International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 9, Issue 2, 2017

Original Article

STUDIES ON *IN VITRO* ANTIDIABETIC ACTIVITIES OF *HOPEA PONGA* AND *VITEX LEUCOXYLON*

ARUN KASHIVISHWANATH SHETTAR[1], ANKALA BASAPPA VEDAMURTHY[1,*]

[1]PG Department of Studies in Biotechnology and Microbiology, Karnatak University, Dharwad 580003, Karnataka, India
Email: vedamurthybt@gmail.com

Received: 22 Nov 2016 Revised and Accepted: 21 Dec 2016

ABSTRACT

Objective: Evaluating antidiabetic property of *Hopea ponga* and *Vitex leucoxylon* extracts by using *in vitro* assays.

Methods: The exhaustive serial extraction was carried out with a series of solvents: chloroform, ethyl acetate, methanol, ethanol and water with increasing polarity using Soxhlet apparatus. The concentrated and dried extracts were evaluated for antidiabetic activity by employing standard *in vitro* techniques (α-amylase and glucose uptake assay using yeast model in which the effects of extracts on α-amylase and glucose uptake was tested by considering the percentage of inhibition of α-amylase and increase in glucose uptake in yeast cells).

Results: *In vitro* antidiabetic studies show that in case of *Hopea ponga* methanol extract showed comparable antidiabetic activity with percentage of α-amylase inhibition 51.7925±0.92794 % and with IC50 value 96.53 µg and it was less on comparison with standard i.e. 71.0907±0.67796% with IC50 value 70.33 µg and in case of glucose uptake assay aqueous extract showed higher activity over all remaining extracts with percentage of inhibition 49.8100±0.62476% and with IC50 value 250.95 µg. whereas in case of *Vitex leucoxylon* aqueous extract exhibited significant activity in both performed assays i. e α-amylase inhibition and glucose uptake assay with percentage 54.6147±0.46397% and 57.1337±0.44201% respectively when compared to other solvent extracts.

Conclusion: Results confirm that aqueous extract of *Vitex leucoxylon* exhibited highest antidiabetic activity among all extracts. Additional studies are needed for purification, characterization and structural elucidation of bioactive compounds from aqueous extract and also confirm its antidiabetic property by *in vivo* studies. The present study provides scientific evidence that the leaves of *Hopea ponga and Vitex leucoxylon* possess anti-diabetic efficacy. Thus, considering its relative antidiabetic potency, these extracts are the useful therapeutic agents for treating and management of diabetes.

Keywords: *Hopea ponga*, *Vitex leucoxylon*, α-amylase assay, Glucose uptake assay, *In vitro* antidiabetic activity

