Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 11, 2015

Original Article

ISOLATION AND CHARACTERIZATION OF SECONDARY METABOLITES FROM *PITHECELLOBIUM DULCE* BENTH FRUIT PEEL

SUKANTHA T. A.[a,*] SHUBASHINI K. SRIPATHI[b]

***[a]Department of Chemistry, K. S. Rangasamy College of Technology, Tiruchengode 637215, Tamil Nadu, India, [b]Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore 641043, Tamil Nadu, India**
Email: tasukantha@gmail.com

Received: 17 Feb 2015 Revised and Accepted: 22 Sep 2015

ABSTRACT

Objective: The present study describes the isolation and characterization of valuable secondary metabolites from the fruit peel of *Pithecellobium dulce*.

Methods: Solvent extraction of the plant material using methanol followed by column chromatographic separation of the extract with solvents (Petroleum ether, ethyl acetate and methanol) of increasing polarity using silica gel yields four compounds (1-4).

Results: The structures of the compounds stigmasterol (1), β-sitosterol (2), quercetin (3) and pinitol (4) were established on the basis of FTIR, ^{1}H and ^{13}C NMR; and mass spectra and in comparison with their literature values.

Conclusion: Two sterols namely stigmasterol (1) and β-sitosterol (2), along with a flavonoid quercetin (3) and a cyclitol pinitol (4) have been isolated from the methanol extract of fruit peel of *Pithecellobium dulce* for the first time.

Keywords: *Pithecellobium dulce,* Pinitol, Sterols, Quercetin.

INTRODUCTION

The plant *Pithecellobium dulce* Benth belongs to *Mimosaceae* family. Previous studies on different parts of this plant such as seeds, leaves, flowers, fruits, bark, heartwood, roots and stems have reported for the presence of steroids, terpenoids, flavonoids, steroids and their glycosides respectively [1-13]. Still there is no such report on the isolation and characterization of bioactive molecules from the fruit peel.

The leaf, seed and bark of the plant *Pithecellobium dulce* have been reported for the anti-diabetic activity [14-16]. The fruit peel of the plant has been used for the control of diabetes by the local people of the northwest region of Tamil Nadu, India. Some people chew raw fruit peel or drink its decoction in water to control blood sugar. But there is no scientific evidence. Researches, hitherto carried on the fruit peel, have reported only for its antibacterial, antioxidant and wound healing potential [17, 18]. Based on ethno knowledge of its use, in the control of diabetes, the fruit peel was taken up for phytochemical investigation. Herein we reports the isolation of four compounds from the fruit peel of *P. dulce* and the same have been characterized as medicinally valuable molecules-stigmasterol, sitosterol, quercetin and pinitol and they are familiar compounds. The isolation of pinitol from this plant and the genus are reported for the first time. All the four compounds were found to possess anti-diabetic activity [19, 20], anti-cancer activity [21], anti-inflammatory activity [22] and cardio protective activity [23]. Compared to other compounds, pinitol is a safe and non-toxic molecule and is available in sufficient quantity in this plant. Hence this first report of isolation of pinitol from the fruit peel of this plant, an agro waste, is of immense significance.

MATERIALS AND METHODS

Collection and identification of plant material

The plant *Pithecellobium dulce* was collected from Namakkal district, Tamil Nadu, India. The plant was taxonomically identified and authenticated by Botanical Survey of India, Coimbatore (Tamil Nadu) and a voucher specimen was deposited in our laboratory for future reference (BSI/SRC/5/23/2011-12/Tech.752).

Extraction of fruit peel of pithecellobium dulce

Air dried pieces of fruit peel of *Pithecellobium dulce* (1 kg) were thoroughly percolated and extracted with petroleum ether (60-80 ^{o}C) for (2 x 6) h, the residual plant material extracted with ethyl acetate for a period of (2 x 6) h and finally with methanol for a period of (2 x 6) h. The petroleum ether extract, ethyl acetate extract and methanol extract obtained were filtered and concentrated under reduced pressure to yield yellowish white, green and greenish brown residues respectively.

