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SYNTHESIS AND EVALUATION OF PIPERONAL CHALCONE AND ITS DERIVATIVES AS ANTI-DIABETIC AGENTS

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ABSTRACT
A series of novel chalcone-piperonal derivatives was synthesized by reaction between 2-chloro-5-acetyl pyridine with heterocyclic aldehyde piperonal in presence of alkali using Claisen-Schmidt reaction. These chalcones on cyclocondensation with hydrazine hydrate in presence of glacial acetic acid give acetyl pyrazolines and also on cyclocondensation with isoniazid in presence of alkali give pyridyl pyrazolines. The synthesized chalcones were screened for their in-vitro antidiabetic activity. The structures of newly synthesized compounds have been established on the basis of their elemental analysis, IR, H^1 and C^{13} NMR spectral data.

KEYWORDS: Cyclocondensation, piperonal, anti-diabetic activity and chalcone.

INTRODUCTION
Piperonal is one of the oxygen containing heterocyclic compound. It is a heterocyclic ring system consisting of a benzene ring fused to a pyran ring. Piperonal, a naturally occurring derivative of piperine compound (the pyrrolidine amide of piperic acid) is an aromatic heterocyclic aldehyde. The choice of piperonal for the aldehyde moiety in chalcone, stemmed from the fact that many compounds containing the 3,4-methylenedioxy group have some biological activity.[1,2] Heterocyclic chemistry is a branch which provides our most of the basic needs. The efficiency of heterocyclic compounds and its derivatives as pharmaceutical and agrochemicals industries are well established.

Chalcone is a class of open-chain flavonoids that is not only biosynthesized by plants but also can be prepared synthetically. Chalcones are secondary metabolites of terrestrial plants, precursors for the biosynthesis of flavonoids, exhibit various biological activities. The Chalcone derivatives are to be used as an important intermediate and also act as precursor for the synthesis of novel cyanopyridines, pyrazolines, isoxazoles, pyrimidines and tetrazole.[3,5]

Chalcones have been reported to posses many useful properties like anticancer[6], anti-inflammatory[7], antimalarial[8], antibacterial[9], antifungal[10] and anti–diabetic activity.[11]

Pyrazolines are an important class of heterocyclic compounds containing two nitrogen atoms were present in adjacent position in the five membered ring. Pyrazoles are the structural isomers of imidazole and pyrazolines are the reduced forms of the pyrazoles. Pyrazoline derivatives are the electron rich nitrogen heterocycles which showed a wide spectrum of biological activities.[12,22] Method for the synthesis of pyrazoline compounds pharmacological from α, β-unsaturated carbonyl compounds (chalcones) is by the cyclization with hydrazine hydrate/substituted hydrazine.

According to reports from the World Health Organization (WHO), around 250 million people are currently living with diabetes. Consequently, the prevention and treatment of Diabetes mellitus (DM) is considered a globally challenging problem. Prevention and control of diabetes with diet, weight control and physical activity are difficult tasks. Treatment of type 2 diabetes mellitus (T2DM) has centered on increasing blood insulin levels, either by direct insulin administration or using oral drugs that promote insulin secretion, decrease insulin resistance, or reduce the rate of carbohydrate absorption from the gastrointestinal tract. Inhibition of α-glucosidase leads to the delay or reduction of increased postprandial blood glucose levels. Thus, α-glucosidase inhibitors have been proposed as a potential therapeutic target for drug discovery in the treatment of T2DM.[23,24] The present study was carried out to synthesize and to screen the newly developed compounds for its anti-diabetic activity.

MATERIALS AND METHODS

All the reagents and solvents for synthesis were obtained from Alfa Aesar and Sigma-Aldrich chemicals. Materials to be used for this experiment are analytical grade. Melting point of the compounds was determined in open capillaries, using Eligo digital melting point apparatus and expressed in degree Celsius and the values were uncorrected. The FT-IR spectra were recorded on a Shimadzu 8201spectrophotometer using KBr and the values are expressed in 4000-400 cm-1. ^{1}H-NMR and ^{13}C- NMR spectra were recorded using a Bruker AV 400 MHz spectrometer and chemical shifts are expressed as δ (ppm) with $SiMe_4$ as internal standard when measured in $CDCl_3$ or DMSO-d_6. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), m (multiplet), and q (quartet). Merck silica gel 60F-254 plates were used for analytical TLC (Merck). The purity of the compound was checked by TLC using silica gel plates with ethyl acetate and n-hexane (3:2) as the solvent system. Spots were visualized using iodine chamber.