DOI: http://dx.doi.org/10.22159/ijpps.2017v9i2.16280

INTRODUCTION

Diabetes mellitus is a complex and diverse group of disorders that disturbs the metabolism of the biomolecules such as carbohydrates, fats and proteins. According to World Health Organization studies, a total of 171 million cases of diabetes were registered worldwide by 2000. It is estimated that the number will significantly rise up to 366 million by 2030 [1]. Basically, diabetes mellitus is classified into two types, insulin dependent diabetes (type1) and Non-insulin dependent diabetes (type 2). Type1 diabetes is an autoimmune disease characterised by a local inflammatory reaction in and around islets that is followed by the selective destruction of insulin secreting β-cells. Type 2 diabetes is characterised by peripheral insulin resistance and impaired insulin secretion [2, 3]. The occurrence and consequences associated with diabetes are found to be high in the countries like India (31.7%), China (20.8%) and USA (17.17%). The rate is expected to rise up to 79.4%, 42.3% and 30.3% respectively by 2030 [4]. Diabetes mellitus is characterized by hyperglycemia that results from an absolute or relative insulin deficiency and is associated with long-term complications affecting eyes, kidneys, hearts and nerves [5]. In modern medicine, there is still no satisfactory effective drug or therapy to cure diabetes [6]. However, there are many synthetic drugs available as oral hypoglycemic agents and as drugs to treat diabetes but continuous use of synthetic drugs cause severe side effects and highly expensive. Recently the hypoglycemic agents from natural products, especially from plants, are gaining more importance due to their lower side effects, and these plants are provided with bioactive compounds called secondary metabolites which are not involved in the growth of plants but possess several biological activities such as antibacterial, antidiabetic, anti-inflammatory, anticancer, etc [7-10]. In ancient Indian literature medicinal properties of several herbal plants have been documented and the preparations have been found to be effective in the treatment of many severe diseases. Medicinal plants play an important role in the development of modern herbal medicines in the treatment of many diseases such as cancer, liver diseases, arthritis and diabetes [11]. Many medicinal plants are reported to be useful in the management and treatment of diabetes too [12]. Currently, there is a growing interest in herbal remedies due to the toxic effects associated with the oral hypoglycemic agents for the treatment of diabetes mellitus [13]. It is estimated that more than thousand plant species are being used as folk medicine for curing diabetes [14]. Herbal products or plant products are rich in flavonoids, phenolic compounds, terpenoids and other constituents which help to reduce blood glucose levels [15].

In the present study *Hopea ponga* and *Vitex leucoxylon* plants were selected for antidiabetic studies. *Hopea ponga* is an endemic tree belonging to Dipterocarpaceae family found in the tropical evergreen forest of western India and it is widely distributed along the western ghat of Karnataka [16]. *H. ponga* is categorized as an endangered tree species under the International Union for Conversation of Nature red list of threatened species. This plant was reported to be used as traditional medicine in the treatment of piles and snake bite [17]. The bark of *Hopea ponga* known to have a high content of tannin and act as astringent [18]. Methanolic extract of seed wings of *Hopea ponga* exhibited antioxidant and antibacterial activity [19]. *Vitex leucoxylon* is commonly known as five-leaved chaste trees and belongs to the verbenaceae family. It is small to large deciduous tree, growing up to 20 m in height. It is widely distributed along the Western Ghats of India. The leaves of *V. leucoxylon* are reported to have medicinal properties like relieving headache, fever and catarrh [20]. Reports indicate that aqueous and ethanolic extracts of *V. leucoxylon* leaves possess antipsychotic, antidepressant, analgesic, anti-inflammatory, anti-parkinsonian and antimicrobial activities [21, 22]. Even the root and bark of *V. leucoxylon* are reported to use as astringent and febrifuge [23]. Many hepatoprotective agents were isolated from leaves and bark of *V.*

leucoxylon which includes β-sitosterol, vitexin, isovitexin and aucubin [24]. The extensive literature survey exposed that only a few reports exist on these plant leaves, but no information are available on anti-diabetic activity in particular so with this background, the present study was undertaken to evaluate antidiabetic properties of *Hopea ponga* and *Vitex leucoxylon* plants collected from Western Ghats region of Karnataka, India by using *in vitro* assays.

MATERIALS AND METHODS

Plants collection

Leaves of *H. ponga* and *V. leucoxylon* were collected from Anashi forest range of Western Ghats, Uttar Kannada District, Karnataka, India during the period of May, 2015. The leaves were identified and authenticated by Dr. Kotresha K, Dept of Botany, Karnatak Science College, Dharwad; Karnataka by referring to the voucher specimen deposited in the Dept of Botany, Karnatak Science College, Dharwad, Karnataka (Voucher specimen No 002 and 003). Fresh plant leaves material was collected and washed under running tap water, shade-dried and then homogenised to coarsely powder. The powder was stored in airtight containers at-20 °C for further use for crude solvent extraction.

Chemicals and reagents

All the solvents and chemicals used were analytical grade and were obtained from Hi-media, India.

