View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.05.014

Anti-diabetic potential of *Urena lobata* leaf extract through inhibition of dipeptidyl peptidase IV activity

Yudi Purnomo[1,2*], Djoko Wahono Soeatmadji[3], Sutiman Bambang Sumitro[4], Mochamad Aris Widodo[5]

[1]*School of Medicine, Brawijaya University, Jl. Veteran, Malang 65145, East Java, Indonesia*

[2]*Pharmacology Department, School of Medicine, Malang Islamic University, Jl. Mayjen, Haryono 193, Malang 65144, Indonesia*

[3]*Internal Department, School of Medicine, Brawijaya University, Jl. Veteran, Malang 65145, East Java, Indonesia*

[4]*Biology Department, Faculty of Science, Brawijaya University, Jl. Veteran, Malang 65145, East Java, Indonesia*

[5]*Pharmacology Department, School of Medicine, Brawijaya University, Jl. Veteran, Malang 65145, East Java, Indonesia*

ARTICLE INFO

Article history:
Received 29 Apr 2015
Received in revised form 11 May 2015
Accepted 28 May 2015
Available online 14 July 2015

Keywords:
Anti-diabetic
Dipeptidyl peptidase IV
In silico
In vitro
Urena lobata

ABSTRACT

Objective: To evaluate the anti-diabetic potential of leaf extract from *Urena lobata* (*U. lobata*) through dipeptidyl peptidase IV (DPP-IV) inhibitory activity.

Methods: *U. lobata* leaf was extracted in hot water and ethanol. The activity of DPP-IV inhibitor was tested by *in vitro* study using gly-pro-p-nitroanilide as substrat of DPP-IV and vildagliptin, as standard reference. A product of the reactions between gly-pro-p-nitroanilide and DPP-IV, was observed by microplate readers with λ = 405 nm. All data were expressed as mean ± SD and the IC_{50} value was determined by non linear regression curve fit. Active substances in leaf extract of *U. lobata* was analyzed by liquid chromatography-mass spectrometry. DPP-IV inhibitory activity of active compounds was evaluated *in silico* using docking server.

Results: The ethanolic extract of *U. lobata* showed stronger DPP-IV inhibitor activity than water extract with the IC_{50} values of 1 654.64 and 6 489.88 μg/mL, respectively. Vildagliptin, based on standard reference for DPP-IV inhibitor activity, has IC_{50} value of 57.44 μg/mL. Based on *in silico* analysis, mangiferin, stigmasterol and β-sitosterol in *U. lobata* extract have a strong inhibitory activity on DPP-IV.

Conclusions: The results showed that DPP-IV inhibitory activity of *U. lobata* is related to its active compounds such as mangiferin, stigmasterol and β-sitosterol.

1. Introduction

Recently, the treatment of type 2 diabetes mellitus is focused on incretin hormone. Glucagon like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are the major incretin hormones which are secreted by intestinal cells. GLP-1 plays a role in regulation of blood glucose level due to their biological actions, such as stimulating the secretion of insulin, increasing β-cell mass, inhibiting the secretion of glucagon, reducing the rate of gastric emptying and inducing satiety [1,2]. However, GLP-1 is rapidly metabolized by the enzyme called dipeptidyl peptidase IV (DPP-IV) into inactive forms. Therefore, the GLP-1 has a short half life, approximately for 1–2 min. Inhibition of DPP-IV maintains the level of endogenous active GLP-1 and prolongs its half life [1,3].

DPP-IV inhibitor has the potential to be a novel, efficient and considerable agent to treat type 2 diabetes mellitus [3]. The usage of DPP-IV inhibitor has less side effects like hypoglycemia, increasing body weight and GIT disorders [4]. The studies of oral glucose tolerance test on animals showed that genetic deletion of DPP-IV have improved glucose tolerance and increased the insulin secretion [5]. In the other hand, the complete data of long term use of synthetic drugs of DPP-IV inhibitor have not been obtained yet, especially on its safety [6]. It induces the research of DPP-IV inhibitor compounds from herbs that are of less side effects, cheaper and easier to get.

*Corresponding author: Yudi Purnomo, Doctoral Student, Pharmacology Department, School of Medicine, Malang Islamic University, Jl. Mayjen, Haryono 193, Malang 65144, Indonesia.