Synthesis

General procedure for the preparation of (2*E*)-3-(2*H*-1,3-benzodioxol-5-yl)-1-(6-chloropyridin-3-yl)prop-2-en-1-one (III)

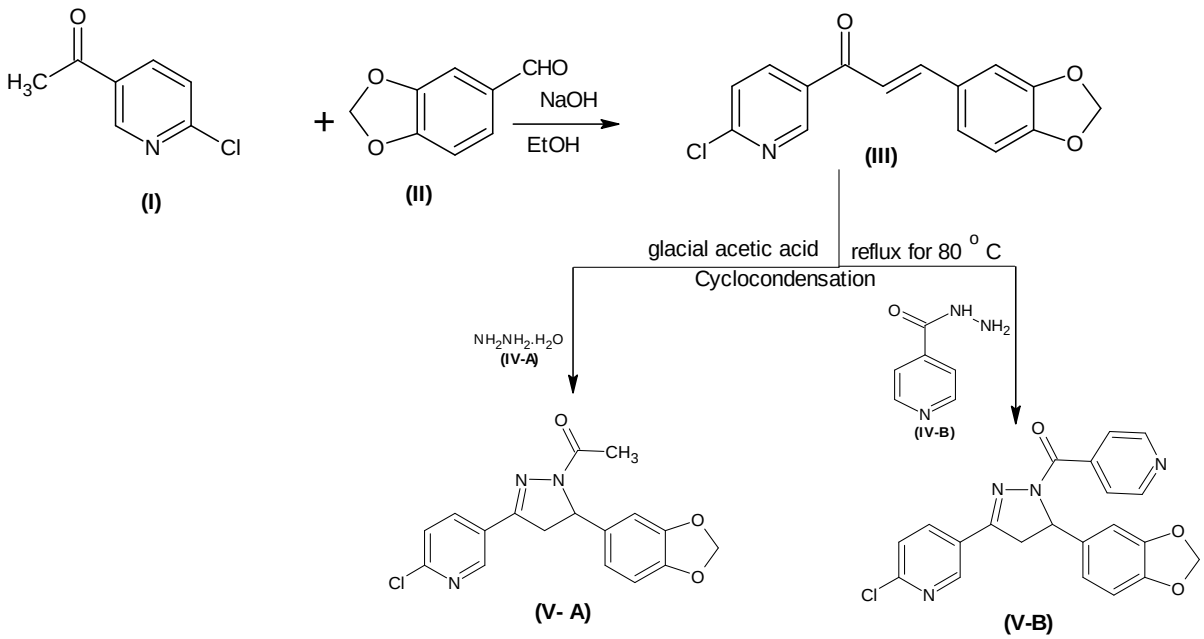
Chalcone were prepared by base catalyzed condensation of a equimolar quantity of the 2-chloro-5-acetyl pyridine **I** (0.01 mol) and piperonal **II** (0.01 mol) in ethanol as a solvent was taken in a 100 mL of RB flask. A solution of 40% NOH (10 mL) solution was added drop wise to this solution with stirring for 5 hrs at 0-5^{0}C. The resulting mixture allowed remaining for 24 hours at room temperature. After completion, content was poured into crushed ice and acidify with conc. HCl. Brown yellow colour solid thus obtained was separated by filtration & recrystallized from proper solvent to get chalcone **III**.