Crude extraction

Coarsely powdered dried leaves of *H. ponga* and *V. leucoxylon* [100g each] were subjected to successive solvent extraction using soxhlet apparatus separately. The extraction of each plant leaf material was done with different solvents in their increasing order of polarity which includes chloroform, ethyl acetate, methanol, ethanol and distilled water. Each time the plant material was dried and later extracted with next high polar solvent (following the strategy of extraction in series of increasing the solvent polarity). All extracts were concentrated in Buchi rotary evaporator, followed by removal of traces of solvent by using desiccator.

Evaluation of antidiabetic activity by using *in vitro* assays

α-amylase inhibitory assay

The α-amylase inhibitory assay for different solvent extracts of *H. ponga* and *V. leucoxylon* were evaluated according to a previously described method by Malik and Singh *et al.* (1980) with slight modification [25]. In brief, 0.5 ml of extract was mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added. The resulting mixture was incubated at room temperature for 10 min, and the reaction was terminated using 1 ml of dinitro salicylic acid colour reagent. At this time, the test tubes were placed in a water bath (100 °C and 5 min) and cooled until room temperature was reached. The mixture was then diluted with 10 ml of deionized water, and absorbance was determined at 540 nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following equation:

$$Percentage\ of\ inhibition\ of\ \alpha - amylase = Abs\ control - Abs\ sample \div Abs\ control \times 10$$

Where $Abs_{control}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α-amylase solution and Abs_{sample} corresponds to the solution with extract and α-amylase solution.

Glucose uptake in yeast cells

Glucose uptake assay by using yeast cells was performed according to the method of Cirillo *et al.*, (1963) [26]. The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of solvents extract of *H. ponga* and *V. leucoxylon* (50-250 µg/ml) were added to 1 ml of glucose solution (5 mmol) and incubated together for 10 min at 37 °C. The reaction was started by adding 100 µl of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and the amount of glucose was estimated in the supernatant. Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake (\%)} = \text{Abs sample} - \text{Abs control} \div \text{Abs sample} \times 10$$

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

Statistical analysis

All experiments were performed in triplicates (n=3) and the data are presented as the mean±standard error. Differences between the means of the individual groups were analysed using the analysis of variance procedure of SPSS software 20 Version (IBM). The significance of differences was defined at the $p<0.05$ and $p<0.01$ level.

RESULTS

In vitro antidiabetic studies show that in case of *Hopea ponga* methanol extract showed comparable antidiabetic activity with percentage of α-amylase inhibition 51.7925±0.92794 % and with IC50 value 96.53 µg (table 1) and it was less on comparison with standard i.e. 71.0907±0.67796% with IC50 value 70.33 µg and in case of glucose uptake assay aqueous extract showed higher activity over all remaining extracts with percentage of inhibition 49.8100±0.62476% and with IC50 value 250.95 µg (table 2). whereas in the case of *Vitex leucoxylon* aqueous extract exhibited significant activity in both performed assays i. e α-amylase inhibition and glucose uptake assay with percentage 54.6147±0.46397% (table 3) and 57.1337±0.44201% (table 4) respectively when compared to other solvent extracts.

Table 1: α-Amylase inhibitory activities and IC50 values by *Hopea ponga* extracts

Samples	Concentration	Inhibition (I %)	IC50 (µg/ml)
Chloroform	100µg/ml	17.3531±0.49582	288.13 µg
Ethyl acetate	100µg/ml	23.8748±1.15430	209.42 µg
Methanol	100µg/ml	51.7925±0.92794**	96.53 µg
Ethanol	100µg/ml	34.6300±0.79637*	144.38 µg
Aqueous	100µg/ml	39.2830±0.80725*	127.28 µg
Standard (Acarbose)	100µg/ml	71.0907±0.67796**	70.33 µg

Results are expressed as mean±SE (n=3); * significant at the $p<0.01$, Correlation is significant at the 0.01 level (2-tailed)**, Correlation is significant at the 0.05 level (2-tailed)*

