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Original Research Article

## IN-VITRO ANTI DIABETIC EFFICACY (POTENTIAL) OF LEAF ETHANOL EXTRACT FRACTIONATES OF PISONIA GRANDIS R.BR

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### ABSTRACT

In this study, the anti diabetic potential of column fractions of leaf ethanol extract of *P.grandis* was assessed by  $\alpha$ -amylase inhibition assay. Acarbose (250 $\mu$ g/mL) used as a standard for reference showed 67% inhibition efficiency. The results of the study disclose the amylase inhibition potential of all fractions of *P.grandis* which can be correlated to anti diabetic potential. The isolated molecule pinitol (250  $\mu$ g/mL), the fraction PGL aq.1 obtained by liquid-liquid extraction of *P.grandis* leaves and the column fractionate PGL1% (both at a concentration of 20  $\mu$ g/mL) exhibited the highest inhibition of  $\alpha$ -amylase. All other fractionates (at concentration of 20  $\mu$ g/mL) also exhibited moderate inhibition of  $\alpha$ -amylase. The promising use of *P.grandis* extracts in formulating anti diabetic agents is obvious from the results.

**Keywords:** *Diabetes mellitus*,  $\alpha$ -amylase, acarbose, pinitol, *Pisonia grandis*

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### INTRODUCTION

*Diabetes mellitus* is a universal disorder with an alarming statistical increase. The Times of India reports 70 million people to be affected by 2015 (Times of India, Jul 30, 2009). This exploding rate necessitates research in this field of study. No single medicine or diet regime is applicable universally as this disorder is dependent on various physiological and genetic factors. Side effects associated with modern medicines warrant use of herbal medicines.

Medicinal plants are widely used for the development of new drugs in the control of diabetes. Several plants have been used in the control of *Diabetes mellitus*. Literature reports reveal use of bitter melon (Joseph and Jini, 2013), *Coccinia indica*, *Azadirachta indica*, *Syzygium cumini*, *Trigonella foenum-graecum*, *Terminalia chebula*, *Ficus racemosa*, *Momordica charantia*, *Swietenia mahagoni* (Ockvirk *et al.*, 2013), *Galega officinalis* (Modak *et al.*, 2007), *Gymnema sylvestre* (Kanetkar *et al.*, 2007) and sweet potato (Dutta, 2015) in the control of blood sugar.

*Pisonia grandis*, a common ornamental plant is reported to possess several biological and pharmacological activities. Recent *in vivo* studies have also established the anti diabetic potential of the ethanolic extract of this plant (Vimalavalli and Sugumar, 2015). Whole plants usually is a blend of several metabolites and elements. The synergistic effect of this hodgepodge of metabolites may be sometimes beneficial

but may reduce the efficacy of an individual metabolite. Hence bio-assay guided fractionation is often preferred. A fraction with comparably significant activity is chosen for making formulations.

There are several screening methods used to ascertain the anti diabetic nature of plants. There are mushrooming research papers on the anti diabetic potential of plants. Screening tools employed conventionally are generally time consuming, expensive and lack scientific evidence. *In vitro* tests play a significant role in the evaluation of anti diabetic activity of drugs and in screening plants for establishing their anti diabetic potential. Anti diabetic activity of few plants studied *in vitro* using  $\alpha$ -amylase assay is reported (Nirmala and Pandian, 2015). The methanolic extracts of *Cinnamomum zeylanicum*, *Artocarpus altilis* and *Artocarpus heterophyllus* are reported to exhibit 50% alpha amylase inhibition activity revealing its potential in control of diabetes (Sindhu et al, 2013). Hence the interest, in exploiting the facile *in vitro* method viz. amylase inhibition assay, to establish the anti diabetic potential of the plant extracts.  $\alpha$ - amylase assay is a simple economic tool to screen medicinal plants for their anti diabetic activity and has been utilized in the present study to screen the leaf ethanol extract fractionates of *P.grandis*.

## **Materials and methods**

### ***Collection of Plant material***

The leaf, stem and roots of *P.grandis* were collected from residential areas in and around Coimbatore and shade dried and pulverised.

### ***Preparation of ethanol extract and dewaxed ethanol extracts of leaves, stem and roots of P.grandis***

The pulverized plant part (leaf, stem and root) was thoroughly percolated and extracted with ethanol for about six hours. The ethanol extract obtained was filtered and concentrated under reduced pressure. A part of the ethanol extract concentrate was sequentially dewaxed with pet-ether and chloroform to obtain the dewaxed extract concentrates. The extract concentrates are designated PGLE, dPGLE and ddPGLE (leaf extracts), PGSE and dPGSE (stem extracts), PGRE and dPGRE (root extracts).

