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IN VITRO NITRIC OXIDE SCAVENGING ACTIVITY AND ALPHA AMYLASE INHIBITORY ACTIVITY OF *PTEROCARPUS MARSUPIUM* EXTRACT

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ABSTRACT

Diabetes mellitus is a metabolic disorder which has emerged as a global public health threat in the present century. The present oral hypoglycemic agents produce undesirable side effects and oxidative stress. Thus there is a need for a safe, cost effective and complementary therapy for Diabetes mellitus. To evaluate the Nitric oxide scavenging activity and Alpha amylase inhibitory activity of *Pterocarpus marsupium*. The antioxidant activity was determined by nitric oxide radical scavenging activity according to the method of Garrat (1964). Alpha-amylase inhibitory activity was evaluated according to the method of Bernfield (1955). % Inhibition of Nitric Oxide by *Pterocarpus marsupium* extract at 20, 40, 60, 80, 100µg/ml was 14.24±0.10, 24.43±0.18, 40.22±0.27, 60.87±0.22, 82.25±0.78 respectively. % inhibition of Alpha amylase by extract of *Pterocarpus marsupium* at 20, 40, 60, 80, 100µg/ml are 22.54±0.18, 30.64±0.52, 40.92±0.56, 56.08±0.07 and 72.25±0.76 respectively. Based on the results we conclude that *Pterocarpus marsupium* as strong in vitro nitric oxide scavenging activity and alpha amylase inhibitory activity. Thus, *Pterocarpus marsupium* is a potential antidiabetic agent.

Key words: In vitro, Nitric oxide, Alpha amylase, *Pterocarpus marsupium*.

INTRODUCTION

Living cells produce free radicals as a byproduct of biochemical processes. The free radicals are the cause for number of disorders in humans like diabetes, tumor, cardiovascular accidents, arthritis etc. Diabetes as emerged as a major health problem worldwide, especially in India. Oral hypoglycemic agents produce adverse effects like hypoglycemia, hypersensitivity, lactic acidosis, weight gain, GI side effects etc. The transition to herbal drugs seems necessary considering their efficacy, safety and cost [1].

Antioxidant property of plants products are mainly attributed to the phytochemical constituents present in the plant. The treatment of diabetes these days is focused on decreasing glucose level by inhibiting the key enzyme (amylase). Therefore ideal antidiabetic properties in herbs are high flavonoids, tannins contents and presence of amylase inhibitory activity [2].

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AIM

The aim of the present study was to evaluate the Nitric oxide scavenging activity and Alpha amylase inhibitory activity of *Pterocarpus marsupium*.

MATERIALS AND METHODS

Material

The plant material (*Pterocarpus marsupium*) was procured from Tamilnadu Medicinal Plant Farms and Herbal Medicine Corporation Ltd. (TAMPCOL), Arumbakkam, Chennai, India. The DPPH was purchased from Sisco Research

Preparation of Plant Extract

Leaves of *Pterocarpus marsupium* was washed with running tap water thoroughly, rinsed with distilled water, sun dried, powdered and made as extract with the

help of chloroform, ethanol, petroleum ether, ethyl acetate and water using soxhlet equipment. The obtained extract was filtered using whatteman no:1 Filter paper, condensed using rotary flash vaporator and stored in a airtight container [3].

Determination of Antioxidant Activity

The antioxidant activity was determined by nitric oxide radical scavenging activity according to the method of Garrat (1964). 2ml of 10mM sodium nitroprusside was added to 0.5 ml of *Pterocarpus marsupium* at various concentrations and incubated at 25oC for 150 minutes. 1ml of sulfanilic acid reagent was added to 0.5ml of incubated mixture and incubated again at room temperature for 5 minutes. Finally, 1ml of naphthylethylene diamine hydrochloride was added and incubated at room temperature for 30 minutes. Absorbance at 540nm was measured using a spectrometer [4].

Extraction of Wheat α-amylase

500mg of wheat flour was added to 1 liter of 0.2% calcium acetate solute at room temperature and was continuously stirred for a while. The suspension was then centrifuged. The obtained extract was stored at 3oC. β-amylase activity was inactivated by heating the extract at 70oC for 10 minutes at a pH of 6.6. The extract was cooled to 4oC until use [5].

Determination of Wheat α-amylase Inhibitory Activity

The mixture containing 20µl of enzyme, 200µl of 0.02M sodium phosphate buffer and plant extract of concentration ranging between 20-100µg/ml was incubated in room temperature for 10minutes followed by adding 200µl of starch in all dilution. The reaction is terminated by adding 400µl of DNS and placed in water bath for 10 minutes, cooled and diluted with 10ml distilled water. Absorbance is measured at 540nm.