Chromatographic analysis of methanol extract

The residue of the methanol extract of *Pithecellobium dulce* fruit peel (about 30 g) was dissolved in minimum quantity of methanol and made into the slurry with minimum amount of silica gel. It was subject to column chromatographic using silica gel (300 g) built in petroleum ether and eluted with increasing amounts of ethyl acetate followed by eluting with ethyl acetate and increasing amount of methanol. Eluates of 200 ml were collected each time and the solvent was distilled on a water bath. The homogeneity of the fractions was examined by TLC on silica gel plates. The spots developed were visualized under UV light and then by exposure to iodine vapour. Similar fractions were combined and purified. Fractions eluted with petroleum ether-ethyl acetate mixture (98:2) yielded a yellowish white solid designated as 1, which on recrystallization yielded a white solid, with an R_f of 0.45 on TLC in petroleum ether-ethyl acetate mixture (4:1). The higher fractions eluted with petroleum ether-ethyl acetate mixture (98:2) yielded a white solid designated as 2, which showed an R_f of 0.47 on TLC in petroleum ether-ethyl acetate mixture (3:2). Both the compounds (1&2) gave positive tests for steroids. Compound 3 was obtained from the column on elution with 100% ethyl acetate, as a yellow solid. It gave a dark grey colour with alcoholic $FeCl_3$ solution and it responded to Shinoda's test (Mg/HCl), giving a pale pink colour, indicating the presence of flavonoids with a chelated hydroxyl group. Thin layer chromatography of compound 3 showed an R_f of 0.65 when developed in methanol-ethyl acetate mixture. Compound 4 was obtained from the column on elution with ethyl acetate-methanol mixture (85:15) as a brown solid which on recrystallization with methanol yielded a white solid. Thin layer chromatography of compound 4 showed an R_f of 0.53 on developing with ethyl acetate-methanol mixture (1:1).

Instruments

Melting points (mp) were expressed in degree centigrade (°C) were determined on Mettler FP 51 apparatus (Mettler Instruments, Switzerland) and are uncorrected. A SHIMADZU, JAPAN-FT-IR spectrophotometer was used to record the IR spectra (4000–400

cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded in Bruker AV 500 (500MHz (^1H) and 125MHz (^{13}C)) spectrometer with tetramethylsilane (TMS) as an internal reference. The chemical shifts are expressed in parts per million (ppm). Mass spectra (MS) were recorded in Auto Spec EI+Shimadzu QP 2010 PLUS GC–MS and Clarus 500 (Auto System XL; Perkin-Elmer, USA) mass spectrometer. The UV-VIS spectra was recorded with UV-2400PC Series, China and Systronics, India model UV spectrometer. Shift reagents were added to the methanol solution of the flavonoidal compounds to study the shift in λmax in UV spectrum and correlate the position of the hydroxyl groups of flavone moiety. The solvent and the reagents used (reagent grade) were purified by standard methods.

Rotatory evaporator was used to dry the solution of organic extracts. Thin layer chromatography (TLC) was performed using glass plates coated with silica gel-G containing 13% calcium sulphate as binder. Ethyl acetate and petroleum ether were used as developing solvents. Spots were visualized under UV light at 254 nm and 365 nm and in iodine vapours. Separation and purification of the crude products was carried out using chromatographic column packed with activated silica gel (60–120 mesh). In case of mixture of solvents used for elution, the ratio of the solvent mixture is given in brackets.

RESULTS

The physical and spectral data of the isolated compounds are given below.

Compound (1)

White amorphous solid; Yield: 0.08%. Mp: 148 ^oC; IR ν_{KBr} cm^{-1}:3425(OH), 2949(C-H), 1454(CH_2), 962(=C-H); ^1H NMR (500MHz, $CDCl_3$, δ ppm) 0.71(3H,m), 0.85(3H,m), 0.95(3H,m), 1.04(3H,s), 3.51(1H,m), 5.05(1H,m), 5.18(1H,m), 5.38(3H,br. s);^{13}C NMR (125 MHz) $CDCl_3$, δ ppm): 37.27(C-1), 28.26(C-2), 71.83(C-3), 39.79(C-4), 140.77(C-5), 121.73(C-6), 31.68(C-7), 31.89(C-8), 50.15(C-9), 36.16(C-10), 21.09(C-11), 40.51(C-12), 42.32(C-13), 56.78(C-14), 24.31(C-15), 26.10(C-16), 56.08(C-17), 12.05 (C-18), 19.82(C-19), 36.52(C-20), 18.79(C-21), 138.32(C-22), 129.30(C-23), 45.83(C-24), 29.17(C-25), 19.04(C-26), 19.4(C-27), 23.08(C-28), 12.25(C-29). GC-MS 70 eV, m/z; 412(M^+) $C_{29}H_{48}O$, 369, 271, 255, 229, 199, 173, 159, 133, 119, 107, 95, 81, 69, 55, 43.