Table 2: Percentage of glucose uptake in yeast cells treated with *Hopea ponga* extracts

Samples	Concentration(µg/ml)	Inhibition (%)	IC50(µg/ml)
Standard	50µg	51.4728±1.00666**	48.56 µg
	100 µg	58.2081±1.05007**	
	150 µg	62.4862±0.50774**	
	200 µg	65.7095±0.28285**	
	250 µg	69.7436±0.25643**	
Chloroform extract	50µg	25.6256±2.35322**	264.55 µg
	100 µg	34.7297±1.88549**	
	150 µg	37.1012±0.72229**	
	200 µg	41.6643±1.02379**	
	250 µg	47.2484±0.33470**	
Ethyl acetate extract	50µg	19.0656±1.19369**	325.51 µg
	100 µg	24.5781±1.05284**	
	150 µg	30.4250±0.58222**	
	200 µg	35.6433±1.50203**	
	250 µg	38.4007±1.36886**	
Methanol extract	50µg	34.7905±1.29548**	276.32 µg
	100µg	38.1814±0.70638**	
	150 µg	42.6166±0.60840**	
	200 µg	41.4576±0.41221**	
	250 µg	45.2360±0.55400**	
Ethanol extract	50µg	28.6667±1.29070*	345.18 µg
	100 µg	32.0634±0.63493*	
	150 µg	36.0456±2.81443*	
	200 µg	35.9924±0.49278*	
	250 µg	36.2123±1.51351*	
Aqueous extract	50µg	38.1814±0.70638**	250.95 µg
	100 µg	42.5946±1.00320**	
	150 µg	44.6185±1.21045**	
	200 µg	47.6185±0.68020**	
	250 µg	49.8100±0.62476**	

Results are expressed as mean±SE (n=3); * significant at the p<0.01., Correlation is significant at the 0.01 level (2-tailed)**, Correlation is significant at the 0.05 level (2-tailed)*

Table 3: α-Amylase inhibitory activities and IC50 values by *Vitex leucoxylon* extracts

Samples	Concentration	Inhibition (I %)	IC50 (µg/ml)
Chloroform extract	100µg/ml	4.2715±0.46400	1170.54 µg
Ethyl acetate extract	100µg/ml	10.5262±0.60544	475 µg
Methanol extract	100µg/ml	26.6209±1.19150	187.82 µg
Ethanol extract	100µg/ml	30.9687±1.00906	161.45 µg
Aqueous extract	100µg/ml	54.6147±0.46397*	91.55 µg
Standard (Acarbose)	100µg/ml	71.0907±0.67796**	70.33 µg

Results are expressed as mean±SE (n=3); * significant at the p<0.01., Correlation is significant at the 0.01 level (2-tailed)**, Correlation is significant at the 0.05 level (2-tailed)*

Table 4: Percentage of Glucose uptake in yeast cells treated with *Vitex leucoxylon* extracts

Samples	Concentration(µg/ml)	Inhibition (%)	IC50(µg/ml)
Standard	50µg	51.4728±1.00666**	48.56 µg
	100 µg	58.2081±1.05007**	
	150 µg	62.4862±0.50774**	
	200 µg	65.7095±0.28285**	
	250 µg	69.7436±0.25643**	
Chloroform extract	50µg	16.7237±1.28448**	345.18 µg
	100 µg	24.5781±1.05284**	
	150 µg	27.2281±1.27374**	
	200 µg	32.9860±1.11015**	
	250 µg	36.2123±1.51351**	
Ethyl acetate extract	50µg	18.6284±0.79641**	307.13 µg
	100 µg	28.9353±2.14780**	
	150 µg	31.7131±1.18220**	
	200 µg	38.3721±1.67544**	
	250 µg	40.6992±1.10100**	
Methanol extract	50µg	27.2281±1.27374**	340.07 µg