1-[5-(2*H*-1,3-benzodioxol-5-yl)-3-(6-chloropyridin-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (V-A)

The above obtained (2*E*)-3-(2*H*-1,3-benzodioxol-5-yl)-1-(6-chloropyridin-3-yl)prop-2-en-1-one **III** (0.01 mol) and hydrazine hydrate **IV-A** (0.01 mol) were refluxed in 30ml ethanol and after half an hour add catalytic amount of glacial acetic acid and reflection was continued for 10 hrs. The reacting mixture was cooled and poured into crushed ice, the solid product was precipitated out, and this was washed twice with cold water and recrystallized from ethanol and give the product **V-A.**

[5-(2*H*-1,3-benzodioxol-5-yl)-3-(6-chloropyridin-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](pyridin-4-yl)methanone (V-B)

The above obtained (2*E*)-3-(2*H*-1,3-benzodioxol-5-yl)-1-(6-chloropyridin-3-yl)prop-2-en-1-one **III** (0.01 mol) and isoniazid **IV-B** (0.01 mol) were refluxed in 30ml ethanol and after half an hour add catalytic amount of glacial acetic acid and reflection was continued for 12 hrs. The reacting mixture was cooled and poured into crushed ice, the solid product was precipitated out, and this was washed twice with cold water and recrystallized from ethanol and give the product **V-B.**

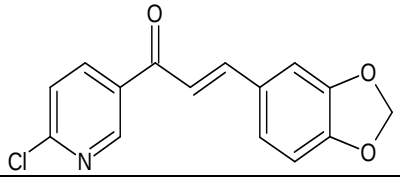
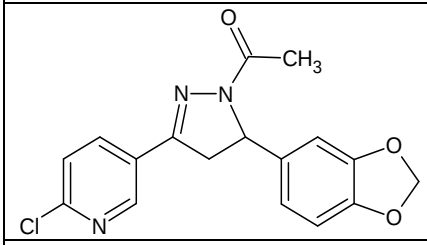
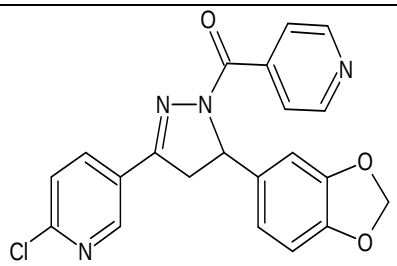


Scheme for the synthetic route of piperonal chalcone and its derivatives.

RESULTS AND DISCUSSION

All the newly synthesized compounds III, V-a, V-b have been characterized by their melting points, CHN analysis and spectroscopic methods such as IR, ^{1}H and ^{13}C-NMR. The results discussed are given below. The physical characteristics data of the synthesized compound was given in the Table-I.

Table – I: Physical Characterization data piperonal chalcones derivatives.

Structure	Molecular Formula	Mol. Wt.	M.P	R_f Value	Elemental Analysis (%)		
					C	H	N
	$C_{15}H_{10}ClNO_3$	287.69	170	0.68	62.62	3.50	12.32
	$C_{17}H_{14}ClN_3O_3$	343.76	210	0.78	59.40	4.10	12.22
	$C_{21}H_{15}ClN_4O_3$	406.82	278	0.72	62.00	3.72	13.77

(2*E*)-3-(2*H*-1,3-benzodioxol-5-yl)-1-(6-chloropyridin-3-yl)prop-2-en-1-one (III)

IR: 1645 Cm^{-1} (C=O), 3540 Cm^{-1} (NH), 1440, 1560, 1678 Cm^{-1} (C=C), 1360 Cm^{-1} (C-N), ^{1}HNMR (300 MHz, DMSO) ppm: 8.2 (s, 1H, -NH-C=O), 6.71-7.44 (m, 15H, aromatic), 9.2 (s, 1H, pyridyl proton), 2.2 (s, 1H, methine), ^{13}CNMR (300 MHz, DMSO) ppm: 161.3 (C=O), 114.3-126.1 (phenyl carbons), 137.5 (pyridyl carbon), 139.2 (methine carbon).

