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Anti-Diabetic Activities of Aqueous Extract from *Actinidia kolomikta* Root Against α-glucosidase

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ABSTRACT

The present study was to evaluate the anti-diabetic activity of the aqueous extract of *Actinidia kolomikta* root against baker's yeast α-glucosidase and rat intestinal α-glucosidase. As a result, the aqueous extract of *Actinidia kolomikta* root exhibited excellent baker's yeast α-glucosidase inhibitory activity which was better than acarbose. It also showed rat intestinal α-glucosidase inhibitory activity not only in maltase, but also in sucrase in a dose dependent manner. With these results, it can be concluded that *Actinidia kolomikta* root existed the anti-diabetic activity depend on their inhibitory activity on glucose absorption in the small intestine. The results also give scientific support to the utilization of *Actinidia kolomikta* root as folklore medicine for the treatment of diabetes.

Keywords: *Actinidia kolomikta*, Anti-diabetic activity, α-Glucosidase

1. Introduction

Diabetes mellitus is a global health problem and the diabetic was constantly increasing. The number of diabetic people is expected to rise to 366 million in 2030 [1], and most of them were type 2 diabetes. As we all know that the hyperglycemia can damage many of the body's organs and systems, and then lead to renal failure, blindness, cerebrovascular disease and so on. One of the therapeutic approaches is to decrease the postprandial hyperglycemia [2]. The postprandial hyperglycemia can be decreased through inhibiting the activity of α-glucosidase enzyme in the brush border of the small intestine, and then delay the digestion and absorption of carbohydrates, reduce the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [3]. Therefore, α-glucosidase inhibitors is one of the choice for diabetic, and now many α-glucosidase inhibitors such as acarbose, miglitol, voglibose as oral anti-diabetic drugs are widely used in clinical trials [4]. However, a lot of clinical and laboratory researches have reported that the α-glucosidase inhibitors has serious side effects such as hepatotoxicity, abdominal pain, flatulence, diarrhea, and hypoglycemia [5, 6, 7, 8]. So it is necessary to search for effective components with low cost, no side effects as a substitute. On the other hand, traditional plant was wildly used for treatment of diabetes throughout the world as it was effective, non-toxic, with less or no side effects [9]. More than 1200 plants have been used to treat diabetes in folk medicine [10], and there are 136 plants clearly showed the anti-diabetic effects [11]. More researchers are trying to search for more effective inhibitors of anti-diabetic compounds from natural plants. A*ctinidia kolomikta* (Rupr. & Maxim.), which is named *Tonh Khidum* (Laotian) in Latin, is a species of deciduous dioecious woody vines in the genus Actinidia. In our lab, various biological activities of *Actinidia kolomikta* root aqueous extract such as antitumor activities [12], antiproliferative activities [13], Immunomodulatory activities [14], anti-oxidant activities [15] have been evaluated. Although the root extract of *Actinidia kolomikta* has been used as a folk remedy for the treatment of diabetes for many years in Laos, there is no scientific evidence to support the anti-diabetic effect of *Actinidia kolomikta*. In the present study, the anti-diabetic effects of *Actinidia kolomikta* root was investigated in vitro by using α-glucosidase inhibitory methods. The bioactive compound was also identified.

2. Material and Methods

2.1 Chemicals

Rat intestinal acetone powder, 4-Nitrophenyl-a-D-glucopyranoside (pNPG), yeast α-glucosidasea (E.C.3.2.1.20) were purchased from Sigma- Aldrich Corp. (St. Louis, MO, USA), Glucose C Ⅱ kit and Acarbose were purchased from Wako Pure Chemical Industries (Osaka, Japan). Other solvents and chemicals were obtained from Wako Pure Chemical Industries.

2.2 Plant Material and Preparation of Extract

The roots of *Actinidia kolomikta* were collected from Laos. Firstly, the roots of *Actinidia kolomikta* were washed thoroughly with distilled water and dried in the shade, shade dried roots were finely powdered. The powder (100 g) was boiled in 1 L distilled water three times and 2 hours for each time. Each extract was then filtered through a Whatman No. 47 paper filter, the filtrate was combined, concentrated by rotary evaporation under reduced pressure, then lyophilized to get powder (yield:10%,w/w). The root aqueous extract was stored at -20 °C until further use.