### ***Fractionation of leaf ethanol extract LLE***

The concentrated greenish black pasty solid was macerated with water and subjected to liquid-liquid extraction (LLE) with chloroform followed by pet-ether to obtain the organic and aqueous fractions designated as PGLC, PGLaq1 and PGLaq2

### **Extraction of Leaves by percolation and heating**

Shade dried leaves of *P.grandis* (10g) were extracted by percolation and heating (1 h) with hydro ethanol solvent of various concentrations [80%, 90% and 100% ethanol. The resulting extracts were concentrated under vacuum and designated as 80% PGLED, 90% PGLED and 100 % PGLED. Similarly fresh leaves of *P.grandis* (10g) were extracted by percolation and heating (1h) with aqueous ethanol [80%, 90%] and 100% ethanol. The resulting extracts were concentrated under vacuum and designated as 80% PGLEF, 90% PGLEF and 100 % PGLEF.

### ***Column chromatographic analysis of leaf-ethanol extract of P.grandis***

The leaf ethanol extract concentrate of *P.grandis* was subjected to column chromatographic analysis over silica gel with a gradient solvent of chloroform and chloroform-methanol mixtures. The eluants were tested for homogeneity by TLC and similar fractions were combined. Eight major fractions were (F I to F VIII) obtained.

F I was eluted with chloroform: methanol (99:1)

F II was eluted with chloroform: methanol (97:3) and yielded a white colored crystalline substance designated as **PG1**

F III eluted with chloroform: methanol (90:10)

F IV was eluted with chloroform: methanol (85:15) and yielded brownish crystalline substance designated as **PG 2**

F V was eluted with chloroform: methanol (75:25)

F VI was eluted with chloroform: methanol (50:50)

F VII was eluted with chloroform: methanol (25:75)

F VIII was eluted with methanol

The leaf ethanol extract concentrate PGLE, the dewaxed ethanol extracts of leaf, stem and roots of *Pisonia grandis* (dPGLE, ddPGLE, dPGSE and dPGRE), the LLE fractionates of the leaf ethanol extract (PGLC, PGLaq1 and PGLaq2), the hydro ethanolic fractions and the column fractionates (F I –F VIII) were tested for their *in vitro* anti diabetic potential.

#### **Pre-preparation of plant extracts for $\alpha$ -amylase inhibition assay**

Each extract sample (1g) was sonicated with 100ml deionised water for 20 min. The solutions were filtered, concentrated at 50°C and dissolved in DMSO to give suitable working solutions. Amylase inhibitory molecules like proteins and glycans gets extracted in more polar solvents like water and hence aqueous extracts were used in the study.

#### **$\alpha$ -Amylase inhibition assay**

The procedure of **Giancarlo *et al.*, (2006)** for  $\alpha$ -amylase inhibition assay was adopted for screening various extract fractions and column fractionates of *P.grandis* for anti diabetic potential. Potato starch (100mg) was added to 100 mL sodium phosphate buffer (pH 6.9) (20 mM) containing 6.7 mM sodium chloride and boiled for 15 min. This solution constitutes starch solution of 0.1% w/v. Sodium potassium tartrate (12 g) dissolved in sodium hydroxide (8 mL, 2 M) and 3, 5-dinitrosalicylic acid (96 mM) under stirring constitutes the colouring reagent. The  $\alpha$ -amylase enzyme (25 mg) was dissolved in 100 mL deionised water and used for further studies.

The mixture of the plant extract (20  $\mu$ L) and starch solution (1 mL) was incubated initially for 20minutes at 20°C and additionally for 3min after the addition of  $\alpha$ -amylase (1 mL). The solution was then heated on a water bath at 75-80°C for 15 min after the addition of 1mL colouring reagent. The colour developed was measured at 540 nm in a photo colorimeter. A similar study was conducted with fractionates, a blank solution and acarbose. The accuracy of the study was ascertained through triplicate measurements.

