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

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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 51.79±0.9279 % and with IC50 value 96.53 µg and it was less on comparison with standard i.e. 71.09±0.67796 % with IC50 value 70.33 µg and in case of glucose uptake assay aqueous extract showed higher hypoglycemic effect exhibiting extracts with percentage of inhibition 49.81±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 54.61±0.46397% and 57.13±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

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). Type 1 diabetes is an autoimmune disease characterized 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 (17.1%). 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 astrigent [18]. Methanolic extract of seed wings of Hopea ponga exhibited antioxidant and antibacterial activity [19]. Vitex leucoxylon is commonly known as five-leaved chastate trees and belongs to the verbenaecae 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 cataract [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 astrigent and febrifuge [23]. Many hepatoprotective agents were isolated from leaves and bark of V.
leucoxyylon 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 Gahs 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 Gahs, Uttar Kannada District, Karnataka, India during the period of May, 2015. The leaves were identified and authenticated by Dr. Kotesha K, Dept of Botany, Karnataka Science College, Dharwad; Karnataka by referring to the voucher specimen deposited in the Dept of Botany, Karnataka 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 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 polarity solvent). 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 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:

\[
\text{Percentage of inhibition of } \alpha\text{-amylase} = \frac{\text{Abs control} - \text{Abs sample} + \text{Abs control}}{\text{Abs control}} \times 10
\]

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 (3,000g, 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:

\[
\text{Increase in glucose uptake} = \frac{\text{Abs sample} - \text{Abs control} + \text{Abs sample}}{\text{Abs control}} \times 10
\]

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 anti diabetic studies show that in case of Hopea ponga methanol extract showed comparable anti diabetic 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.

| Samples       | Concentration | Inhibition (%) | IC50 (µg/ml) |
|---------------|---------------|----------------|--------------|
| Chloroform    | 100 µg/ml     | 17.3531±0.49582| 288.13 µg    |
| Ethyl acetate | 100 µg/ml     | 23.8748±1.15430| 209.42 µg    |
| Methanol      | 100 µg/ml     | 51.7925±0.92794| 96.53 µg     |
| Ethanol       | 100 µg/ml     | 34.6300±0.79637| 144.38 µg    |
| Aqueous       | 100 µg/ml     | 39.2830±0.80725| 127.28 µg    |
| Standard (Acarbose) | 100 µg/ml | 71.9070±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                  | 51.47±1.00666" | 48.56 µg     |
|                    | 100 µg                | 52.01±1.05007" |              |
|                    | 150 µg                | 62.48±0.50774" |              |
|                    | 200 µg                | 65.79±0.28285" |              |
|                    | 250 µg                | 69.74±0.25643" |              |
| Chloroform extract | 50µg                  | 25.62±2.35322" | 264.55 µg    |
|                    | 100 µg                | 34.73±1.88549" |              |
|                    | 150 µg                | 37.10±0.72229" |              |
|                    | 200 µg                | 41.66±0.02379" |              |
|                    | 250 µg                | 47.24±0.33470" |              |
| Ethyl acetate      | 50µg                  | 19.07±1.19369" | 325.51 µg    |
|                    | 100 µg                | 24.58±0.05284" |              |
|                    | 150 µg                | 30.42±0.58222" |              |
|                    | 200 µg                | 35.64±0.50083" |              |
|                    | 250 µg                | 38.40±0.36890" |              |
| Methanol extract   | 50µg                  | 34.79±0.70638" | 276.32 µg    |
|                    | 100 µg                | 42.16±0.60840" |              |
|                    | 150 µg                | 41.45±0.41221" |              |
|                    | 200 µg                | 45.23±0.55460" |              |
| Ethanol extract    | 50µg                  | 28.66±0.29070" | 345.18 µg    |
|                    | 100 µg                | 32.06±0.63493" |              |
|                    | 150 µg                | 36.04±0.81443" |              |
|                    | 200 µg                | 35.99±0.49278" |              |
| Aqueous extract    | 50µg                  | 38.18±0.70630" | 250.95 µg    |
|                    | 100 µg                | 42.59±0.00320" |              |
|                    | 150 µg                | 44.61±0.21045" |              |
|                    | 200 µg                | 47.18±0.68020" |              |
|                    | 250 µg                | 49.81±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 (µg/ml) | Inhibition (%) | IC50 (µg/ml) |
|------------------|-----------------------|----------------|--------------|
| Chloroform extract | 100µg/ml              | 4.27±0.64400" | 1170.54 µg    |
| Ethyl acetate     | 100µg/ml              | 10.52±0.60544" | 475 µg       |
| Methanol extract  | 100µg/ml              | 26.60±1.19150" | 187.82 µg    |
| Ethanol extract   | 100µg/ml              | 30.96±0.09906" | 161.45 µg    |
| Aqueous extract   | 100µg/ml              | 54.61±0.46397" | 91.55 µg     |
| Standard (Acarbose) | 100µg/ml            | 71.09±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                  | 51.47±1.00666" | 48.56 µg     |
|                  | 100 µg                | 52.01±1.05007" |              |
|                  | 150 µg                | 62.48±0.50774" |              |
|                  | 200 µg                | 65.79±0.28285" |              |
|                  | 250 µg                | 69.74±0.25643" |              |
| Chloroform extract | 50µg                  | 25.62±2.35322" | 264.55 µg    |
|                  | 100 µg                | 34.73±1.88549" |              |
|                  | 150 µg                | 37.10±0.72229" |              |
|                  | 200 µg                | 41.66±0.02379" |              |
|                  | 250 µg                | 47.24±0.33470" |              |
| Ethyl acetate    | 50µg                  | 19.07±1.19369" | 325.51 µg    |
|                  | 100 µg                | 24.58±0.05284" |              |
|                  | 150 µg                | 30.42±0.58222" |              |
|                  | 200 µg                | 35.64±0.50083" |              |
|                  | 250 µg                | 38.40±0.36890" |              |
| Methanol extract | 50µg                  | 16.72±1.28448" | 345.18 µg    |
|                  | 100 µg                | 24.58±0.05284" |              |
|                  | 150 µg                | 27.22±1.27374" |              |
|                  | 200 µg                | 32.98±1.11015" |              |
|                  | 250 µg                | 36.21±1.51351" |              |
| Ethanol extract  | 50µg                  | 18.62±0.79641" | 307.13 µg    |
|                  | 100 µg                | 28.93±2.14780" |              |
|                  | 150 µg                | 31.71±1.18220" |              |
|                  | 200 µg                | 38.21±1.67544" |              |
|                  | 250 µg                | 40.69±1.10100" |              |
| Aqueous extract  | 50µg                  | 27.22±1.27374" | 340.07 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)
The concentrated and dried extracts of the plant could show its antidiabetic activity. In the present study, the aqueous extract showed higher activity among all other extracts as well as methanol extract over all remaining extracts with the percentage of inhibition of five solvent extracts, methanol extract exhibited higher activity with IC50 value 96.53 µg and it was less on comparison with standard i.e. 71.0907±0.67796% with IC50 value 51.7925±0.92794 % and with IC50 value 96.53 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.

### 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 sugar 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 Acrabose 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 anti-diabetic 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.

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