2.3 Assay for yeast α-glucosidase Inhibitory Activity

The assay was according to a report [16, 17] using p-nitrophenyl-a-D-glucopyranoside as substrate. Briefly, a mixture of 50 µl of different concentrations of the extracts and 100µl of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase solution (1.0 U/ml) was incubated in 96-well plates at 25 °C for 10 min. After pre-incubation, 50 µl of 5 mM p-nitrophenyl-α-d-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Absorbance was recorded at 405 nm before and after incubation with a micro-plate reader (BIO-RAD, Japan) and compared to that of the control, which had 50 µl 0.1 M phosphate buffer instead of the extracts. The α-glucosidase inhibitory activity was expressed as % inhibition and was calculated as follows:

$$\%\text{Inhibition} = (\Delta A_C - \Delta A_S)/\Delta A_C \times 100$$

Where ΔAs represent the changes in absorbance for the sample, ΔAc represent the changes in absorbance for the control, respectively.

2.4 Assay for Rat Intestinal Maltase Inhibitory Activity

Rat intestinal maltase inhibitory activity was determined using a literature method [18] with a slight modification. Enzyme solutions were prepared using rat intestinal acetone powder as a source of α-glucosidase. One gram of rat intestinal acetone powder was homogenized with 10 ml of 0.1 M phosphate buffer (pH 6.9), followed by centrifugation at 1000 g for 10 min at 4 °C. The supernatant was diluted with 0.1 M phosphate buffer by 20 times, and was used as the enzyme solution for maltase reactions, respectively. *Actinidia kolomikta* root aqueous extracts (final concentration: 0.125, 0.25, 0.5, 1.0 mg /ml) were diluted with 0.1M phosphate buffer, acarbose as a positive control, was prepared in the same concentration with the same method. Next, in a 96-wells micro-plate, 20 µl of sample solution and 20 µl of maltose substrate solution (2% w/v in phosphate buffer) was mixed, and pre-incubated at 37 °C for 5 min. The enzyme reaction was initiated by adding 20 µl of the enzyme solution, followed by incubation at 37 °C for 60 min. After 60 min incubation, the mixture of 10 µl filtrate and 200 µl glucose kit solution (Glucose CII-Test Wako, Japan) was incubated at 37 °C for 30 min in a new 96-wells micro-plate. The optical density (OD) of the wells was measured at 490 nm with a micro-plate reader (Cambridge, UK) and the inhibitory activity was calculated using following formula:

$$\%\text{Inhibition} = (A_{CB} - A_C) - (A_{SB} - A_S)/(A_{CB} - A_C) \times 100$$

where A_{CB} was the absorbance with a-glucosidase and without the sample, A_C was the absorbance without a-glucosidase and the sample, A_{SB} was the absorbance with a-glucosidase and the sample, and A_S was the absorbance with the sample but without a-glucosidase.

2.5 Assay for rat Intestinal Sucrase Inhibitory Activity

Rat intestinal sucrase inhibitory activity was determined using a literature method [19] with a slight modification. The assay was carried out in the same manner as the assay for rat intestinal maltase inhibitory activity, except for the supernatant was diluted with 0.1 M phosphate buffer by 2 times and sucrose substrate solution (2% w/v in phosphate buffer).

2.6 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FT-IR spectrum was recorded on a FTIR 300 spectrometer (Jasco, Japan).The root extract was mixed with potassium bromide (KBr) and was made into a pellet by using a hydraulic press. The scans were made in a wave number range from 4000 and 400 cm^{-1}. All determinations were performed in triplicate.

2.7. UV–vis spectra analysis

The UV–Vis spectrophotometer UV–1800 (shimadzu, Japan) was used to measure UV–Vis absorbance spectra of *Actinidia kolomikta* root aqueous extract. The absorbance measurements were made in 200 –400 nm range with a 1 nm step

3. Result and Discussion

3.1 Assay for yeast a-glucosidase inhibitory activity

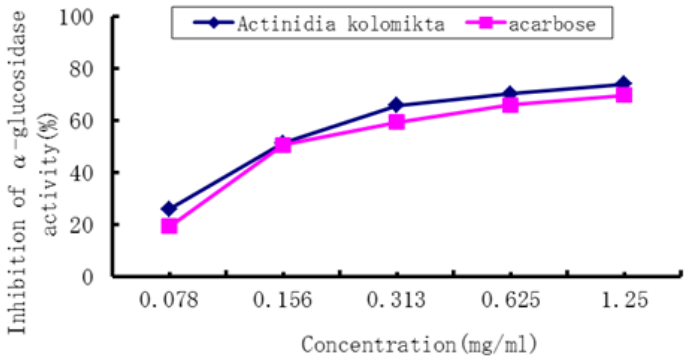

Fig 1: Yeast α-glucosidase inhibitory activity of *Actinidia kolomikta* root aqueous extract at different concentration