The percentage inhibition was calculated using the formula.

$$\text{Percentage inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} * 100$$

Acarbose was used as the reference α-amylase inhibitor [6].

DISCUSSION

Antioxidant Activity

Antioxidant present in herbal plants is accountable for prevention of damage caused by free radicals. Flavonoids and tannins present in plants are potent free radical scavengers [7]. Nitric oxide is widely used chemical compound for assessing free radical scavenging activity. The above results shows that *Pterocarpus marsupium* has significant antioxidant property under in vitro condition. Moreover the graph indicates a dose dependent inhibition of nitric oxide.

α-Amylase Inhibition Activity

α-amylase is a key enzyme in carbohydrate metabolism. Inhibition of α-amylase is one of the strategy of treating diabetes. Inhibiting α-amylase will lower post prandial blood sugar [8]. The result suggests that ethanoic extract of *Pterocarpus marsupium* exhibit good α-amylase activity under in vitro condition. Dose dependent % inhibitory activity against α-amylase was noted.

Our study indicate that *Pterocarpus marsupium* could be useful in the treatment of post prandial hyperglycaemia. The Antioxidant and anti-diabetic activity may be attributed to the presence of flavonoids, tannins & anti α-amylase activity.

RESULTS

Antioxidant activity

% Inhibition of Nitric Oxide by Quercetin at20, 40, 60, 80, 100µg/ml was 49.66±1.12, 63.09±0.16, 76.05±0.36, 85.04±2.47, 95.32±1.07 respectively.% Inhibition of Nitric Oxide by *Pterocarpus marsupium* extract at 20, 40, 60, 80, 100µg/ml was 14.24±0.10, 24.43±0.18, 40.22±0.27, 60.87±0.22, 82.25±0.78 respectively (Table 1)

α-Amylase Inhibition Activity

% inhibition of Alpha amylase by extract of *Pterocarpus marsupium* at 20, 40, 60, 80, 100µg/ml are 46.66±0.10, 59.54±0.52, 68.25±0.12, 76.14±1.01, 82.69±1.02 respectively. % inhibition of Alpha amylase by extract of *Pterocarpus marsupium* at 20, 40, 60, 80, 100µg/ml are 22.54±0.18, 30.64±0.52, 40.92±0.56, 56.08±0.07 and 72.25±0.76 respectively.(Table 2).

DISCUSSION

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Table 1. % Inhibition Nitric Oxide - *Pterocarpus marsupium* Vs Quercetin

Concentration (µg/ml)	% Inhibition Quercetin	% Inhibition *Pterocarpus marsupium*
20	45.86 ± 1.14	14.24 ± 0.12
40	61.29 ± 0.14	24.43 ± 0.18
60	74.09 ± 0.26	40.22 ± 0.27
80	84.04 ± 2.48	60.87 ± 0.22
100	94.14 ± 1.07	82.25 ± 0.78

Table 2. % Inhibition α-amylase - *Pterocarpus marsupium* Vs Acarbose

Concentration (µg/ml)	% Inhibition Acarbose	% Inhibition *Pterocarpus marsupium*
20	46.66 ± 0.10	22.54 ± 0.18
40	59.54 ± 0.52	30.64 ± 0.52
60	68.25 ± 0.12	40.92 ± 0.56
80	76.14 ± 1.01	56.08 ± 0.07
100	82.69 ± 1.02	72.25 ± 0.76

