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Biochemical activity of endangered medicinal plant *Kingiodendron pinnatum*

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ABSTRACT

Kingiodendron pinnatum, a vulnerable species in Western Ghats of Karnataka, is used in gonorrhoea, catarrhal conditions of genito-urinary and respiratory tracts. In the present study crude leaves extract of this plant have been screened and tested for antioxidant, lipoprotein lipase and alpha-amylase inhibitory activities. The bioassay results have shown the potential biochemical activity of the leaf extract.

Keywords: *KingiodendronPinnatum*, traditional medicine, maceration, ethyl acetate, diabetic, obesity.

INTRODUCTION

Kingiodendronpinnatum is a vulnerable and endangered medicinal plant belonging to the family Fabaceae. The oleo-gum-resin of this plant species is used in gonorrhoea, catarrhal conditions of genito-urinary and respiratory tracts [1]. It is also used in curing sores of elephants. The population of this species has declined considerably because of overexploitation and habitat degradation. Investigations relevant to phytochemistry may add new knowledge in traditional medicinal practices. It is well established fact that plant derived compounds offers numerous sources of antioxidant, antidiabetic and antiobesity and antimicrobial agent [2]. The scientific and pharmacological formulation of *Kingiodendronpinnatum* has not been established though it is being traditionally used by tribes of the region.Ehanobotnical search reveals use of many traditional herbs in treatment of various diseases, which are usually free from side effects, are economical and also accessible to humans and provide significant potential for the development of novel biomolecules [3-4]. Therefore there is a need for the biological evaluation of bioactive compounds in this plant especially for medicinal properties. In recent years many plants have been screened for their antioxidant, antibacterial, antifungal activities.

In the present study investigations have been made to identify the medicinal properties of *Kingiodendronpinnatum* and its applications.

MATERIALS AND METHODS

Collection of the Plant material

Evergreen forest of the Western Ghats region of Hassan district, Karnataka, India was explored to locate the population of *Kingiodendronpinnatum*. The leaves of this plant were carefully exercised from this plant, shade dried and placed in polythene bags. Herbarium of this plant is kept in the Department of Environmental Science, University of Mysore.

Preparation of crude methanol extract

The leaves were shade dried at room temperature in a clean environment to avoid contamination for 14 days and powdered in a domestic grinder. Extracts of these plant was made by maceration method. Two hundred grams of shade dried leaf powder was extracted with 90% methanol for 72hrs at room temperature. The extract was first filtered through cheese cloth then through Whatman filter paper No.1. The filtrate was evaporated to 50ml at room temperature and then in a vacuum concentrator. The dried powder was used for different bioassays.

Test chemicals

Chemicals such as soluble starch, DNS (3, 5-dinitrosalysilic acid), methanol, DPPH (1.1 – Diphenyl-2-picryl-hydrazil), ascorbic acid, ammonium molybdate. Solvent and reagents used were of analytical grade and obtained from Merck Company, India.

DPPH Radical scavenging activity

The antioxidant activity of different extracts was measured in terms of radical scavenging ability by DPPH method [5]. Methanolic solution (0.1 ml) of plant extracts at 10µg/ml concentration was added to 1 ml methanolic solution of DPPH (2 mg/ml)".The absorbance was measured at 517 nm after 10 min. This method was used as a principle antioxidant as fast test (H-donor method) [6]. The results were evaluated as percentage scavenging of radical (% scavenging of DPPH• = Abs. of blank - Abs. of sample/ Abs. of blank x 100). IC50 value (concentration of sample where absorbance of DPPH decreases 50% with respect to absorbance of blank) of extracts were determined. Methanol was used as negative control. DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. DPPH is relatively stable nitrogen centred free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agent as a result of which electron become paired off forming the corresponding hydrazine [7]. The solution therefore loses colour stochiometrically depending on the number of electrons consumed which is measured spectrometrically at 517 nm [8-9].

Phosphomolybdate assay

Total antioxidant activity of extracts was evaluated by the formation of phosphomolybdenum complex [10]. One hundred microliters of methanolic solution of extracts (10 µg/ml) was added to 1.9 ml of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mMammoniummolybdate). The blank solution contained only 2 ml of reagent solution. The absorbance was measured at 695 nm after 90 min.