E-mail: y_purnomo92@yahoo.com

Peer review under responsibility of Hainan Medical University.

Foundation Project: Supported by a grant of doctoral dissertation research from Education Ministry of Indonesia. below this sentence.

2221-1691/Copyright © 2015 Hainan Medical University. Production and hosting by Elsevier (Singapore) Pte Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Urena lobata (*U. lobata*) is the plant that can be found in Indonesia and has been used to cure many diseases. Based on experiences, Nigerian people used *U. lobata* to treat diabetes mellitus because of their biology activities [7]. The study showed that administration of *U. lobata* roots extract had anti-hyperglycemic effect on rat induced by streptozotocin before [8]. It related to active substances in *U. lobata* such as sterol groups, alkaloid and flavonoid [9,10]. Anti-diabetic potential of *U. lobata* has not been evaluated yet, especially the inhibition of DPP-IV activity. Therefore it is an opportunity to expand herbs that can become candidate of phytopharmaca. The aim of this study was to know the anti-diabetic potential of *U. lobata* leaf extract through inhibition of DPP-IV activity.

2. Materials and methods

2.1. Chemicals used

DPP-IV was obtained from porcine kidney. Gly-pro-p-nitroanilide and Tris–HCl buffer were used. All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

U. lobata leaf powder was obtained from Materia Medika Batu Malang with certificate number 074/027/101.8/2015. Then, 50 g of the powdered plant materials were extracted in 250 mL hot water at 90 °C for 30 min. Similarly, 50 g *U. lobata* powder was extracted in 250 mL ethanol for 4 h by waterbath shaker and repeated 2 times with fresh ethanol. Both of the extracts were then evaporated.

2.3. Identification of active compounds

Both water and ethanol extract were analyzed on a semi qualitative scale by liquid chromatography–mass spectrometry (LC–MS) Accela 1250 pump for identification of active compounds. Mobile phase contained 0.1% formic acid in a mixture of methanol and water.

2.4. DPP-IV assays

The assay was performed in 96 micro well plates. A pre-incubation solution (50 μL) contained 35 μL Tris–HCl buffer, 15 μL DPP-IV enzyme and various concentration (625, 1 250, 2 500, 5 000 and 10 000 μg/mL) of the extracts or standard (6.25, 12.5, 25, 50 and 100 μg/mL). This mixture was incubated at 37 °C for 10 min, followed by addition of 50 μL gly-pro-p-nitroanilide as substrate. The reaction mixture was incubated for 30 min at 37 °C and the absorbance was measured by microplate readers at λ = 405 nm every 10 s. Vildagliptin was used as the standard DPP-IV inhibitor [11]. % Inhibition was calculated by the following formula:

$$\%\ \text{Inhibition} = \frac{\text{DPP}-\text{IV activity (with extract)}}{\text{DPP}-\text{IV activity (without extract)}} \times 100$$

2.5. Molecular docking studies

DPP-IV inhibitory activity of active compounds in *U. lobata* leaf extracts was evaluated by *in silico* study using a web-based software application (www.dockingserver.com) for protein and ligand molecular docking. Free energy binding, inhibition constant and surface interactions were analyzed by this method to measure the DPP-IV inhibitory activity of active compounds.

2.6. Statistical analysis

All data are expressed as mean ± SD. The IC_{50} was determined by non-linear regression curve fit. The statistical data were analyzed by SPSS One-way ANOVA test followed by least significant difference test with significant value at $P < 0.05$.

3. Results

3.1. DPP-IV inhibitory activity of U. lobata

Both water and ethanol *U. lobata* leaf extracts were tested on DPP-IV inhibitory assay by *in vitro* method. The DPP-IV inhibitory activity is shown in Table 1.

The results obtained in the DPP-IV inhibitory assay showed that ethanolic extract of *U. lobata* showed stronger activity in DPP-IV inhibition, about 4 times folds, compared to water extract ($P < 0.05$). However, the DPP-IV inhibitory activity of both water and ethanolic *U. lobata* extracts are still lower, approximately 30–100 times folds, compared to vildagliptin as reference drugs of DPP-IV inhibitor ($P < 0.05$).