#### **2.5 Calculation of inhibition efficiency**

The  $\alpha$ -amylase inhibitory activity, expressed as % inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \left[ \frac{A_{540}^{(\text{blan})} - \left( \frac{A_{540}^{(\text{sampl})} - A_{540}^{(\text{blan})}}{k} \right)}{A_{540}^{(\text{blan})}} \right] \times 100$$

## **Results and Discussion**

Herbal medicines are on an increase to control diabetes. The many available traditional herbs for control of diabetes require scientific evidence for their activity. In the present study an indigenous plant well-known for its anti diabetic potential has been screened with reference to commercially prescribed anti diabetic drug acarbose. Though there are reports on the anti diabetic activity of the ethanol extract of leaves of plant *Pisonia grandis* (Vimalavalli and Sugumar 2015) there is no work on the anti diabetic study of extract fractionates which is of recent interest in research. Crude extracts are often a complex mixture of several metabolites. Fractionates contain bioactive agents responsible for activity. The quantity of bioactive agent varies from fraction to fraction. Hence it is of prime concern to employ suitable techniques to arrive at fractionate rich in

bioactive compounds. In this study open column chromatography was employed to collect fractions of *P.grandis* in a sequential manner with both non-polar and polar solvents of increasing polarity.

In the process of alpha amylase inhibition primarily a complex is formed between starch-iodine and plant extract. The hydrolysis of starch to sugars is aided by  $\alpha$ -amylase, the inhibition of which setbacks carbohydrate digestion and hence the rate of glucose absorption (Lordan *et al.*, 2013).

The results of the *in vitro* anti diabetic effect of the various extracts of *P.grandis* and isolated compounds PG1 and PG2 is summarized in Table 1.

The inhibition efficiency of whole ethanol extract of leaves was higher (c.a.66%) compared to that of the dewaxed extracts dPGLE and ddPGLE (c.a 31% and 36% respectively).The results of *in vitro* anti diabetic assay of the fractions indicate that the anti diabetic effect of the leaf extracts of *P.grandis* might be bestowed by its non-polar and polar constituents. The polar main constituents identified in the ethanol extract being the bioactives allantoin and pinitol. The compounds PG1 and PG2 isolated from fractionates II and IV, were identified as pinitol and allantoin respectively based on comparison with authentic samples. The presence of pinitol and allantoin as major constituents of *Pisonia grandis* is earlier reported (Shubashini *et al*, 2011). Both pinitol and allantoin are reported to be anti diabetic molecules. Pinitol was reported first by Narayanan *et al.*,(1987) as an anti diabetic agent and proven to exert acute and chronic insulin-like anti hyperglycemic effect in diabetic mice (Bates *et al.*, 2000) and can aid in transporting glucose into the muscle and increase glycogen storage. It has been also shown to increase whole body creatine uptake and retention. The efficacy and safety of pinitol as a dietary supplement for human consumption has been well documented and its safety has been reviewed by the FDA in 1998. D-Pinitol was first marketed in 1998 by Humanetics Corporation. Allantoin, a ureide molecule best known for its keratolytic action and use in numerous skin creams, also is recently reported to decrease plasma glucose in diabetic rats (Niu *et al.*, 2010)

The results of the amylase inhibition analysis prove the enhancement of anti diabetic activity of fractionates containing the active constituents allantoin and pinitol. These bioactive components in the plant extract, due to the binding competence might have induced conformational changes at the active site of the alpha amylase enzyme initiating inhibition.

In clinical treatments, acarbose and migitol, are used as enzyme inhibitors for type 2 diabetes (Van de Laar *et al.*, 2005). These inhibitors produce side effects like diarrhoea, flatulence and liver disorder necessitating use of herbs in the control of blood sugar.

In the present study the efficacy obtained from the standard drugs such as acarbose against  $\alpha$ -amylase enzyme was 67% at a concentration of 250  $\mu\text{g/mL}$ . The  $\text{IC}_{50}$  value of acarbose is 128  $\mu\text{g/mL}$  as reported by Andrade-Cetto *et al* (2008), which is on par with the percent inhibition (67%) obtained at 250  $\mu\text{g/mL}$ . The delay in the digestion of carbohydrates is accomplished by acarbose and consequently inhibiting the pancreatic amylase action (Shai *et al.*, 2010; Narkhede *et al.*, 2011). This revealed that the lowering of glucose level can be achieved effectively by the use of food-derived enzyme inhibitors in *P.grandis*.

### **Conclusion**

The isolated molecule pinitol (at a concentration of 250  $\mu\text{g/mL}$ ), the LLE fraction PGL aq.1 and the column fractionate PGL1% (both at a concentration of 20  $\mu\text{g/mL}$ ) exhibited the highest inhibition of  $\alpha$ -amylase. All other fractionates (at concentration of 20  $\mu\text{g/mL}$ ) also exhibited moderate inhibition of  $\alpha$ -amylase

Prospects of the use of *P.grandis* extracts in formulating anti diabetic agents are obvious from the results of this study.

### **Conflict of Interest**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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