In vitro *α*-amylase inhibitory activity

Amylase and amylase inhibitor activity assays were based on Bernfeld's method for amylase assay [11]. Amylase inhibitor extracts were mixed with amylase and incubated for 30 min at 37°C. The reaction was started by adding extract-enzyme mixture to test tubes containing buffered starch solution (2 mg starch in 20 mM phosphate buffer of pH 6.9 containing 0.4

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mMNaCl) and was incubated for 20 min. This reaction was terminated by adding 3,5-dinitrosalicyclic acid (DNS) reagent to the assay mixture. The assay tubes were kept in a boiling water bath for 5 min, cooled under tap water and the colour formed by maltose oxidation was measured at 530 nm. Controls without inhibitor were run simultaneously. One amylase unit is defined as the amount of enzyme that will liberate 1 μmol of maltose from starch under the assay conditions (pH 6.9, 37°C, 5 min). Inhibitory activity is expressed as the percentage of inhibited enzyme activity out of the total enzyme activity used in the assay.

***In vitro* lipoprotein lipase (LPL)**

Five hundred microliters (25 units) of Diluted LPL (Sigma-Aldrich) and five hundred microliters of each sample were mixed in 15 mL glass test tubes and pre incubated at 4°C for 30 min. One milliliter of prepared substrate emulsion was then added into the mixture and incubated in a water bath at 37°C to initiate hydrolysis. Reaction was stopped after 30 min by the addition of 2ml 1 M NaCl (Sigma-Aldrich). Control samples were carried out simultaneously with mixture of enzymes and substrate only, without the plant extract. Liberated free fatty acids were titrated with standardized 0.01 M NaOH until pH 9.2 was reached. The amount of free fatty acid liberated into the system was calculated from the amount of base added [12].

RESULTS AND DISCUSSION

Antioxidant activity

The leaves of *Kingiodendronpinnatum* were screened and analysed to unravel the present bioactive compounds possessing medicinal properties. The methanolic extract of the leaves was tested for antioxidant potentialities. DPPH assay results have shown that hydrogen donors scavenge free radical DPPH at a concentration of 0.01mg/ml. the leaves extract has also shown 36%, 52%, 93%, 95% of dose dependent inhibition (Fig. 1A). DPPH radical scavenging activity of methanolc extract of leaves in *Kingiodendronpinnatum*. DPPH is generally recognised to be the wide-ranging assay to use for antioxidant determination, and therefore was applied in this study. However, there are several other assays to determine the total antioxidant capacity.Antioxidant property was also confirmed by phophomolybdate assay using ascorbic acid as standard. Phosphomolybdate method has been routinely used to evaluate the total antioxidant capacity of the extract [8]. In the presence of the extract, the Mo (VI) is reduced to Mo (V) and forms a green coloured phosphomolybdenum V complex which shows maximum absorbance at 695 nm (Fig.1B). Methanol extract showed 88%, 80%, 60%, 50%, 40% activity at the concentration of 500µg/ml, 400µg/ml, 300µg/ml, 200µg/ml, 100µg/ml. This assay confirmed that methanol extract of *kingiodendronpinnatum* showed significant results compared to the standard antioxidant ascorbic acid.

Reactive oxygen species (ROS) are produce in the cells by cellular metabolism and other exogenous environmental agents. They are generated by a process known as redox cycling and are catalysed transition metals, such as Fe^{2+} and Cu^{2+} [13]. Over production of ROS can damage cellular biomolecules such as enzymes, lipids, proteins, nucleic acids and carbohydrates resulting in several diseases such as inflammation, atherosclerosis, stroke, heart diseases, diabetes mellitus, multiple sclerosis, cancer, Parkinson's disease Alzheimer's disease etc. Antioxidants scavenge free radicles and are associated reduced risk of cardiovascular disorder [14].

