabetes.[6]

Diabetes mellitus is a chronic metabolic disorder affecting approximately 4% of the world population. The demand for herbal medicine for treatment of diabetes is increasing as it does not cause any harmful side effects.[7] The inhibition of carbohydrate hydrolyzing enzymes such as α-Amylase and Glucoamylase is important to lower the post parandial blood glucose level. Such inhibitors have many gastrointestinal side effects. Hence it is necessary to identify and explore Glucoamylase and α-Amylase inhibitors from natural sources having very few side effects.[8]

Phytosterols (plant sterols and stanols) are naturally occurring compounds that have similar function and structure as that of cholesterol. Phytosterols are present in various plant and marine sources. In our present study we have isolated (24*R*) Stigmast-5-en-3-ol from cassia fistula bark extract and have also carried out its antidiabetic and antioxidant study.

2. MATERIALS AND METHODS

2.1 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 future reference. It was air dried for a week and was ground to a coarse powder in a grinder. The powdered bark (50g) was extracted in various solvents like pet-ether, chloroform, ethyl acetate and butanol. The Ethyl acetate extract was further subjected to Hydrolysis.

2.2 Isolation of compound

2.2.1 Acidic Hydrolysis: 30 mg of Ethyl acetate extract was refluxed for 5 hrs in 10% H_2SO_4-MeOH (1:2,15 ml) then poured in ice-water and extracted with ethyl acetate. The ethyl acetate layer was washed with Water and dried over Sodium sulphate and concentrated to give crude product.[9]

2.2.2 Column chromatography: The Hydrolyzed crude product was subjected to column chromatography in Hexane: Ethyl acetate system. Various fractions were collected using 100% Hexane, Hexane: Ethyl acetate in the ratio 95:5, 90:10, 85:15 and so on. A crystalline compound was obtained in Hexane :Ethyl acetate system (95:5). The compound obtained was washed with pet ether and analyzed by spectroscopic methods.

2.3 Identification of compound

2.3.1 Liebermann-Buchards Test: A few crystals were dissolved in $CHCl_3$ and few drops of conc H_2SO_4 was added to the solution followed by addition of 2-3 drops of acetic anhydride. The solution turned violet blue and finally green. (Harbone 1984).

2.3.2 Salkowski reaction: A few crystals were dissolved in CHCl3. To the CHCl3 layer few drops of conc H2SO4 was added slowly along the sides of the test tube. A reddish colour was seen in the upper chloroform layer. (Harbone 1984).

2.4 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 (5 mg/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 15 minutes. 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.

2.5 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} x 100

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

IC_{50} values of Acarbose and (24*R*) Stigmast-5-en-3-ol was determined from the plots of percentage inhibition versus concentration (µg/ml).

2.6 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} x 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 average.

2.7 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 & ^{13}C -NMR spectra were recorded using CDC_{l3} as solvent on broker Avance II 300 MHz and 75 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.

2.8 Spectral Data

UV: λmax = 248 nm.

MELTING POINT: 137 – 139 °C.

NATURE: The compound is white crystalline solid.

FT-IR: 3456 cm^{-1}, 2916.01 cm^{-1}, 2848.54 cm^{-1}, 1703.91 cm^{-1}, 1463.15 cm^{-1}, 1376.89 cm^{-1}, 1260.72 cm^{-1}, 1206.53 cm^{-1} , 1097 cm^{-1}, 1018.83 cm^{-1}.

^{1}H-NMR: δ5.34 (1H,d,5.1Hz,H6), δ3.50 (1H,m,H-3), δ1.184 (3H,s,H-18), δ0.609 (3H,s,H-19), 0.936 (3H,d,J=6.9Hz,H-21), 0.841 (3H,d,J=6.3Hz,H-26), 0.775 (3H,d,J=6.9 Hz,H-27).

^{13}C-NMR: 32.42 (C-1), 35.81 (C-2), 71.79 (C-3), 42.30 (C-4), 140.773 (C-5), 121.7 (C-6), 30.3 (C-7), 28.9 (C-8), 42.334 (C-9), 39.79 (C-10), 20.216(C-11), 31.922 (C-12), 40.49 (C-13), 50.161 (C-14), 21.229 (C-15), 21.094 (C-16), 51.25 (C-17), 19.824 (C-18), 19.402 (C-19), 29.185 (C-20), 18.714 (C-21), 33.7 (C-22), 29.185 (C-23), 45.860 (C-24), 31.482 (C-25), 18.997 (C-26), 18.907 (C-27), 25.410 (C-28), 12.250 (C-29).

EI-MS(m/z): 414 (M+), 396, 381, 354, 329, 273, 213, 173, 159, 145, 107, 95, 81.

SPECIFIC ROTATION

The compound showed λmax=248 nm and the specific rotation of the compound is $[\alpha]_D$= 38. The Cotton effect curve of the compound is given in Fig.1 signifies (+) rotation i.e rotation to the right which confirms that the compound is (24R) Stigmast-5-en-3-ol.