Compound (2)

White amorphous solid; Yield: 0.02%. Mp: 136 ^oC; IR ν $^{KBr}cm^{-1}$:3439(OH), 2949(C-H), 1456(CH_2), 962(=C-H); ^1H NMR (500MHz, $CDCl_3$, δ ppm) 0.71(3H,s), 0.85(3H,m), 0.95(3H,m), 1.04(3H,s), 3.51(1H,m), 5.38(3H,br. s);^{13}C NMR (125 MHz) $CDCl_3$, δ ppm): 37.27(C-1), 31.90(C-2), 71.83(C-3), 42.29(C-4), 140.77(C-5), 121.73(C-6), 31.68(C-7), 31.92(C-8), 50.13(C-9), 36.52(C-10), 21.09(C-11), 39.78(C-12), 42.32(C-13), 56.78(C-14), 24.31(C-15), 28.26(C-16), 56.07(C-17), 11.86 (C-18), 19.40(C-19), 36.15(C-20), 18.79(C-21), 33.97(C-22), 26.07(C-23), 45.86(C-24), 29.17(C-25), 19.83(C-26), 19.04(C-27), 23.08(C-28), 11.99(C-29). GC-MS 70 eV, m/z; 414(M^+) $C_{29}H_{50}O$, 396, 381, 329, 303, 273, 255, 231, 213, 173, 161, 145, 119, 107, 95, 81, 69, 55, 43.

Compound (3)

Yellow amorphous solid; Yield: 0.41%. Mp: 310 ˚C; IR (KBr) ν_{max} cm^{-1}:3408,3315,1664,1608,1521,1386,1261,1021,1010,819,680;^1H NMR (500MHz, DMSO, δ): H-3,10.85(1H,s), H-5, 12.47(1H,s), H-6, 6.18(1H. d,J=2.0Hz), H-7, 9.44(1H,s), H-8, 6.42(1H,d,J=2.0Hz), H_2', 7.66 (1H,d,J=2.0Hz), $H_3'$and H_4', 9.36(2H,s), H_5', 6.87(1H,d,J=8.5Hz), H_6', 7.54(1H,dd,J=8.5,2.0 Hz); ^{13}C NMR (125 MHz, DMSO, δ): C-2, 146.8, C-3, 135.7, C-4, 175.8, C-5, 160.7, C-6, 98.2, C-7, 163.8, C-8, 93.4, C-9, 156.1, C-10, 103.0, C_1', 121.9, C_2', 115.0, C_3', 145.0, C_4', 147.6, C_5', 115.6, C_6', 120.0. GC-MS 70 eV, m/z; 301.4(M+); $C_{15}H_{10}O_7$, 301.4.

Compound (4)

White amorphous solid; yield: 2.3%. Mp: 184-186 ˚C; IR (KBr) ν_{max} cm^{-1}:2800, 1672,1448,1361,1193, 1128, 1064, 939, 894, 580;^1H NMR (500MHz, DMSO, δ): 3.445 (3H,s), 2.97-3.016 (1H,m), 3.352(1H,br. s), 3.072-3.109 (2H, br. s), 3.673-3.679 (1H,q), 3.194-3.232 (1H, m), 4.52(1H,m), 4.58(1H,m), 4.38(1H, m), 4.643-4.652(1H, m), 4.54(1H,m); ^{13}C NMR (125 MHz, DMSO, δ):C-1, 83.9, C-2, 75.0, C-3, 73.4, C-4, 72.0, C-5, 73.4, C-6, 71.7, C-Methoxy, 60.2, EI-MS, m/z; 194(M+) calculated for $C_7H_{14}O_6$.