3.2. Identification of active compounds in U. lobata leaf extracts

Ten active substances from alkaloid, fitosterol and flavonoid groups were identified in extracts of *U. lobata*. The active compounds, both in water and ethanol leaf extract of *U. lobata*, can be seen in Table 2. The semi-qualitative analysis by LC–MS showed that the most abundant active compounds both in water and ethanolic extract of *U. lobata* were stigmasterol, gossypetin and β-sitosterol. Active compounds such as mangiferin and chrysoeriol were also identified in both water and ethanolic extracts of *U. lobata* with less content.

Table 1
DPP-IV inhibitory activity of *U. lobata* leaf extracts and vildagliptin.

Sample (n = 3)	Concentration (μg/mL)	% Inhibition	IC_{50} (μg/mL)
Water extract of *U. lobata*	625.00	00.00 ± 0.00	6 489.88[a]
	1 250.00	13.33 ± 0.00	
	2 500.00	26.67 ± 0.00	
	5 000.00	42.22 ± 3.85	
	10 000.00	62.22 ± 3.85	
Ethanolic extract of *U. lobata*	625.00	36.17 ± 0.00	1 654.64[b]
	1 250.00	48.94 ± 0.00	
	2 500.00	55.32 ± 0.00	
	5 000.00	61.70 ± 0.00	
	10 000.00	74.47 ± 0.00	
Vildagliptin	6.25	8.93 ± 0.00	57.44[c]
	12.50	16.07 ± 4.12	
	25.00	37.50 ± 0.00	
	50.00	46.63 ± 3.85	
	100.00	60.71 ± 0.00	

[a, b, c]: Different letters showed the differences of the potency ($P < 0.05$, Least Significant Difference test).

Table 2
Active compounds in *U. lobata* leaf extracts.

Active compounds	Molecule weight (Dalton)	Water extract	Ethanolic extract
Stigmasterol	413	+++	++
β-Sitosterol	415	+	+
Mangiferin	423	+	+
Quercetine	303	–	–
Kaempferol	286	–	–
Hypolaetin	302	–	–
Gossypetin	318	+	++
Luteolin	286	–	–
Apigenin	270	–	–
Chrysoeriol	300	+	+

+: Weak; ++: Moderate; +++: Strong; –: Negative.

Table 3
Molecular docking of active compounds in *U. lobata* leaf extracts.

Active compounds	Estimation of free energy of binding (Kcal/mol)	Estimation of inhibition constant (μmol/L)	Interaction surface
Stigmasterol	–7.42	3.62	962.48
β-Sitosterol	–6.59	14.67	886.91
Mangiferin	–7.66	2.43	742.75
Gossypetin	–5.20	153.42	552.29
Chrysoeriol	–4.66	386.05	539.84

3.3. Molecular docking of active compounds in U. lobata leaf extracts

Inhibitory activity of *U. lobata* leaf extracts on DPP-IV was evaluated by *in silico* study. Active compounds identified in *U. lobata* as ligand were docked with DPP-IV as protein target and the results can be seen at Table 3.

Docking studies showed that mangiferin, stigmasterol and β-sitosterol have a low value in both the binding free energy and the inhibition constant but the surface interaction was high. However, gossypetin and chrysoeriol have a higher value in binding free energy and inhibition constant than other substances above. The differences in each parameter value caused the distinction in inhibitory activity on DPP-IV.

4. Discussion

4.1. Identification of active compounds in U. lobata leaf extracts

Five active compounds were identified in *U. lobata* leaf extract and had been found in both water and ethanol extract. It is only different in the quantity or amount of active compounds in both extracts. The active compounds are stigmasterol, gossypetin, β-sitosterol, mangiferin and chrysoeriol. All of them are classified into secondary metabolite groups and have biological activity that can be used to cure diseases. Stigmasterol is one of a group of plant sterols or phytosterols that are chemically similar to animal cholesterol. Phytosterols are insoluble in water but soluble in most organic solvents and contain one alcohol functional group. Stigmasterol is an unsaturated plant sterol in the plant fats or oils of soybean, calabar bean, rape seed, and in various medicinal herbs. Studies about laboratory animals treated by stigmasterol found that both cholesterol and sitosterol absorption decreased 23% and 30%, respectively, over a 6-week period. It also possesses potential antioxidant, hypoglycemic and thyroid inhibiting properties [12,13].