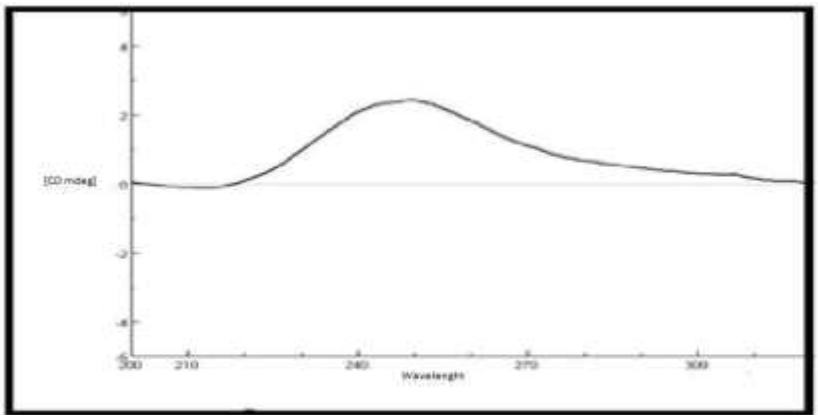


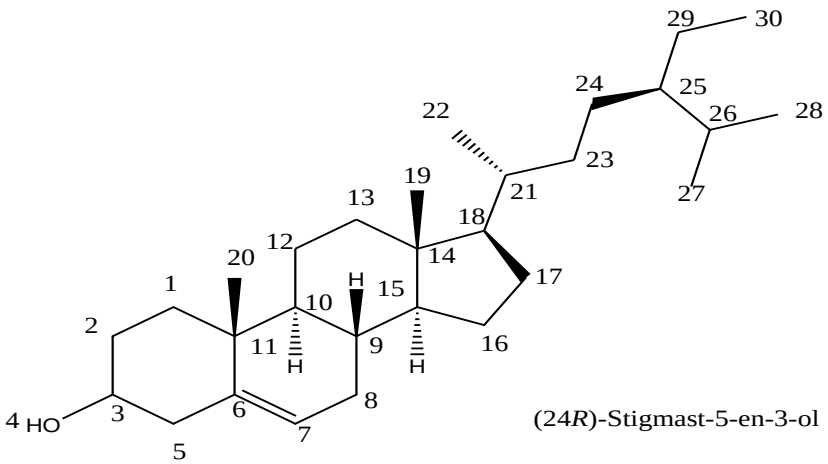
Fig.1

3. RESULTS AND DISCUSSION

The compound was crystalline in nature with melting point of 137 to 139 °C. The compound gave a positive result for libermann buchard and Salkowski's test for steroids. The IR spectra of the compound gave absorption band at 3456 cm^{-1} which is the characteristic of –OH stretching. Absorption at 2916.01 cm^{-1} and 2848.54 cm^{-1} is due to aliphatic C-H stretching of methylene and methyl groups. Absorption frequency at 1703 cm^{-1} due to C=C absorption, peak at 1463.15 cm^{-1} is bending frequency for cyclic compounds. 1376.89 cm^{-1} indicates signal for –CH3 group, 1260.72 cm^{-1} represents absorption frequency for bending –C-OH group, 1206.53 cm^{-1} represents –C-O stretching frequency and 1097.73 cm^{-1} signifies absorption frequency for cycloalkanes.

The ^{1}H NMR spectra revealed that the compound has a steroidal nucleus at 0.69 to 2.2 ppm indicates overlapping of methyl, methylene and methine protons. A multiplet at 3.162 ppm represents oxymethine proton at position 3 and a single unsaturated proton gave a doublet at 5.34 ppm. The ^{13}C NMR spectrum of the compound revealed a total of 29 carbon signals,out of which 6 were methyl signals, 11 were methylene signals, 9 were methine and 3 were quaternary carbon signals. The signal between 12.250 and 50.161 ppm represents region of overlapping of methyl, methylene and methine carbon atoms, a signal at 71.79 ppm represents the oxymethine signal at position 3 and finally the unsaturated carbon signals at 121.704 and 140.773 ppm representing the two carbon atoms in the olefinic system.

The EI-Mass spectrum showed the molecular ion at m/z 414 with fragments at m/z (relative intensity) 414.2 (44), 396 (36), 381.22 (50), 354 (11), 329.17 (54), 273.09 (22), 213.15 (100) (Base peak), 173 (52),159.2 (76), 145.17 (90), 107 (36), 95 (48), 81 (48). These data were compared with the data reported in the literature of this compound.



(24*R*)-Stigmast-5-en-3-ol

Fig.2

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 was used as reference glucosidase inihibitor.