DISCUSSION

The IR spectrum of the compound (1) showed characteristic absorptions for hydroxyl group (3425 cm^{-1}). The mass spectrum of the compound showed molecular ion peak at m/z 412 associated with the molecular formula $C_{29}H_{48}O$. The ^1H NMR spectrum of the compound (1) showed the presence of six methyl groups that appeared at δ 0.71, 0.85, 0.95 and 1.04 respectively. The multiplet that appeared at δ 3.51 indicated the hydroxyl proton at C-3 carbon. The olefin protons were exhibited at δ 5.38, 5.18 and 5.05. The two olefin protons which appeared at δ 5.05 and 5.18 have been attributed to the H-23 and H-22 respectively of the steroid side chain.

The third olefin proton that resonated at δ 5.38 was assigned to the olefin proton at H-6. The ^{13}C NMR spectrum of the compound (1) indicated twenty nine carbon signals. Olefin carbons were seen at 140.23(C-5), 121.67(C-6), 138.04(C-22) and 129.15(C-23). The DEPT 90 spectra of compound (1) showed eleven signals indicating the presence of eleven methylene carbons in compound (1). The carbinolic carbon was seen at δ 71.83(C-3). The DEPT-135 spectra of compound (1) showed seventeen positive signals and nine negative signals indicating the presence of nine methane carbons and six methyl carbons in compound (1). The ^1H-^1H COSY spectrum expressed the proton connectivity and the HSQC experiment expressed the expected connectivity between carbons and their respective protons.

The presence of steroid skeleton was confirmed by the ^{13}C NMR signals at δ 129.15 and 138.04 characteristic of Δ[22] sterol and at δ 140.23 and 121.67 characteristic of Δ^5 sterol. Based on preliminary tests and spectral data and in comparison (table 1) with the literature data [24, 25], compound (1) has been identified as the known compound stigmasterol and its structure is given in fig. 1.

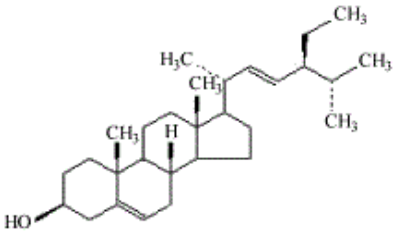

Fig. 1: Stigmasterol

The IR spectrum of compound (2) showed characteristic absorption for hydroxyl group (3439 cm^{-1}). The mass spectrum of the compound showed molecular ion peak at m/z 414 associated with the molecular formula $C_{29}H_{50}O$. The ^1H NMR spectrum of compound (2) showed the presence of six methyl protons that appeared at δ 0.71, 0.85, 0.95 and 1.04. The multiplet that appeared at δ 3.52-3.56 indicated the hydroxyl proton at C-3 carbon. The peak resonated at δ 5.38 and was assigned to olefin proton at H-6. The ^{13}C NMR indicated twenty nine carbon signals. Olefin carbons were seen at δ 140.77(C-5) and 121.73(C-6). The hydroxyl bearing carbon was seen at δ 71.83(C-3). Based on its spectral data and comparison of its physical properties and spectroscopic data with that of compound (2) with literature values (table 1), the compound (2) was identified as the known compound β-sitosterol [24, 25], and its structure is given in fig. 2.