1-[5-(2*H*-1,3-benzodioxol-5-yl)-3-(6-chloropyridin-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (V-A)

IR: 1635 Cm^{-1} (C=O), 3345 Cm^{-1} (NH), 1480, 1450, 1568 Cm^{-1} (C=C), 1330 Cm^{-1} (C-N), ^{1}HNMR (300 MHz, DMSO) ppm: 3.4 (s, 1H, C-NH-C), 6.55-7.45 (m, 15H, aromatic), 9.4 (s, 1H, pyridyl proton), 2.2 (s, 1H, methylene), ^{13}CNMR (300 MHz, DMSO) ppm: 160.3 (C=O), 120.3-123.9 (phenyl carbons), 137.5 (pyridyl proton), 40.2 (methine carbon).

[5-(2*H*-1,3-benzodioxol-5-yl)-3-(6-chloropyridin-3-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](pyridin-4-yl)methanone (V-B)

IR: 1545 Cm^{-1} (C=O), 3480 Cm^{-1} (NH), 1450, 1490, 1568 Cm^{-1} (C=C), 1340 Cm^{-1}(C-N), ^{1}HNMR (300 MHz, DMSO) ppm: 3.6 (s, 1H, -NH-C), 6.41-7.64 (m, 15, aromatic), 9.4 (s, 1H, pyridyl proton), 1.4 (s, 1H, methylene), ^{13}CNMR (300 MHz, DMSO) ppm: 165.3 (C=O), 112.3-128.1 (phenyl carbons), 147.5, 142.3 (pyridyl carbons), 41.2 (methine carbon).

IN-VITRO ANTI DIABETIC ACTIVITY METHODOLOGY

Non-enzymatic glycosylation of haemoglobin method

Antidiabetic activity of given samples were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. 25, 50 and 100 µg/ml of each samples were added to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Metformin was used as a standard drug for assay. % inhibition was calculated.[25,26]

$$\% \text{ inhibition} = \frac{As - Ac}{As} \times 100$$

Glucose uptake in Yeast cells method

The commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (25, 50 and 100 µg/ml) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Glinose was taken as standard drug.[27,28]

α- Amylase Inhibition method

1ml of substrate-potato starch (1% w/v), 1ml of drug solution (GLINIL drug/ethanol extract) of 4 different concentrations such as 25, 50 and 100 µg/ml. 1ml of α-amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2pH) was added. The mixture was incubated for 1hr. then 0.1 ml iodine-iodide indicator (635mg iodine and 1gm potassium iodide in 250ml distilled water) was added in the mixture. Absorbance was taken at 565nm in UV-Visible spectroscopy.[29,30]

The percentage increase in glucose uptake by yeast cells and % of α- amylase inhibition were calculated using the following formula-

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Table-II: Non enzymatic glucosylation of haemoglobin method.

SAMPLE		Concentrations (µg/ml)		
	Abs	25 µg/ml	50 µg/ml	100 µg/ml
Blank	0.076±0.002	% of inhibition	% of inhibition	% of inhibition
STANDARD		66.5	82.7	88.3
III		68.9	73.7	80.4
V-A		55.1	68.0	73.7
V-B		40.8	48.5	54.9

Table-III: Glucose uptake in yeast cells.

SAMPLE		Concentrations (µg/ml)		
	Abs	25 µg/ml	50 µg/ml	100 µg/ml
Blank	0.136±0.020	% of inhibition	% of inhibition	% of inhibition
STANDARD		80.5	83.7	90.0
III		77.5	86.5	88.9
V-A		63.4	67.2	72.6
V-B		65.3	69.5	72.6

Table-IV: α -Amylase Inhibition Method.

SAMPLE		Concentrations (µg/ml)		
	Abs	25 µg/ml	50 µg/ml	100 µg/ml
Blank	0.136±0.020	% of inhibition	% of inhibition	% of inhibition
STANDARD		72.6	77.5	88.0
III		76.3	82.1	84.2
V-A		63.4	67.2	72.6
V-B		67.2	72.6	79.1

CONCLUSION

In this present work an attempt has been made to synthesize some novel piperonal derivatives and to study their invitro anti-diabetic activity. Both analytical and spectral data (IR, MS, ^{1}HNMR) of all the synthesised compounds were in full agreement with the synthesized structure. The results of this study revealed that most of the compounds showed moderate to good anti-diabetic activity. Structure and biological activity relationship of the title compounds showed the electron donating groups enhanced the activity.