Table 1: Effect of *(24R)* Stigmast-5-en-3-ol on Glucoamylase :

Concentration (µg/mL)	% Inhibition ((24*R*) Stigmast-5-en-3-ol)		Concentration (µg/mL)	% Inhibition (Acarbose)	
2	35.5	IC_{50} 5.8	20	41.59	**IC_{50}** 40.87
4	42.2		40	48.38	
6	56.8		60	57.47	
8	64.8		80	69.59	
10	79.7		100	76.49	

Table 2: Effect of *(24R)* Stigmast-5-en-3-ol on α-Amylase

Concentration (µg/mL)	% Inhibition ((24*R*) Stigmast-5-en-3-ol)		Concentration (µg/mL)	% Inhibition (Acarbose)	
2	38.2	IC_{50} 7.19	20	38.12	IC_{50} 48.23
4	41.5		40	41.59	
6	46.2		60	48.38	
8	51		80	57.47	
10	58.3		100	69.59	

The percentage inhibition at 2, 4, 6, 8 and 10 µg/mL concentrations of (24*R*) Stigmast-5-en-3-ol showed concentration dependent increase in percentage inhibition. As a result the highest concentration of 10 µg/mL showed a maximum inhibition of 58.3% whereas Acarbose showed inhibition of 76.49% in maximum concentration of 100 µg/mL. Biological evaluation with glucoamylase showed IC_{50} values of 5.8 µg/mL and 40.48 µg/mL for (24*R*) Stigmast-5-en-3-ol and Acarbose respectively. α-Amylase showed maximum inhibition of 58.3% by (24*R*) Stigmast-5-en-3-ol and 69.59% by the standard drug Acarbose. The IC_{50} value of 7.19 µg/mL for (24*R*) Stigmast-5-en-3-ol and 48.23 µg/mL for Acarbose revealed excellent inhibitory action of isolated compound over standard Acarbose.

Antioxidant activity of (24*R*) Stigmast-5-en-3-ol was tested against DPPH radical at varying conentrations of 20, 40, 60, 80 & 100 µg/mL. Results obtained are shown in the Table 3. The activity of the standard (Ascorbic acid) was much higher than (24*R*) Stigmast-5-en-3-ol.

Table 3: DPPH Radical scavenging activity of *(24R)* Stigmast-5-en-3-ol and Ascorbic acid.

Sample	Radical scavenging activity (µg/mL)
(24R) Stigmast-5-en-3-ol	71.42
Ascorbic acid	5.30

4. CONCLUSION

The compound (24*R*) Stigmast-5-en-3-ol isolated from *Cassia fistula* bark reportedly reduces carcinogen-induced cancer of the colon[10]& prostate disorder occurring mostly in ageing men. It is also used in treatment of benign prostatic hyperplasia (BPH).[11]

The aim of this study was to isolate and analyze the hypoglycemic activities of (24*R*) Stigmast-5-en-3-ol. Our study confirms antioxidant and antidiabetic potency of (24*R*) Stigmast-5-en-3-ol.

5. ACKNOWLEDGEMENT

The authors are thankful to Department of chemistry, University of Mumbai for providing the facilities. This work was financially supported by UGC Non-NET fellowship provided by UGC, New Delhi.

6. REFERENCES

1. Leena S, Priyanka S, Anupma M, Sheema B. Phtochemical evaluation HPTLC fingerprint profile of Cassai fistula. IJAPBC. 2014; 3(3): 604-611.
2. Jignasu P.Mehta. Seperation and Characterization of Antraquinone derivatives from Cassia Fistula using chromatographic and spectral techniques. IntJ.Chem.Sci. 2012; 10(1): 306-316.
3. Olubukanla T, Olusola O. Natural seed germination and seedling dynamics in cultivated population of Cassia fistula Linn in Nigeria. AJAR. 2015; 10(8): 865-870.
4. Chauhan Neelam, Bairwa Rajhan. Review on Cassia Fistula. IJRAP. 2011; 2(2): 426-430.
5. Chauhan Neelam, Bairwa Rajhan. Antimicrobial Activity of Cassia fistula Linn. IRJP. 2011; 2(10): 100-102.
6. Hermien Noorhajati, Mulyadi T, NanikSiti A, Ami S. Antioxidant Activities of extracts of Trengguli Stem bark (Cassia fistula L.). IJBAS-IJENS. 2012; 12(4): 85-89.
7. Nirmala A , Eliza J, Rajalakshmi M , Priya E, Daisy P. Effect of Hexane Extract of Cassia Fistula Barks on Blood Glucose and Lipid profile in streptozotocin in Diabetic Rats. Int. Journal of Pharmacology. 2008; 4(4): 292-296.

8. Bhutkar M.A ,Bhise S.B. Invitro Assay of alpha amylase inhibitory activity of some indigenous plants. Int J. Chem. Sci. 2012; 10(1): 457-462.
9. Akira I, Masaaki Y, Hiroko M, Mari K, Harumasa T. Phytochemical studies of seeds of medicinal plants. Two sulphated triterpenoid glycosides sulfapatrinosides from seeds of patriniascabiosaefoliafischer. Chem Pharm. Bull. 1988; 36(11): 4269-4274.
10. Bulama J.S, Dangoggo S.M, Mathias S.N. Isolation and Characterization of Beta-(24R) Stigmast-5-en-3-ol from ethyl acetate extract of root bark of Terninaliaglaucescens. Int. Journal of Scientific and Research Publications. 2015; 5(3): 1-3.
11. Berges R.R, Kassen .A, Senge .T. Treatment of symptomatic benign prostatic hyperplasia with beta-(24R) Stigmast-5-en-3-ol: an 18 month follow-up. BJU International. 2000; 85: 842-846.