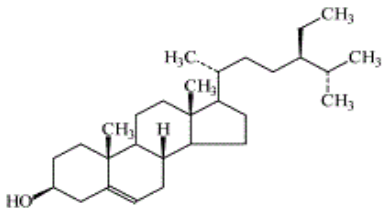

Fig. 2: β-sitosterol

Table 1:^{13}C NMR chemical shifts δ of compounds 1and 2

Carbon number	Chemical Shift δ ppm			
	Compound1	Literature data alma *et al.,* (1996)	Compound2	Literature data alma *et al.,* (1996)
C-1	37.3	37.06	37.3	37.36
C-2	28.3	31.64	31.9	31.90
C-3	71.8	71.77	71.8	71.77
C-4	39.8	41.50	42.3	42.29
C-5	140.8	140.23	140.8	140.75
C-6	121.7	121.69	121.7	121.69
C-7	31.7	31.72	31.7	31.65
C-8	31.9	31.95	31.9	31.93
C-9	50.2	50.10	50.1	50.13
C-10	36.2	36.72	36.5	36.50
C-11	21.1	20.80	21.1	21.09
C-12	40.5	39.59	39.8	39.78
C-13	42.3	42.50	42.3	42.32
C-14	56.8	56.67	56.8	56.77
C-15	24.3	24.01	24.3	24.31
C-16	26.1	28.55	28.3	28.26
C-17	56.1	55.85	56.1	56.06
C-18	12.1	11.61	11.9	11.86
C-19	19.8	19.23	19.4	19.40
C-20	36.5	40.13	36.2	36.15
C-21	18.8	20.48	18.8	18.79
C-22	138.3	138.04	34.0	33.95
C-23	129.3	129.15	26.1	26.07
C-24	45.8	51.06	45.9	45.83
C-25	29.2	32.00	29.2	29.16
C-26	19.0	19.00	19.8	19.83
C-27	19.4	21.21	19.0	19.04
C-28	23.1	25.41	23.1	23.07
C-29	12.3	12.00	12.0	11.99

The IR spectrum of the compound (3) showed an absorption band at 3408 cm^{-1} due to hydroxyl group. Another band at 1664 $cm^{-1}$is attributed to stretching frequency of carbonyl group. The UV spectrum of the compound (3) shows an absorption maximum at 370 nm characteristics of flavones. By the use of shift reagents, the position of hydroxyl groups was ascertained. A shift 60 nm was observed in the UV band on addition of $AlCl_3$. This suggested that the carbonyl group of the compound is chelated with a hydroxyl group at C-3 position. Addition of sodium acetate shifts the absorption maxima by 10 nm indicating an unsubstituted C-7 hydroxyl group. The ^{1}H NMR spectrum of the compound (3) revealed the presence of a chelated hydroxyl signal at δ 12.47. A broad singlet at δ 9.36 which integrated for two protons is characteristic of C_3'and C_4'hydroxyl groups. Two singlet's at δ 6.18 and 6.42 which integrated for one proton each are characteristic of phenyl ring protons of flavonoid nucleus (H-6 and H-8). Two doublets at δ 7.66 and δ 6.87 were attributed to H_2'and H_5'protons. The signal appeared as a doublet of doublet at δ 7.54 is due to C_6' protons. The ^{13}C NMR spectrum showed fifteen signals. The signal at δ 175.78 implies characteristic absorption of carbonyl carbon at C-4. The signal at δ 146.79 was indicative of C-2 carbon of flavonoid. The other characteristic signals were observed at δ 156.11 and δ 102.96 and attributed to C9 and C10 carbons. The phenolic carbons were seen at δ 135.65, 160.65, 163.83, 145.0 and 147.64 and assigned respectively to C-3, C-5, C-7, C_3'and C_4' of the flavonoid skeleton.

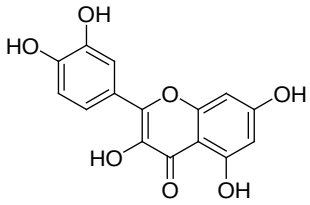

Fig. 3: Quercetin

Table 2: ^{13}C NMR chemical shifts δ of compounds 3 and 4

Carbon number	Chemical shift δ ppm			
	Compound3	Literature data wagner *et al.* (1976)	Compound 4	Literature data narda *et al.* (2008)
C-1	-	-	83.87	83.80
C-2	146.79	146.9	75.01	70.00
C-3	135.65	135.8	73.37	71.90
C-4	175.78	175.9	72.04	72.48
C-5	160.65	160.8	73.39	70.90
C-6	98.16	98.3	71.71	72.50
C-7	163.83	164.0	60.15(OCH_3)	59.63(OCH_3)
C-8	93.35	93.5	-	-
C-9	156.11	156.2	-	-
C-10	102.96	103.1	-	-
C-11	121.92 C_1'	122.1	-	-
C-12	115.0 C_2'	115.2	-	-
C-13	145.0 C_3'	145.1	-	-
C-14	147.64 C_4'	147.7	-	-
C-15	115.55 C_5'	115.7	-	-
C-16	119.99 C_6'	120.1	-	-