The yeast α-glucosidase inhibition of *Actinidia kolomikta* root extract at final concentrations of 0.078, 0.156, 0.313, 0.625 and 1.25 mg/ml were shown in Fig. 1. The results suggest that *Actinidia kolomikta* root aqueous extract showed a dose dependent inhibition of yeast α-glycosidase activity. *Actinidia kolomikta* root aqueous extract showed superior α-glucosidase inhibitory activity compared to acarbose, which is used as anti-diabetic drug. Accoding to the results, *Actinidia kolomikta* root aqueous extract exhibited remarkable potential anti-diabetic properties. The results also give scientific support to the utilization of *Actinidia kolomikta* root as folklore medicine for the treatment of diabetes.

3.2 Assay for rat Intestinal Maltase Inhibitory Activity

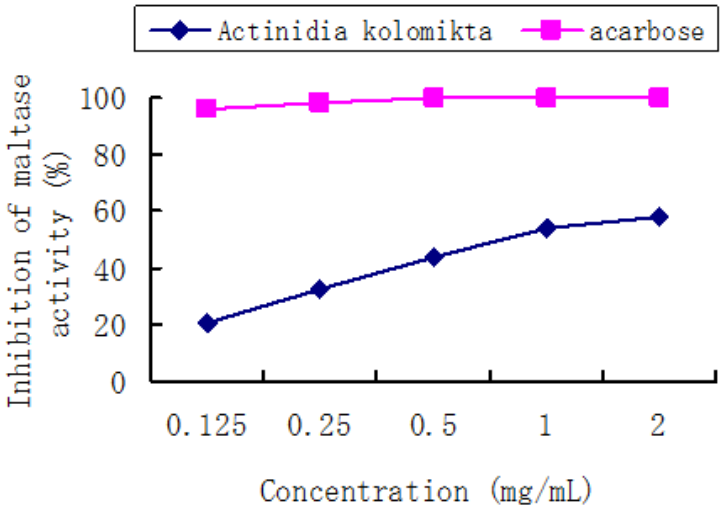

Fig 2: Rat intestinal Maltase Inhibitory Activity of *Actinidia kolomikta* root Aqueous Extract at Different Concentration

Recent years, yeast α-glucosidase is widely used to search α-glucosidase inhibitors, but the results do not always agree with those obtained in mammals [20]. Therefore, the rat intestinal acetone powder as a source of α-glucosidase was used to evaluate the α-glucosidase inhibitory activity of *Actinidia kolomikta* root aqueous extract. The rat intestinal maltase inhibitory activity of *Actinidia kolomikta* root aqueous extract was presented in Fig. 2. The result showed that the extract inhibited rat intestinal maltase activity dose-dependently (0.125–2mg/mL), and the rat intestinal maltase inhibition was 57.9% at the concentration of 2 mg/ml. Acarbose as a positive control showed strong rat intestinal maltase inhibitory activity, even at the concentration of 0.125 mg/ml, the rat intestinal maltase inhibition was reached to 96.2%. However, as shown in Fig.1. acarbose showed a weaker yeast a-glucosidase inhibitory activity than *Actinidia kolomikta* root aqueous extract. This result was consistent with the past reports [21]. The high rat intestinal maltase activity of *Actinidia kolomikta* root aqueous extract maybe is bound up with its high content of phenolic compounds [13].

3.3 Assay for rat Intestinal Sucrase Inhibitory Activity

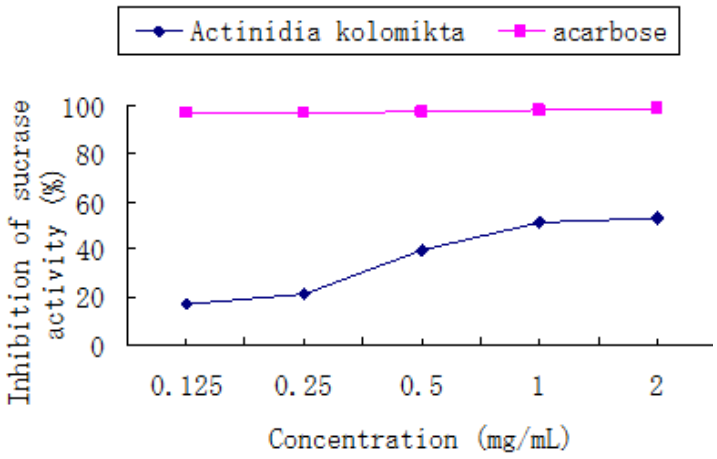

Fig 3: Rat intestinal sucrase inhibitory activity of *Actinidia kolomikta* root aqueous extract at different concentration