Gossypetin is flavonol or flavone, a type of flavonoid. It has been isolated originally from the flowers and the calyx of *Hibiscus* species. Gossypetin shows potential antioxidant, antimicrobial, anti-mutagenic and anti-atherosclerotic activities [14]. This compound is very soluble in chloroform and benzene, and also moderately soluble in ethanol and ether, but insoluble in water.

β-sitosterol is one of several phytosterols or plant sterols with chemical structure similar to that of cholesterol. Sterols are isoprenoid-derived molecules that have essential functions typically in eukaryotes, and especially in higher plants. β-sitosterol are white, waxy powder with characteristic odor. They are hydrophobic and soluble in ethanol and chloroform but insoluble in water [15]. It can be found in avocados, cucurbita pepo, corn oil and soy beans; it also showed anti-cholesterol, anti-inflamatory and immunomodulator effects [16].

Mangiferin is a xanthonoid, and a glucoside of norathyriol. It was found in mangoes, *Iris unguicularis* and *Anemarrhena asphedelous*. Mangiferin is soluble in hot diluted ethanol and methanol but insoluble in water. Laboratory study has identified a variety of pharmacology effect that associated with mangiferin including anti-microbial, antioxidant activity, and anti-diabetic effect in rodent [17,18].

Chrysoeriol is a flavon, one of major flavonoid classes. They exhibit many activities such as anti-inflammation and anti-histamine activities. It is soluble in alkalies solution and sufficiently soluble in water [19].

The presence of active compounds in extract was influenced by polarity and extract solvent. Type of extract solvent impacts the amount of active compounds in extract due to the difference of their solubility in solvent. Secondly, polarity of active compound also contributes to their solubility in solvent. Alkaloid, terpenoid and steroid are soluble in non polar solvent like acetone, diethyl ether and hexane. Meanwhile, flavonoid, phenol and glycoside dissolve in polar solvent such as water and methanol [20,21]. It is appropriate with the determinate solubility theory “like dissolve like” that polar substances will dissolve in polar solvent and vice versa [20,22].

Generally, plants contain two major substances; they are nutrition and non nutrition compounds. Primary metabolite or nutrition compounds such as carbohydrate, protein, fatty acids and phytosterol can be found in a huge proportion but they do not have pharmacology effect. On the other hand, non nutrition compounds or secondary metabolite like alkaloid, terpenoid, flavonoid and steroid are found in a small concentration but it have pharmacology effect at certain dose [20]. Secondary metabolites are derived from metabolism of primary metabolite in plant but sometimes they have a toxic effect especially if it is used in high dose. Most of flavonoid and terpenoid in herbs have a potency as antioxidant, antiseptic and anti-inflammatory whereas steroid as anti-inflammatory and sex hormone. But, the pharmacology effect of alkaloid is difficult to be predicted in medicinal plants because they have so many biological activities [23].

Anti-diabetic effect of herbs is indicated by their potency to decrease blood glucose level. The hypoglycemia effect is controlled by active compounds like terpenoid, steroid, alkaloid and flavonoid but their mechanisms of work are different. Some herbs work as anti-diabetes by mechanisms such as insulin

sensitizers, insulin secretory, DPP-IV inhibitor and α-glucosidase inhibitor [24]. Anti-diabetic herbs have many active compounds so that they have a possibility to work by multiple action and result in interactions either synergistic or antagonistic. Sometimes the interactions have both negative and positive pharmacology effect [4].