	100µg	31.7131±1.18220**	
	150 µg	33.8680±1.58547**	
	200 µg	34.3361±2.64282**	
	250 µg	36.7567±1.62163**	
Ethanol extract	50µg	14.2127±1.77001*	365.79 µg
	100 µg	20.4082±1.15458*	
	150 µg	24.6000±0.40000*	
	200 µg	30.6782±1.78932*	
	250 µg	34.1724±1.56321*	
Aqueous extract	50µg	36.8129±0.96036**	199.37 µg
	100 µg	43.0174±1.36312**	
	150 µg	45.6311±0.88967**	
	200 µg	50.1573±0.62776**	
	250 µg	57.1337±0.44201**	

Results are expressed as mean±SE (n=3); * significant at the p<0.01., Correlation is significant at the 0.01 level (2-tailed)**, Correlation is significant at the 0.05 level (2-tailed)*

DISCUSSION

Diabetes is the major health problem and continues to be one of the major causes of the death all over the world. Various therapeutic agents are available in medicine to treat diabetes, but they are toxic, expensive and associated with many side effects [27]. The alpha-amylase enzyme is known as one of the key enzymes in a human digestive system which converts starch to monosaccharide and causes the rise in the blood glucose [28]. Amylase acts upon large polysaccharides (starch) at internal bands. The inhibition of alpha-amylase has been suggested as a strategy for diabetes and obesity management by reducing sugars levels in the blood. Although modern medicines have introduced many synthetic therapeutic agents like insulin, biguanides, sulfonylureas and thiazolidinedione are to treat diabetes but still there are no any satisfactory drugs to avoid diabetic complications [29]. Traditional medicinal plants having anti-diabetic properties can provide useful sources for the discovery of safer hypoglycemic agents [30]. These plants are the major source for discovering new compounds with therapeutic value for drug development against most common and very prevalent disease, diabetes mellitus. More than 1200 plants were identified experimentally to be used in the treatment of diabetes due to several biological activities of their constituents [31]. Enzyme inhibition assay for plant extracts determines the inhibitory potency of the sample against the enzyme, and it is one of the mechanisms through which plant could show its antidiabetic activity. In the present study, the concentrated and dried extracts *H. ponga* and *V. leucoxylon* were evaluated for antidiabetic activity by employing standard *in vitro* techniques (Alpha-amylase and glucose uptake assay using yeast model). In the Alpha-amylase inhibitor assay, the known concentration (100µg) of different solvent extracts of *H. ponga* and *V. leucoxylon* were subjected to α-amylase inhibitory assay along with Acarbose as a standard. In the case of *H. ponga* out of five solvent extracts, methanol extract exhibited higher activity over all remaining extracts with the percentage of inhibition 51.7925±0.92794 % and with IC50 value 96.53 µg and it was less on comparison with standard i.e. 71.0907±0.67796% with IC50 value 70.33 µg. In the case of *V. leucoxylon* the aqueous extract showed higher activity among all other extracts as well as methanol extract of *H. ponga* with the percentage of inhibition 54.6147±0.46397% and with IC50 value 91.55 µg. In Glucose uptake in Yeast cells model the different solvent extracts of *H. ponga* and *V. leucoxylon* leaves at different concentrations (50µg-250 µg) are subjected to *in vitro* glucose uptake assay using yeast as a model. The percentage of glucose uptake in yeast cells by the extract was compared with Metronidazole standard drug. In the case of *H. ponga* out of five solvent extracts, aqueous extract showed higher activity over all remaining extracts with the percentage of glucose uptake 49.8100±0.62476% and with IC50 value 250.95 µg. In the case of *V. leucoxylon* the aqueous extract showed higher activity among all other extracts as well as an aqueous extract of *H. ponga* with the percentage of inhibition 57.1337±0.44201% and with IC50 value 199.37 µg and both plant extracts exhibited lesser activity on comparison with Metronidazole standard drug. Already over 400 traditional plants for diabetes has been reported, although only a small number of these have received a scientific and medical evaluation to assess their efficacy [32, 33]. Based on the results obtained from different *in vitro* anti-diabetic assays, there is a significant difference in anti-diabetic activity of different extracts evaluated and the results revealed that these plant extracts might possibly reduce the blood glucose level in diabetes patients by inhibiting the action of the alpha-amylase enzyme.