The rat intestinal sucrase inhibition of *Actinidia kolomikta* root aqueous extract at final concentrations of 2, 1, 0.5, 0.25, 0.125 mg/ml was showed in Fig. 3. *Actinidia kolomikta* root aqueous extract elicited a dose dependent inhibition of rat intestinal sucrase activity. The extract at 2 mg/ml had a percentage inhibition of 53.3% while acarbose at the same concentration inhibited 98.5% of the enzyme activity. Many studies confirmed that phenolic compounds had anti-diabetic activity [22, 23, 24]. According to previous research [12], the high content of phenolic compounds was found in *Actinidia kolomikta* root aqueous extract, maybe it can explain its high rat intestinal sucrase activity.

3.4 Fourier Transform Infrared Spectroscopy (FTIR) analysis

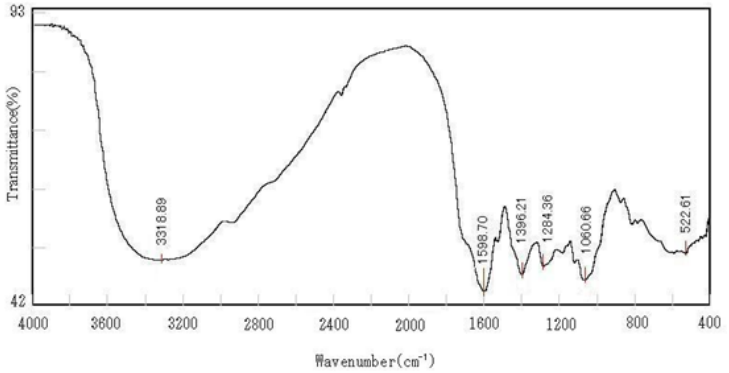

Fig 4: FTIR spectrum of *Actinidia kolomikta* root aqueous extract

FTIR spectra and the functional group of compounds present in the root aqueous extract of *Actinidia kolomikta* was shown in Fig.4. The FTIR spectrum shows a Strong and broad absorption peak at 3318.89 cm^{-1} corresponding to the overlapping of –OH peaks [25]. Another peak located at 1608–1580 cm^{-1} was assigned to C–C ring vibration [26]. The peak at 1284.36 cm^{-1} represents the C–O stretching, the peak at 1060.66 cm^{-1} represents the O–H stretching [27]. It has been reported that phenolic compounds exhibited an absorption peak at 1650–1450cm^{-1} and 1420–1330cm^{-1} [28]. Therefore, the FTIR spectrum of *Actinidia kolomikta* root aqueous extract was similar to phenolic compounds. It has been reported that phenolic compounds play a major role in anti-diabetic activity.

3.5 UV–vis Spectra Analysis

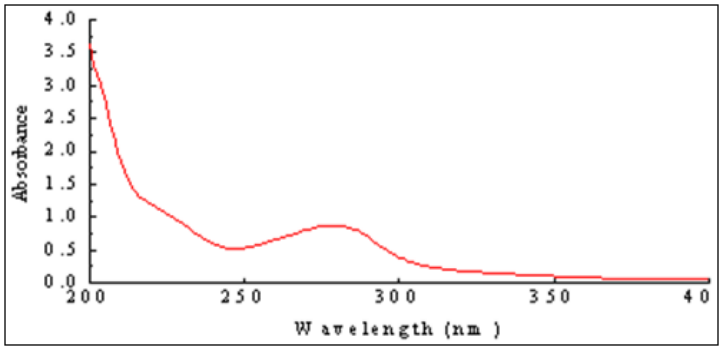

Fig 5: UV–vis electronic spectra of *Actinidia kolomikta* root aqueous extract

Actinidia kolomikta root aqueous extract were examined by UV-VIS spectrophotometry, and the results are shown in Fig. 5. As we all know that phenolic compounds including phenolic acids, phenols, phenyl propanols, flavonoids, minor flavonoids, quinines and tannins. Phenolic acid derivatives showed a single peak at 270-290 nm [29], flavonoids showed double maximum absorption peaks at 240-280nm and 330-400nm [30]. The spectrum of *Actinidia*

kolomikta root aqueous extract showed a single peak at 278 nm, which suggests to be related to the presence of phenolic acids. It has been reported that phenolic acids such as Chlorogenic Acid [31], Caffeic acid [32], 3-5-dicaffeoylquinic acid methyl ester [33] exhibited anti-diabetic activity.