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REFERENCE

1. Vidhya KR, Syed Shafi S. Synthesis characterization and antimicrobial studies of novel 2-pyrozoline derivatives. Der Pharma Chemica, Scholar research library, 2014; 6(2): 283-287.
2. Vidhya KR, Syed Shafi S. Synthesis, Characterization and Antimicrobial studies of novel 2-Pyrazoline derivatives. Int. J. of Chem. Tech. Research, 2014; 6(4): 2558-2563.
3. Nebras M.Jamel, Dhuha F. Hussein, Jumbad H.Tomma. Synthesis and Characterization New Schiff Bases, Pyrazole and Pyrazoline Compounds Derived From Acid Hydrazide Containing Isoxazoline Ring. Ibn Al-Haitham Jour. for Pure & Appl. Sci., 2014; 27(3): 435-447.
4. Elarfi M, Al-Difar, H. Synthesis of some heterocyclic compounds derived from Chalcones. Scientific Reviews and Chemical Communications, 2012; 2(2): 103-107.
5. Selvakumar S, Babu I, Chidambaranathan N. Pharmacological evaluation of some potent 2-substituted benzimidazolyl chalcones for analgesic, antiinflammatory, anthelmintic and central

nervoussystem activities. International Journal of Phytopharmacology, 2012; 3(2): 163-172.
6. Syam S, Abdelwahab SI, Al-Mamary MA, Mohan S. Synthesis of chalcones with anticancer activities. Molecules, 2012; 17: 6179–6195.
7. Motta LF, Gaudio AC, Takahata Y. Quantitative structure–activity relationships of a series of chalcone derivatives (1,3-Diphenyl-2-propen-1-one) as anti plasmodium falciparum agents (anti malaria agents). Internet Electron J. Mol. Des., 2006; 5: 555–569.
8. Yadav HL, Gupta P, Pawar RS, Singour PK, Patil UK. Synthesis and biological evaluation of anti–inflammatory activity of 1,3 diphenyl propenone derivatives. Med. Chem. Res, 2011; 20: 461–465.
9. Asrar Ahamed A, Mohamed Sihabudeen M. Synthesis and biological evaluation of some novel heterocyclic Chalcone derivatives. Asian J. of Pharmacy and Pharmacology, 2017; 3(6): 247-250.
10. Lahtchev KL, Batovska DI, Parushev SP, Ubiyvovk VM, Sibirny AA. Antifungal activity of chalcones: A mechanistic study using various yeast strains. Eur. J. Med. Chem., 2008; 43: 2220–2228.
11. Hsieh CT, Hsieh TJ, El–Shazly M, Chuang DW, Tsai YH, Yen CT, Wu SF, Wu YC, Chang FR. Synthesis of chalcone derivatives as potential anti–diabetic agents. Bioorg. Med. Chem. Lett, 2012; 22: 3912–3915.
12. Mohamad Yusuf, Payal Jain. Synthetic and biological studies of pyrazolines and related heterocyclic compounds. Arabian Journal of Chemistry, 2014; 7: 553–596.
13. Johnson M, Younglove B, Lee L, LeBlanc R, Holt H Jr. Design, synthesis and biological testing of pyrazoline derivatives of combretastatin-A4. Bioorg Med Chem Lett, 2007; 17: 5897-5901.
14. Sameena B, Kalim J, Shamim A, Rathish IG, Surender S, Alam MS. Synthesis and biological evaluation of some new 2-pyrazolines bearing benzene sulfonamide moiety as potential anti-inflammatory and anti-cancer agents. Eur J Med Chem, 2011; 46: 5763-5768.
15. Ratkovic Z, Juranic ZD, Stanojkovic T, Manojlovic D, Vukicevic RD, Radulovic N, Joksovic MD. Synthesis, characterization, electrochemical studies and antitumor activity of some new chalcone analogues containing ferrocenyl pyrazole moiety. Bioorg Chem, 2010; 38: 26-32.