CONCLUSION

In the present study in both *in vitro* methods both plant extracts showed antidiabetic activity but among tested extracts methanol extract of *H. ponga* and aqueous extract of *V. leucoxylon* exhibited higher antidiabetic activity overall extracts with a good percentage of inhibition. The result of this study are encouraging which may offer a safe method or supplement treatment strategy to control diabetes through its alpha-amylase inhibition, Therefore; their derived products may be an important source of nutrition and therapy. Further, purification of the specific active constituents needs to be carried out; that can serve in the development of new pharmaceuticals to treat diabetes mellitus.

CONFLICT OF INTERESTS

We wish to confirm that there are no known conflicts of interest associated with this publication.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
2. Foulis AK. The pathogenesis of β cell destruction in Type-1 (insulin dependent) diabetes mellitus. J Pathol 1987;152:141-8.
3. Gepts W, LeCompte PM. The pathology of type 1 (juvenile) diabetes. In: BW Yolk, ER Arquilla. Eds. The diabetic pancreas, Plenum, New York; 1985. p. 337-65.
4. Trease A, Evans WC. Pharmacognosy. 14th Edn. WB Saunders Company, limited, London; 1996. p. 35-7, 219, 224-8.
5. Gispen WH, Biessels GJ. Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci 2000;23:542-9.
6. Piedrola G, Novo E, Escober F, Garcia -Robles R. White blood cell count and insulin resistance in patients with coronary artery disease. Annee Endocrinol 2001;62:7-10.
7. Stamp N. Out of the quagmire of plant defence hypotheses. Quarterly Rev Biol 2003;78:23-55.
8. Aeschbacher HU, Meier H, Ruch E. Nonmutagenicity *in vivo* of the food flavonol quercetin. Nutr Cancer 1982;2:90.
9. Reinhold U, Seiter S, Ugurel S, Tilgen W. Treatment of progressive pigmented purpura with oral bioflavonoids and ascorbic acid: an open pilot study in 3 patients. J Am Acad Dermatol 1999;41:207-8.
10. Knekt P, Jarvinen R, Seppanan R, Heliovaara M, Teppo L, Pukkala E, *et al.* Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 1997;146:223-30.
11. Prashanth KN, Neelam S, Chauhan S, Harishpadhi B, Ranjani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. J Ethnopharmacol 2006;107:182-8.