The DEPT 90 and DEPT 135 spectra that showed five signals indicated the presence of five methylene groups C-6,C-8, $C_{2'}$, $C_{5'}$and $C_{6'}$respectively. The ^{1}H-^{1}H COSY spectrum showed the correlation between protons signals. The HSQC spectrum established the connectivity between the carbons and respective protons. The HMBC spectrum showed the 3-bond correlations (table 1). The mass spectrum gave the molecular ion peak at 301.4 associated with the molecular formula $C_{15}H_{10}O_7$. Based upon the above spectral details and comparing the spectral data with literature [26], the compound (3) was identified as the known compound Quercetin (3, 5, 7, 3', 4'- pentahydroxyflavone) and its structure is given in fig. 3.

The compound (4) did not answer any color tests meant for the common class of natural products. It was also negative to Lassaigne's test for nitrogen. The compound did not show good solubility in organic solvents like chloroform, acetone etc. But it was soluble in water and DMSO thus indicating the high polar nature of the compound. Its IR spectrum indicated an intense absorption around 2800-2900 cm^{-1} due to considerable C-H stretching absorption which revealed the organic nature of the molecule. However, the IR spectrum lacked any carbonyl absorption.

The ^{1}H NMR spectrum of the compound (4) was recorded in a high resolution NMR spectrometer operating at 500 MHz frequency. The spectrum expressed very clear and conspicuous signals. It showed a three proton singlet at δ 3.45 indicating the presence of a methoxyl group. Further, a group of 5 signals were observed in the downfield region of δ 4.37 to δ 4.65 integrating totally to 5 protons. Each of these signals was a clear intense doublet. In the upfield region of δ 2.97-3.68 a group of 5 signals appearing as three multiplets and two intense broad signals were observed.

The ^{13}C NMR spectrum of compound (4) revealed seven distinct signals. One signal appeared up field at δ 60.15 and another at a distinct downfield position of δ 83.86. The other signals appeared as a group of 5 lines at δ 72.04, 73.39, 73.36, 71.71 and 75.09 (Table1). This region corresponds to absorption of carbinolic carbons. Thus the ^{13}C NMR spectrum indicated that the compound (4) possesses 7 carbons. The ^{1}H-^{13}C HETCOR (HSQC) spectrum indicated the correlation between carbons and the attached proton. The signal at δ 60.15 correlated with methoxy proton signal at δ 3.45. The proton signal at δ 3.07-3.11 correlated with the downfield carbon signal at δ 83.87 and also to the carbon signal at δ 72.04. The group of 5 signals in the downfield region did not show any contour. Hence they may be protons attached to hetero atoms which should be oxygen atom in this case be 5 hydroxyl substituent's one on each of the 5 carbons giving the signals at δ 72.04, 73.39, 73.36, 71.71 and 75.09. The ^{1}H-^{1}H COSY spectrum revealed that the signal at δ 3.07-3.11 due to two methine protons one on each carbon is flanked by the signals at δ 2.97-3.02 and δ 3.19-3.23 and is correlated to both signals. The COSY spectrum also clearly reveals the splitting interaction of hydroxyl proton signals with the geminally attached protons of 5 carbons. The HMBC spectrum assisted in confirming the assigned positions of carbons and hydrogens in the compound (4). It reveals 2 bond and 3 bond connectivity as contours and suppresses one bond interactions in the spectrum.

A thorough analysis of the COSY and HETCOR (HSQC) spectra helped in unambiguously assigning the NMR signals to the respective protons and subsequently to the respective carbons. Thus it may be proposed that the compound (4) possesses five–CHOH groups and one methoxyl group is attached to a methine carbon. Based on this and by comparison with literature [27] the structure of compound (4) was established as that of a pinitol.