4.2. Molecular docking of U. lobata leaf extracts

Molecular docking is now widely used to discover new ligands for target of known structure. Potential compound can be screened by free energy binding. The score of free energy binding represents binding affinity of ligand to the target protein; the lower free energy binding, the higher binding affinity [25]. In addition, inhibition constant can be predicted using bioinformatics approach. The lowest inhibition constant indicates the most potential compound. Other parameter is surface interaction. It represents the molecular recognition between ligand and target protein. The higher value of surface interaction, the higher interaction possibilities of compounds interacting with the target protein [26]. Based on the findings in the present study, mangiferin have the lowest value of inhibition constants followed by stigmasterol. It is related to binding free energy and surface interaction of these compounds. In this study, stigmasterol has the highest value of surface interaction followed by β-sitosterol and mangiferin. A great result of surface interaction showed a stronger binding between ligand and protein target, so that the biology activity is higher. Based on the *in silico* analysis, mangiferin has the lowest value in binding free energy while stigmasterol and β-sitosterol were in the second and third position. The lowest value of binding free energy produces a strong binding molecule and then causes the potential biology activity. Free energy binding and surface interaction between ligand and protein target affects the inhibitory activity of *U. lobata* leaf extract on DPP-IV.

Molecular docking studies are widely used to predict the potential candidates of drugs in the pharmaceutical industry. Binding orientation of these small molecules or active compounds to their protein targets reveals their affinity and activity as possible candidates of drugs.

4.3. DPP-IV inhibitory activity of U. lobata

DPP-IV inhibitory activity of ethanolic extract of *U. lobata* is stronger than that of water extract. It is regulated by the differences of both active compounds and their proportions in these extracts. Semi qualitative test of *U. lobata* leaf extract by LC–MS showed the contents of stigmasterol, β-sitosterol, gossypetin and chrysoeriol which are higher than that of mangiferin, quercetine and hypolaetin. Active compounds such as stigmasterol, β-sitosterol and gossypetin are soluble in semi-polar solvents like alcohol but mangiferin and hypolaetin are insoluble. The differences of solubility of active compounds in the solvents will affect the percentages of active compounds in the extracts.

Both ethanolic and water extracts of *U. lobata* contain the same active compounds but different in amounts. Content of gossypetin is lower in water extract meanwhile content of stigmasterol is higher in water extract than that in ethanolic extract, but the proportions of chrysoeriol, mangiferin and β-sitosterol are similar in both water and ethanolic extract. Non-polar compounds such as stigmasterol, β-sitosterol and gossypetin could be extracted in water solvent even though in small amount. When the water is boiled, their polarity will decrease so that it could be extracted from semi-polar until non-polar compounds [27].

Molecular docking study of *U. lobata* leaf extract showed inhibitory activity on DPP-IV. Three active compounds such as mangiferin, stigmasterol and β-sitosterol showed a low value in binding free energy. It means that the binding between ligand and molecule target is easy so that cause a strong DPP-IV inhibitory activity. It is also supported by a low value from inhibitions constant of mangiferin, stigmasterol and β-sitosterol which showed a high DPP-IV inhibitory activity. The lower value of inhibitions constant means that these compounds with low doses are able to inhibit the DPP-IV activity. Surface interaction between DPP-IV and three compounds above showed a high score (stigmasterol: 962.48, β-sitosterol: 886.91, and mangiferin: 742.75). The compound with higher value of surface interaction has the potential to binding ligand and molecule target, predicting a stronger biological activity.

DPP-IV or CD26 is a membrane-associated peptidase of 766 amino acids that is widely distributed in numerous tissues. DPP-IV is hydrolase enzyme and also exists with a soluble circulating form in plasma, and significant DPP-IV-like activity is detectable in plasma from humans and rodents. DPP-IV (CD26) exerts its biological effects via two distinct mechanisms of action. First, as a membrane-spanning protein, it binds adenosine deaminase and when activated, conveys intracellular signals independent of its enzymatic function via dimerization and activation of intracellular signaling pathways. The signaling properties of membrane-associated CD26 have been most extensively characterized in T cells [27]. The second principal biological activity of CD26 (DPP-IV) is its enzymatic function. The enzymatic activity of CD26 is exhibited by the membrane-spanning form of the molecule, and by the slightly smaller circulating soluble form [27,28].

The substrates of CD26/DPP-IV are not specific to a particular peptides. The substrates of CD26/DPP-IV are proline or alanine containing peptides and include growth factors, chemokines, neuropeptides and vasoactive peptides. DPP-IV prefers substrates with an amino-terminal proline or alanine at position 2, but may also cleave substrates with non-preferred amino acids at position 2. The structure of incretin hormone such as GLP-1 and GIP reveals a highly conserved alanine at position 2, rendering these peptides ideal putative substrates for the aminopeptidase DPP-IV [29].