16. Rafia B, Syed O, Shafiya Y, Hinna H, Alam MS, Mohammad S, Surender S, Kalim J. Synthesis of some new 1, 3, 5-trisubstituted pyrazolines bearing benzene sulfonamide as anticancer and anti-inflammatory agents. Bioorg Med Chem Lett, 2011; 21: 4301-4305.
17. Zuhal OH, Burak KBGX, Unsal C, Alisx A, Altan B. Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. Eur J Med Chem, 2007; 42: 373-379.
18. R.athish IG, Kalim J, Shamim A, Sameena B, Alam MS, Pillai KK, Surender S, Vivek B. Synthesis and anti-inflammatory activity of some new 1, 3, 5-trisubstituted pyrazolines bearing benzene sulfonamide. Bioorg Med Chem Lett, 2009; 19: 255-258.
19. Sakthinathan SP, Vanangamudi G, Thirunarayanan G. Synthesis, spectral studies and antimicrobial activities of some 2-naphthyl pyrazoline derivatives. Spectrochim Acta A, 2012; 95: 693-700.
20. Jadhav SB, Shastri RA, Gaikwad KV, Gaikwad SV. Synthesis and antimicrobial studies of some novel pyrazoline and isoxazoline derivatives. Eur J Chem, 2009; 6: 183-188.
21. Abdel-Wahab BF, Abdel-Aziz HA, Ahmed EM. Synthesis and antimicrobial evaluation of 1-(benzofuran-2-yl)-4- nitro-3-arylbutan-1-ones and 3-(benzofuran-2 yl)-4, 5-dihydro –5-aryl-1-[4-(aryl)-1, 3-thiazol-2-yl]-1H-pyrazoles. Eur J Med Chem, 2009; 44: 2632-2635.
22. Mashooq Ahmad Bhat1, Abdul Arif Khan, Mohamed A. Al-Omar1, Azmat Ali Khan. Synthesis and anti-candidal activity of some new pyrazoline derivatives. Biomedical Research, 2017; 28(7): 3082-3087.
23. Israili ZH. Advances in the treatment of type 2 Diabetes Mellitus. Am. J. Therap, 2011; 18: 117-52.
24. Chi-Ting Hsieh, Tusty-Jiuan Hsieh, Mohamed El-Shazly, Da-Wei Chuang, Yi-Hong Tsai, Chiao-Ting Yen, Shou-Fang Wu, Yang-Chang Wu, Fang-Rong Chang. Synthesis of chalcone derivatives as potential anti-diabetic agents. Bioorg. Med. Chem. Lett, 2012; 22: 3912–3915.
25. Machius M, Vertesy L, Huber R, Wiegand G. Carbohydrate and protein-based inhibitors of porcine pancreatic α-amylase: structure analysis and comparison of their binding characteristics. J Mol Biol, 1996; 260: 409-421.
26. Kim JS, Kwon CS, Son KH. Inhibition of α-glucosidase and amylase by luteolin, a flavonoid. Biosci Biotechnol Biochem, 2000; 64: 2458-2461.
27. Rosak C, Mertes G. Effects of acarbose on proinsulin and insulin secretion and their potential significance for the intermediary metabolism and cardiovascular system. Curr Diabetes Rev., 2009; 5: 157-164.
28. Yamagishi S, Nakamura K, Takeuchi M. Inhibition of postprandial hyperglycemia by acarbose is a promising therapeutic strategy for the treatment of patients with the metabolic syndrome. Med Hypotheses, 2005; 65: 152-154.
29. Ogawa H, Ohno M, Baba K. Hypotensive and lipid regulatory actions of 4-hydroxy derricin, a chalcone from Angelica keiskei, in stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol Physiol, 2005; 32: 19-23.
30. Yoon SH, Robyt JF. Study of the inhibition of four α amylases by acarbose and its 4IV-α-maltohexaosyl and 4IV-α-maltododecaosyl analogues. Carbohydr Res, 2003; 338: 1969-1980.