The cyclitols are sugar alcohol type of molecules and some of them have significant bio activity. The physical properties, TLC analysis and a thorough spectral analysis of compound (4), led to its characterization as the bioactive molecule Pinitol (Fig.4). The spectral data were also compared with that reported for pinitol in recent literature. This molecule has been primarily identified as a safe and non toxic anti diabetic agent. It mimics insulin in action. The present isolation of this molecule from the fruit peel of *P. dulce* more than substantiates the anti diabetic potential of the extracts of this plant material. There are also ethno reports on the use of the aqueous extract of the fruit peel in alleviating diabetes conditions. It is found to control high blood sugar.

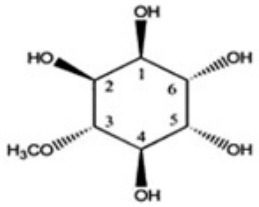

Fig. 4: Pinitol

It is noteworthy that, (+) pinitol, which is well known for its anti-diabetic [28], anti-inflammatory [29], and anti-asthmatic [30] potential, has been isolated for the first time from the fruit peel of *P. dulce*. Pinitol is used in the treatment of diabetes mellitus and related chronic complications-obesity, hyperlipidemias, dyslipidemias, atherosclerosis, hypertension, cardiovascular disorders, AIDS, cancer, malnutrition, stress, lupus, autoimmune disorders, endocrine disorders and complications arising from athletic activity or from inactivity.

CONCLUSION

The isolation of four compounds from the fruit peels of *P. dulce* and the same have been characterized as medicinally valuable molecules such as stigmasterol, sitosterol, quercetin and pinitol. These molecules have been reported for their pharmacological potential especially the anti-diabetic activity. The present study has exposed medicinally valuable molecules from a fruit peel which is usually an agro waste for the first time. The isolation of pinitol from the fruit peel of the common plant *Pithecellobium dulce* for the first time bestows immense significance to the study owing to the enormous pharmacological potential of pinitol. Thus the characterization of anti-diabetic metabolites of *Pithecellobium dulce* Benth fruit peel is a viable drug target for diabetes mellitus.

ACKNOWLEDGMENT

We thank Avinashilingam University for Women, Coimbatore-43 and the Management of K. S. Rangasamy College of Technology, Tiruchengode, India, for providing support and infrastructural facilities. Support in documentation work rendered by Ms. K. Prabha, and Ms. M. Umai Arasi are kindly acknowledged.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Nigam SK, Gupta RK, Mitra CR. *Pithecellobium dulce*. I. Isolation and characterization of the constituents of the legume. J Pharm Sci 1962;52:459-62.
2. Nigam SK, Mitra CR. *Pithecellobium dulce*. IV. Constituents of flowers, heartwood, and root bark. Planta Med 1968;163:335-7.
3. Nigam SK, Mitra CR. *Pithecellobium dulce*. V. Chemistry of the seed saponin and constituents of the leaves. Planta Med 1970;18:44-50.
4. Adinarayana D, Ramachandraiahchetty P. Chemical investigation of some medicinal plants occurring in southern India. Indian J Chem 1985;24B:453.
5. Zapesochnaya GG, Yarosh EA, Syanidze NV, Yarosh GI. Flavanoids of the leaves of *Pithecellobium dulce*. Khim Prir Soedin 1980;2:252-3.
6. Sahu NP, Mahato SB. Anti-inflammatory triterpene saponins of *Pithecellobium dulce*: Characterization of echinocystic acid bisdesmoside. Phytochemistry 1994;375:1425-7.
7. Nigam SK, Misra G, Uddin R, Yoshikawa K, Kawamoto M, Arihara S. Pithedulosides A-G, Oleanane glycosides from *Pithecellobium dulce*. Phytochemistry 1997;44:1329-34.