A number of study showed that the importance of DPP-IV mediated inactivation of GLP-1 as a key determinant of GLP-1 and GIP bioactivity [30]. DPP-IV inhibition prevents the degradation of active GLP-1 but does not increase the levels of circulating total GLP-1 and does not prevent the kidney from rapidly clearing GLP-1. DPP-IV inhibition also acutely decreases L cell secretion of GLP-1, likely via negative feedback on the L cell. The biological activities of GLP-1 are stimulating the secretion of insulin, increasing β-cell masses, inhibiting the secretion of glucagon, reducing the rate of gastric-emptying and inducing satiety that contribute to maintain blood glucose level in type 2 diabetes mellitus [1,2].

Using of DPP-IV inhibitors, primarily for the treatment of diabetes, relates to the potential effects of these inhibition on immune function. DPP-IV/CD26 is expressed on T cells, plays a functional role in T cell activation, and activates CD26 as signaling cascade in the T cell. CD26 associates with CD45, and

modulation of CD26 activity is frequently associated with enhanced T cell proliferation in immune system [29]. CD26/DPP-IV plays an important role in tumor biology, and is useful as a marker for various cancers, with its levels either on the cell surface or in the serum increased in some neoplasms and decreased in others [31].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This study was funded by Doctorate Research Grant of Directorate General of Higher Education Indonesia (No. 053/B.07/U.III/LPPM/2014).