4. Conclusion

In conclusion, anti-diabetic activities of *Actinidia kolomikta* root aqueous extract were firstly established through α-glucosidase inhibitory activity. The results give scientific support to the use of *Actinidia kolomikta* root in folklore medicine for the treatment of diabetes. Further studies were needed to isolate the active compounds and make clear the mechanism of anti-diabetic activities.

5. Acknowledgement

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6. Reference:

1. Sarah W, Gojka R, Anders G, Richard S, Hilary K. Global Prevalence of Diabetes. Diabetes Care 2004; 27:1047-1053.
2. Megh RB, Nilubon JA, Gao H, Jun K. a-Glucosidase and a-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). Food Chemistry 2008; 106:247-252.
3. Elsnoussi AHM, Mohammad J AS, Lee FA, Amirin S, Sue H C, Soo CT et al. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. BMC Complementary and Alternative Medicine 2012; 12:176.
4. Ana T, Amadeu L. A Prospect for Pyrrolidine Iminosugars as Antidiabetic α-Glucosidase Inhibitors. Journal of Medicinal Chemistry 2012; 55:10345-10346.
5. Coniff RF, Shapiro JA, Seaton TB, Bray GA. Multicenter, placebocontrolled trial comparing acarbose (BAY g 5421) with placebo, tolbutamide, and tolbutamide-plus-acarbose in non–insulin-dependent diabetes mellitus. American Journal of Medicine 1995; 98: 443- 451.
6. Coniff RF, Shapiro JA, Seaton TB, Hoogwerf BJ, Hunt JA. A doubleblind placebo-controlled trial evaluating the safety and efficacy of acarbose for the treatment of patients with insulin-requiring type II diabetes. Diabetes Care 1995; 18: 928- 932.
7. Carrascosa M, Pascual F, Arest S. Acarbose-induced acute severe hepatotoxicity. Lancet 1997; 349: 698-699.
8. Charpentier G, Riveline JP, Varroud-Vial M. Management of drugs affecting blood glucose in diabetic patients with renal failure. Diabet Med 2000; 26:73- 85.
9. Clifford B, Caroline D. Traditional Plant Medicines as Treatments for Diabetes. Diabetes Care1989; 12:553-564.
10. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine 1995; 2(2):137-89.
11. Kavishankar GB, Lakshmidevi N, Murthy SM, Prakash HS, S.R. Niranjana SR. Diabetes and medicinal plants-A review. International Journal of Pharmacy and Biomedical Sciences 2011; 2(3):65-80.
12. Guan D, Zhang ZY, Yang YN, Sugiura N, Hu HH, Xing GQ et al.. Antioxidant and antitumor activities of water extracts from the root of *Actinidia kolomikta*. Experimental and Therapeutic Medicine 2011; 2:33-39.
13. Liu JQ, Zhang ZY, Xing GQ, Hu HH, Sugiura N, Intabon K. Potential antioxidant and antiproliferative activities of a hot-water extract from the root of Tonh khidum. Oncology letters 2010;1: 383-387.
14. Guan D, Zhang ZY, Yang YN, Xing GQ, Liu JQ. SImmunomodulatory Activity of Polysaccharide from the Roots of *Actinidia kolomikta* on Macrophages. International Journal of Biology 2011; 3: 2.
15. 15. Sun HY, Wang SF, Li SH, Yuan X, Ma J, Zhang ZY. Antioxidant activity and Immunomodulatory of Extracts from Roots of *Actinidia kolomikta*. International Journal of Biology 2013; 5: 3.
16. Apostolidis E, Li LY, Lee C, Seeram NP. In vitro evaluation of phenolic-enriched maple syrup extracts for inhibition of carbohydrate hydrolyzing enzymes relevant to type 2 diabetes management. Journal of Functional Foods 2011; 3:100-106.
17. Wan CP, Yuan T, Cirello AL, Seeram NP. Antioxidant and α-glucosidase inhibitory phenolics isolated from highbush blueberry flowers. Food Chemistry 2012; 135:1929-1937.
18. Ikarashi N, Takeda R, Ito K, Ochiai W, Sugiyama K. The inhibition of lipase and glucosidase activities by acacia polyphenol. Evidence-Based Complementary and Alternative Medicine 2011; ID 272075, doi:10.1093/ecam/neq043.
19. Sakulnarmrat K, Konczak I. Composition of native Australian herbs polyphenolic-rich fractions and in vitro inhibitory activities against key enzymes relevant to metabolic syndro. Food Chemistry 2012; 134:1011-1019.
20. Nimal CIV, Praveen KP, Sunil C, Vajravijayan S, Lakshmi SR, Jenifer SS *et al*.. In vitro studies on a-glucosidase inhibition, antioxidant and free radical scavenging activities of *Hedyotis biflora* L. Food Chemistry2013; 138:1689-1695.
21. Yoshikawa M, Pongpiriyadacha Y , Kishi A ,kaeura T , Wang T, Morikawa T , Matsuda H. Biological Activities of *Salacia chinensis* Originating in Thailand: The Quality Evaluation Guided by α-Glucosidase Inhibitory Activity. Yakugaku Zasshi 2003; 123 :871-880.
22. 22. You Q, Chen F, Wang X, Jiang YM, Lin SY. Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipas. LWT - Food Science and Technology 2012; 46:164-168.
23. Gandhi GR, Ignacimuthu S, Paulraj MG. *Solanum torvum* Swartz. fruit containing phenolic compounds shows antidiabetic and antioxidant effects in streptozotocin induced diabetic rats. Food and Chemical Toxicology 2011; 49:2725–2733.
24. Hossam MA, Maha MS, Enas HA, Shohda AEM. Antidiabetic activity of phenolic compounds from Pecan bark in streptozotocin induced diabetic rats. Phytochemistry Letters 2011; 4: 337-341.
25. Raya A, Gupta SD, Ghosh S. Evaluation of anti-oxidative activity and UV absorption potential of the extracts of *Aloe vera* L. gel from different growth periods of plant. Industrial Crops and Products 2013; 49:712-719.
26. Guan XH, Shang C, Chen GH. ATR-FTIR investigation of the role of phenolic groups in the interaction of some NOM model compounds with aluminum hydroxide. Chemosphere2006; 65: 2074-2081.
27. Jadav MM, Teraiya SR, Patel KN, Patel BA, Patel PA. Formulation and Evaluation of Oral Controlled Porosity Osmotic Pump Tablet of Zaltoprofen. International Journal for