The large generation of free radicals, particularly reactive oxygen species and their high activity play an important role in the progression of great number of pathological disturbances like inflammation, atherosclerosis, stroke, heart diseases, diabetes mellitus, multiple sclerosis, cancer, Parkinson's diseases, Alzheimer's diseases, etc [15].

Sedentary life style, unhealthy dietary habits, genetic predispositions, lacking exercise, consumption of energy rich diets are some of the key factors that have conspired to create the current worldwide epidemic type 2 diabetes and obesity. In 2005, 246 million people worldwide suffered from type 2 diabetes and this number is projected to grow to 366 million by 2030. Very few medicinal plants provide potential drugs to cure Diabetics [16]. Potential size of the markers for antidiabetic and antiobesity drugs is still unsatisfactory [17].

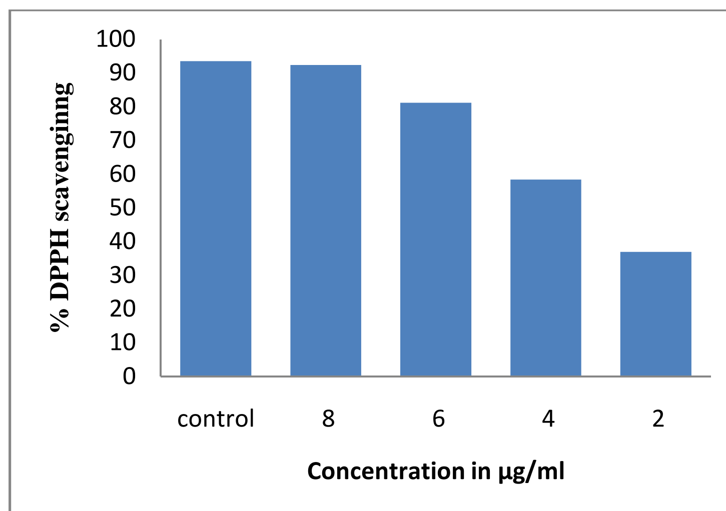

Fig.1A DPPH activity of *KingiodendronPinanatum* leaf methanol extract
Values are the mean of triplicates (±SD).

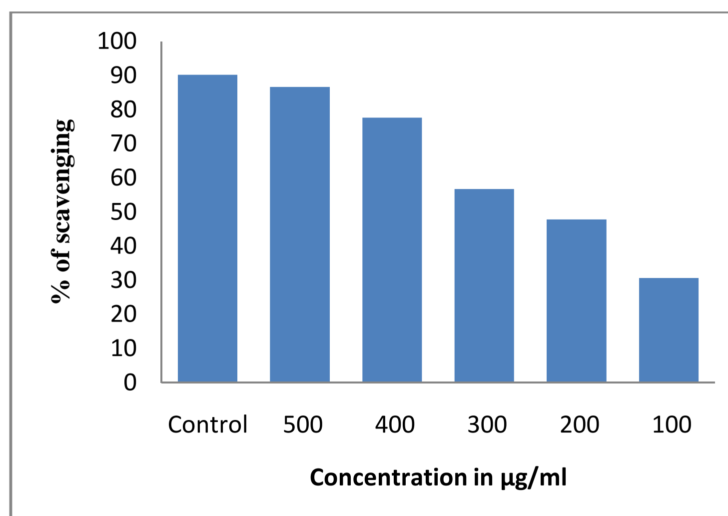

Fig.1B Phosphomolybdate activity of *Kingiodendronpinnatum* leaf methanol extract
Values are the mean of triplicates (±SD).

In vitro α-amylase inhibitory activity

In vitro α-amylase inhibitory studies showed that the methanol extract of *Kingiodendronpinnatum leaves exhibit* potent inhibitory activity at the concentration of 1mg/ml. Fig.2 shows the inhibitory activity. α-Amylase is one of the main enzymes in human that is responsible for the breakdown of starch to more simple sugars, thus the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Consequently, postprandial rise in blood glucose is decreased [18]. Hence, they have long been though to improve glucose tolerance in diabetic patients [19].