8. Yoshikawa K, Suzaki Y, Tanaka M, Arihara S, Nigam SK. Three acylated saponins and a related compound from *Pithecellobium dulce.* J Nat Prod 1997;6012:1269-74.
9. Saxena VK, Singal M. Geinstein 4'-O-alpha-L rhamnopyranoside from *Pithecellobium dulce.* Fitoterapia 1998;694:305-6.
10. Niranjan PS, Kazuo K, Zhonghua J, Sukdeb B, Basudeb A, Tamotsu N. A minor acylated triterpenoid saponin from the seeds of *Pithecellobium dulce*. J Chem Res 1999;32:558-9.
11. Banerjee A. Studies on alkylated resin from *Pithecellobium dulce*. J Indian Chem Soc 2005;82:186-7.
12. Shanmugakumaran SD, Amerjothy S, Balakrishna K. Pharmacognostical, Antibacterial and antifungal potentials of the leaf extracts of *Pithecellobium dulce* Benth. Pharmacogn Mag 2006;72:163-7.
13. Chandran PGR, Balaji S. Phytochemical investigation and pharmocological studies of the flowers of *Pithecellobium dulce.* Ethnobotanical Leaflets 2008;12:245-53.
14. Sugumaran M, Vetrichelvan T, Darline Quine S. Anti-diabetic potential of aqueous and alcoholic leaf extracts of *Pithecellobium dulce.* Asian J Res Chem 2009;2:83-5.
15. Nagmoti DM, Kothavade PS, Bulani VD, Gawali NB, Juvekar AR. Antidiabetic and antihyperlipidemic activity of *Pithecellobium dulce* (Roxb.) Benth seeds extract in streptozotocin-induced diabetic rats. Eur J Integrative Med 2015;7:263–73.
16. Katekhaye DS, Nagmoti DM. α-Glucosidase and α-amylase inhibitory activities of *Pithecellobium dulce* bark and leaves. Phytopharmacology 2013;4:123-30.
17. Sukantha TA, Subashini KS, Ravindran NT, Balashanmugam P. Evaluation of *in vitro* antioxidant and antibacterial activity of *Pithecellobium dulce* Benth fruit peel. Int J Curr Res 2011;3:378-82.
18. Sukantha TA, Subashini KS, Ravindran NT. Antibacterial activity of selected medicinal plant in traditional treatment of wound infection in Southeast India. Int J Pharm Pharm Sci 2014;6:511-3.
19. Kim MJ, Yoo KH, Kim JH, Seo YT, Ha BW, Kho JH, *et al*. Effect of pinitol on glucose metabolism and adipocytokines in uncontrolled type 2 diabetes. Diabetes Res Clin Pract 2007;77 Suppl 1:S247-51.
20. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia 2009;80:123-6.
21. Thamaraiselvan R, Natarajan Nandakumar, Maruthaiveeran PB. D-pinitol a low-molecular cyclitol prevents 7,12-Dimethylbenza. anthracene induced experimental breast cancer through regulating anti-apoptotic protein Bcl-2, mitochondrial and carbohydrate key metabolism enzymes. Biomed Nutr 2012;2:25-30.
22. Singh RK, Pandey BL, Tripathi M, Pandey VB. Anti-inflammatory effect of (+)pinitol. Fitoterapia 2001;72:168-70.
23. Kim JI, Kim JC, Kang MJ, Lee MS, Kim JJ, Cha IJ. Effects of pinitol isolated from soybeans on glycaemic control and cardiovascular risk factors in Korean patients with type II diabetes mellitus: a randomised controlled study. Eur J Clin Nutr 2005;56:456-8.
24. Alma MS, Chopra N, Ali M, Niwa M. Oleanen and sitgmasterol derivatives from *Ambroma augusta*. Phytochem 1996;414:1197-200.
25. Kamboj A, Saluja AK. Isolation of Stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (*Asteraceae*). Int J Pharm Pharm Sci 2011;3:94-6.
26. Wagner H, Chari VM, Sonnenbichler J. C-13 NMR spectra of natural flavonoids, Tetrahedron Lett 1976;21:1799-802.
27. Narda Blanco, Yonny Flores, Giovanna R Almanza. Secondary metabolites from *Senna versicolor*. Rev Boliv Quim 2008;25:36-42.
28. Narayanan CR, Joshi DD, Mujumdar AM, Dhekne VV. Pinitol-a new anti-diabetic compound from the leaves of *Bougainvillea spectabilis.* Curr Sci 1987;563:139-41.
29. Singh RK, Pandey BL, Tripathi M, Pandey VB. Anti-inflammatory effect of (+)-pinitol. Fitoterapia 2001;72:168-70.
30. Lee JS, Jung ID, Jeong YI. D-Pinitol inhibits Th1 Polarization via the suppression of dendritic cells. Int Immunopharmacol 2007;7:791-804.