12. Joy KL, Kuttan R. Antidiabetic activity of *Picorrhiza kurroa* extract. J Ethnopharmacol 1999;167:143-8.
13. Bhalodi M, Shukla S, Saluja AK. *In vitro* antioxidant activity of the flowers of *Ipomoea aquatic forsk*. Pharmacogn Mag 2008;4:220-6.
14. Bhandari MR, Anurakkun NJ, Hong G, Kawabata J. Alpha glucosidase and alpha-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). Food Chem 2008;106:247-52.
15. Jung M, Park M, Chul HL, Kang Y, Seok-Kang E, Ki-Kim S. Antidiabetic agents from medicinal plants. Curr Med Chem 2006;13:1-16.
16. Shiddamallayya N, Azra Y, Gopakumar K. Medico botanical survey parvatha kkuke subramanaya mangalore Karnatak. Indian J Tradi Med 2008;9:96-9.
17. Muralikrishnan H, Chandrashekar KR. Regeneration of *hopea ponga*: influence of wing loading and viability of seeds. J Trop Forest Sci 1997;10:58-65.
18. Shivaprasad PV, Vasanthraj BK, Chandrashekar KR. Dipterocarps of the Western Ghats of Karnataka. Indian J Forest 1999;9:201-6.
19. Sukesh, Syed Hidayath, Haneef M, Arunkumar K, Chandrashekar KR. Phytochemical evaluation, antioxidant and antibacterial activity of seed wings of *Hopea ponga* (Dennst). Mabberly Int J Pharm Pharm Sci 2011;8:2593-5.
20. Chanda YR. The wealth of India: a dictionary of Indian raw materials and Industrial products. Publication and Information Directorate, CSIR, New Delhi; 1982. p. 520-21.
21. Makwana HG, Ravishankar B, Shukla VJ, Vijayan NP, Sasikala CK, Saraswathy VN, *et al*. General pharmacology of *Vitex leucoxylon* linn leaves. Indian J Physiol Pharmacol 1994;38:95-100.
22. Sarma SP, Aithal KS, Srinivasan KK, Udupa AL, Kumar V, Kulkarni DR, *et al*. Anti-inflammatory and wound healing activities of the crude alcoholic extract and flavonoids of *Vitex leucoxylon*. Fitoterapia 1990;61:263-5.
23. Meena AK, Uttam Singh, Yadav AK, Singh B, Rao MM. Pharmacological and phytochemical evidence for the extracts from plants of the genus vitex–a review. Int J Pharm Clin Res 2010;2:1-9.
24. Rao RVK, Satyanarayana T, Jena R. Phytochemical studies on *Vitex leucoxylon* L. Indian Drugs 1997;34:50-1.
25. Malik CP, Singh MB. Plant Enzymology and Histoenzymology, Kalyani Publishers: New Delhi; 1980. p. 278.
26. Cirillo VP. Sugar transports in psychrophilic yeast. J Bacteriol 1963;84:485–91.
27. Apostolidis E, Lee CM. *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant-mediated-glucosidase and-amylase inhibition. J Food Sci 2010;75:97–102.
28. Oboh G, Ademiluyi AO, Akinyemi AJ, Henle TH, Saliu JA, Schwarzenbolz U. Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorus olitorius*) on key enzyme linked to type 2 diabetes (alpha amylase and alpha glucosidase) and hypertension (angiotensin I converting) *in vitro*. J Funct Foods 2012;4:450–8.
29. Yao X, Ling Zhu L, Chen Y, Tian J, Wanga Y. *In vivo* and *in-vitro* antioxidant activity and a-glucosidase, a-amylase inhibitory effects of flavonoids from *Cichorium glandulosum* seeds. Food Chem 2013;139:59–66.
30. Sunila C, Agastian P, Kumarappan C, Ignacimuthu S. *In vitro* antioxidant, antidiabetic and antilipidemic activities of *Symplocos cochinchinensis* (Lour.) S. Moore bark. Food Chem Toxicol 2012;50:1547-53.
31. Kambouche N, Merah B, Derdour A, Bellahouel S, Bouayed J, Dicho A, *et al*. Hypoglycemic and antihyperglycemic effects of *Anabasis articulata* (Forssk) Moq (Chenopodiaceae), an Algerian medicinal plant. Afr J Biotechnol 2009; 8:5578e83.
32. Ajithadas Aruna, Ramraj Nandhini, Venkatachalam Karthikeyan, Pandi Bose, Kannappan Vijayalakshmi. Comparative anti-diabetic effect of methanolic extract of insulin plant (costus pictus) leaves and its silver nanoparticle. Indo Am J Pharm Res 2014;4:3217-30.
33. Mani Rupeshkumar, Kunchu Kavitha, Pallab Kanti Haldar. Role of herbal plants in the diabetes mellitus therapy: an overview. Int J Appl Pharm 2014;6:1-3.

How to cite this article

- Arun Kashivishwanath Shettar, Ankala Basappa Vedamurthy. Studies on *in vitro* antidiabetic activities of *Hopea ponga* and *Vitex leucoxylon*. Int J Pharm Pharm Sci 2017;9(2):263-267.