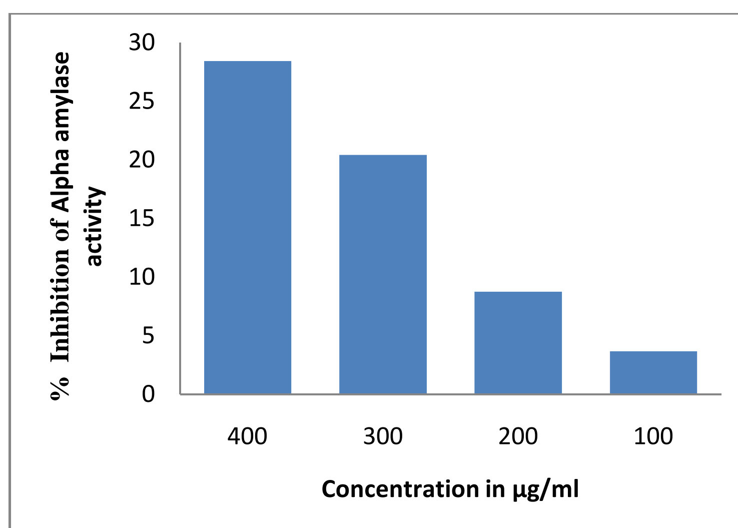

Fig.2 Alpha-amylase inhibitory activity of *Kingiodendronpinnatum* leaf methanol extract
Values are the mean of triplicates (±SD).

In vitro lipoprotein lipase (LPL)

Result of our study showed that the leaf extract of the plant tested inhibited Lipoprotein lipase at a concentration of 1mg/ml. Fig.3. Shows the inhibitory activity. LPL is a factor that contributes to the development of obesity [20]. This is the first report about the potential antioxidant, antiobesity and antidiabetic effects of this plant.

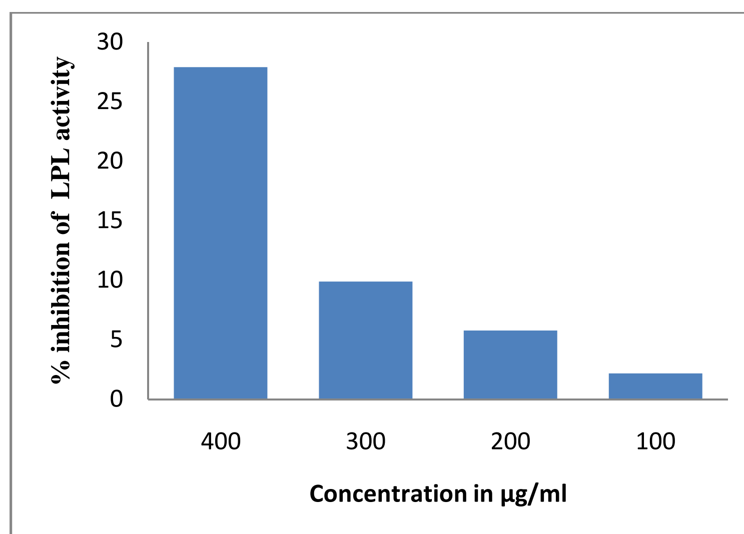

Fig.3 lipoprotein lipase inhibitory activity of *Kingiodendronpinnatum* leaf methanol extract
Values are the mean of triplicates (±SD).

CONCLUSION

The present study indicated that the methanolic leaves extract of *Kingiodendronpinnatum* possesses various bioactive compounds which have antioxidant, antiobesity and antidiabetic properties. This medicinal plant can be considered as promising sources of natural antioxidants for medicinal and commercial uses.An assessment of the toxicity and kinetic studies, as well as the function of these extracts in food and biological systems also needs to be investigated.These results suggest that DPPH, phosphomolybdate, LPL activity, Alpha amylase determination is of

interest for a comparative evaluation of in vitro antioxidant, antiobesity and antidiabetic potential, but it needs to be combined with in vivo data for adequate assessment of the antioxidant capacity of medicinal plant extracts.This work offers scope for further research in phytochemical analysis and development of novel drugs. Owing to the medicinal potentiality of this plant, the conservation of this vulnerable species should be given prior attention.

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