Fig 1. *Pterocarpus marsupium* plant

Fig 2. Dried leaves of *Pterocarpus marsupium*

Fig 3. *Pterocarpus marsupium* extract

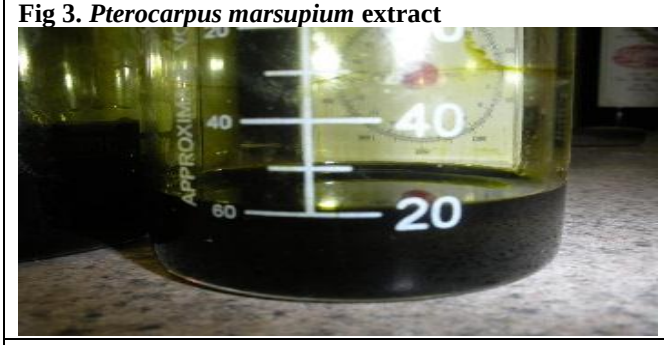


Fig 4. Wheat α-amylase extract

Fig 5. Principle of Nitric Oxide assay

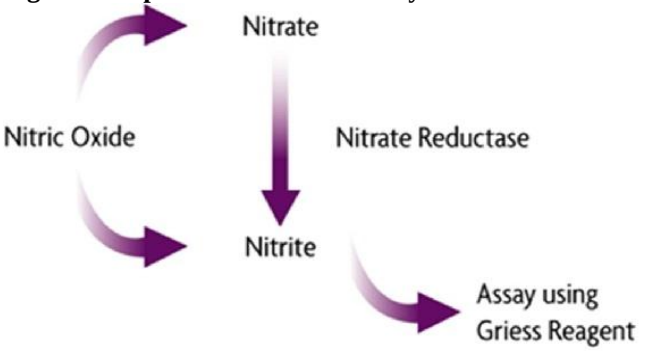


Fig 6. α-amylase inhibitory activity

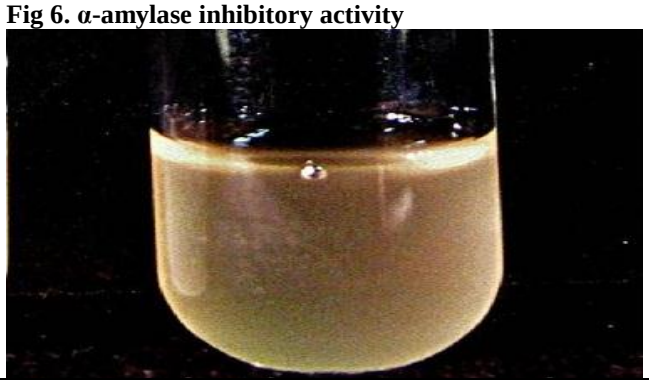


Fig 7. % Inhibition Nitric Oxide - *Pterocarpus marsupium* Vs Quercetin

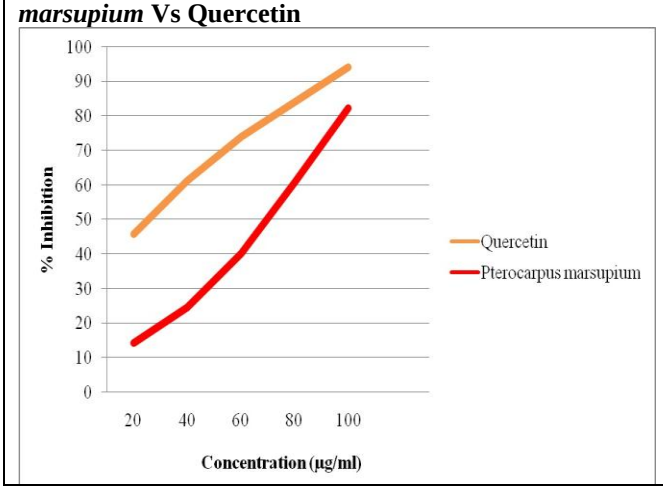
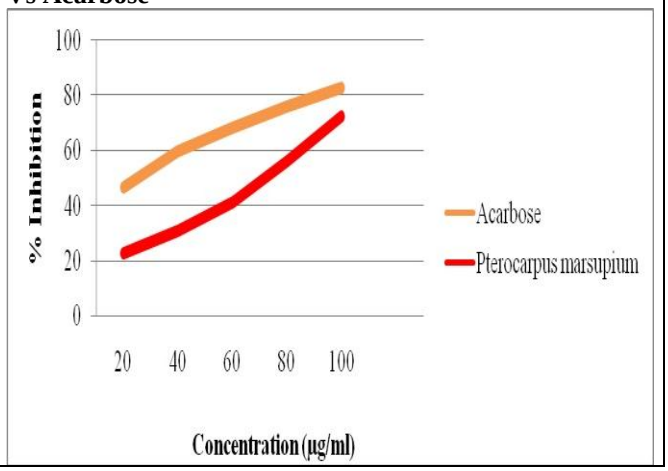


Fig 8. % Inhibition α-amylase - *Pterocarpus marsupium* Vs Acarbose



CONCLUSION

Based on the above results we conclude ethanolic leaf extract of *Pterocarpus marsupium* possesses both antioxidant and antidiabetic activity. However pharmacokinetic and safety profile of *Pterocarpus marsupium* requires pre-clinical testing prior to its application on humans.

FUNDING

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CONFLICT OF INTEREST

Nil

ETHICAL APPROVAL

Not required

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