References

[1] Wang Y, Li L, Yang M, Liu H, Boden H, Yang G. Glucagon-like peptide-1 receptor agonist versus insulin in inadequately controlled patients with type 2 diabetes mellitus: a meta-analysis of clinical trials. *Diabetes Obes Metab* 2011; **13**: 972-81.
[2] Saraiva FK, Sposito AC. Cardiovascular effects of glucagon-like peptide 1 (GLP-1) receptor agonists. *Cardiovasc Diabetol* 2014; **13**: 142.
[3] Singh AK. Dipeptidyl peptidase-4 inhibitors: novel mechanism of actions. *Indian J Endocrinol Metab* 2014; **18**(6): 753-9.
[4] Ábel T. A new therapy of type 2 diabetes: DPP-4 inhibitors. In: Rigobelo EC, editor. *Hypoglycemia-causes and occurrences*. Rijeka: Intech; 2011.
[5] Duez H, Smith AC, Xiao C, Giacca A, Szeto L, Drucker DJ, et al. Acute dipeptidyl peptiase-4 inhibition rapidly enhances insulin-mediated suppression of endogenous glucose production in mice. *Endocrinology* 2009; **150**(1): 56-62.
[6] Sharma A, Paliwal G, Upadhyay N, Tiwari A. Therapeutic stimulation of GLP-1 and GIP protein with DPP-4 inhibitors for type-2 diabetes treatment. *J Diabetes Metab Disord* 2015; **14**: 15.
[7] Omonkhua AA, Onoagbe IO. Preliminary proximate and phytochemical analyses of some medicinal plants used to treat diabetes mellitus in Nigeria. *Inven Impact Ethnopharmacol* 2010; **1**: 68-70.
[8] Onoagbe IO, Negbenebor EO, Ogbeide VO, Dawha IH, Attah V, Lau HU, et al. A study of the anti-diabetic effects of *Urena lobata* and *Sphenostylis stenocarpa* in streptozotocin-induced diabetic rats. *Eur J Sci Res* 2010; **43**: 6-14.
[9] Islam MH, Rahman KMH, Rahman S, Rahmatullah M. Preliminary antihyperglycemic, antinociceptive activity, phytochemical analysis and toxicity studies on leaves of *Urena lobata* L. *J Chem Pharm Res* 2015; **7**(4): 559-63.
[10] Sosa A, Rosquete C. Flavonoids from *Urena sinuata* L. *Av Quím* 2010; **5**(2): 95-8.
[11] Bharti SK, Sharma NK, Kumar A, Jaiswal SK, Krishnan S, Gupta AK, et al. Dipeptidyl peptidase IV inhibitory activity of seed extract of *Castanospermum australe* and molecular docking of their alkaloids. *Topclass J Herb Med* 2012; **1**: 29-35.
[12] Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, anti-peroxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoterapia* 2009; **80**(2): 123-6.
[13] Kanimozhi D, Ratha bai V. Evaluation of phytochemical antioxidant antimicrobial activity determination of bioactive components of ethanolic extract of aerial and underground parts of Cynodon dactylon L. *Int J Sci Res Rev* 2012; **1**(2): 33-48.
[14] Chen JH, Tsai CW, Wang CP, Lin HH. Anti-atherosclerotic potential of gossypetin via inhibiting LDL oxidation and foam cell formation. *Toxicol Appl Pharmacol* 2013; **272**(2): 313-24.
[15] Saeidnia S, Manayi A, Gohari AR, Abdollahi M. The story of beta-sitosterol-a review. *Eur J Med Plants* 2014; **4**(5): 590-609.
[16] Pumpkin Patel S. (*Cucurbita* sp.) seeds as a nutraceutic: a review on status quo and scopes. *Med J Nutr Metab* 2013; http://dx.doi.org/10.3233/s12349-013-0131-5.
[17] Matkowski A, Kuś P, Góralska E, Woźniak D. Mangiferin-a bioactive xanthonoid, not only from mango and not just antioxidant. *Mini Rev Med Chem* 2013; **13**(3): 439-55.
[18] Sellamuthu PS, Arulselvan P, Kamalraj S, Fakurazi S, Kandasamy M. Protective nature of *Mangifera* on oxidative stress and antioxidant status in tissues of streptozotocin-induced diabetic rats. *ISRN Pharmacol* 2013; http://dx.doi.org/10.1155/2013/750109.
[19] Chahar MK, Sharma N, Dobhal MP, Joshi YC. Flavonoids: a versatile source of anticancer drugs. *Pharmacogn Rev* 2011; **5**(9): 1-12.
[20] Çitoğlu GS, Acikara ÖB. Column chromatography for terpenoids an flavonoids. In: Dhanarasu S, editor. *Chromatography and its applications*. Rijeka: Intech; 2012.
[21] House JE. *Inorganic chemistry*. Massachusetts: Academic Press; 2008.
[22] Gupta A, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive plant extracts. *Int J Appl Nat Sci* 2012; **1**(1): 8-26.
[23] Evans WC. *Trease and Evans' pharmacognosy*. 15th ed. London: W.B Sauders Company Ltd; 2002.
[24] Chang CL, Lin Y, Bartolome AP, Chen YC, Chiu SC, Yang WC. Herbal therapies for type 2 diabetes mellitus: chemistry, biology, and potential application of selected plants and compounds. *Evid Based Complement Alternat Med* 2013; http://dx.doi.org/10.1155/2013/378657.
[25] Utomo DH, Widodo N, Rifa'i M. Identifications small molecules inhibitor of p53-mortalin complex for cancer drug using virtual screening. *Bioinformation* 2012; **8**: 426-9.
[26] Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J Cheminform* 2009; **1**: 15.
[27] Stevens MM, Honerkamp-Smith AR, Keller SL. Solubility limits of cholesterol, lanosterol, ergosterol, stigmasterol and β-sitosterol in electroformed lipid vesicles. *Soft Matter* 2010; **6**: 5882-90.
[28] Kanchanamala P, Rao AA, Rao PS, Sridhar GR. Drug design studies on dipeptidyl peptidase IV using auto dock tools. *J Pharm Res* 2011; **4**(11): 4113-6.
[29] DDP-4. Toronto: Glucagon.com; 2012. [Online] Available from: http://www.glucagon.com/dpp4.html [Accessed on 21th April, 2015]
[30] Gopalan B, Ravi D, Rasheed M, Hosamanesreedhara SHK, Ishtiyaque A, inventors. *Novel dipeptidyl peptidase IV inhibitors and process for their preparation and pharmaceutical composition containing them. WO2007113634A1*. 2010.
[31] Prabavathy N, Vijayakumari M, Minil M, Sathiyaraj U, Kavimani S. Linagliptin-a novel DPP-IV inhibitor. *Int J Pharma Bio Sci* 2011; **2**(1): 438-42.