Pharmaceutical Research Scholars2012; 1(2):254-267.
28. 28. Vazquez G, Fontenla E, Santos J, Freire MS, Gonzalez-Alvarez J, Antorrena G. Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. Industrial Crops and Products 2008; 28:279-285.
29. Kiss AK, Naruszewicz M. Polyphenolic compounds characterization and reactive nitrogen species scavenging capacity of *Oenothera paradoxa* defatted seed extracts. Food Chemistry 2012; 131:485-492.
30. Chen Y, Wu CM, Dai RJ, Li L, Yu YH, Li Y *et al*.. Combination of HPLC chromatogram and hypoglycemic effect identifies isoflavones as the principal active fraction of *Belamcanda chinensis* leaf extract in diabetes treatment. Journal of Chromatography B2011; 879:371-378.
31. Hunyadi A, Martins A, Hsieh TJ, Seres A, Zupko I. Chlorogenic Acid and Rutin Play a Major Role in the *In Vivo* Anti-Diabetic Activity of Morus alba Leaf Extract on Type II Diabetic Rats. PLoS ONE 2012; 7(11): e50619.
32. Celik S, Erdogan S, Tuzcu M. Caffeic acid phenethyl ester (CAPE) exhibits significant potential as an antidiabetic and liver-protective agent in streptozotocin-induced diabetic rats. Pharmacological Research 2009; 60:270–276.
33. Tan C, Wang QX, Luo C, Chen S, Li QY, Li P. Yeast α-Glucosidase Inhibitory Phenolic Compounds Isolated from *Gynura medica* Leaf. International Journal of Molecular Sciences 2013; 14:2551-2558.