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ANTI-DIABETIC AND ANTI MICROBIAL ACTIVITY OF *PTEROCARPUS SANTALINUS* HEART WOOD

Chandra Sekhar Challa[1], T. Lokesh[2], Devanna Nayakanti[1], N.CH. Varadacharyulu[2*]

1. Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Anantapur, 515 002, India.
2. Department of Biochemistry, Sri Krishnadevaraya University, Anantapur, 515 003, India.

ABSTRACT: Diabetes mellitus is a chronic illness, and the management of diabetes is a global problem. Successful treatment is required to prevent complications and organ damages. Herbal medicines are having minimal adverse effects when compared to the available synthetic drugs to treat such chronic diseases and disorders. The present study was aimed to evaluate the antidiabetic and antimicrobial activity of methanolic extract of *Pterocarpus santalinus* Heartwood. The *in vitro* antidiabetic activity of methanolic extract was evaluated in C2C12 cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (cell viability method) and glucose uptake assay. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method used for the evaluation of *in vitro* antioxidant activity. Methanolic extract of *Pterocarpus santalinus* Heartwood had shown better antimicrobial activity and cytotoxic activity in C2C12 cell line. From our study results, we concluded that *Pterocarpus santalinus* Heartwood had shown better antidiabetic activity and antimicrobial activity under *in vitro* models.

KEYWORDS: Antimicrobial activity, Antidiabetic activity, *Pterocarpus santalinus* L, C2C12 Cell lines.

Corresponding Author: Prof. N. CH. Varadacharyulu* Ph.D.
Department of Biochemistry, Sri Krishnadevaraya University, Anantapur, 515 003, India.

1.INTRODUCTION

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with

irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs [1-3]. Before onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases. With introduction of scientific procedures the researchers, were able to understand about toxic principles present in the green flora. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active. Aconitine, Atisine, Lobeline, Nicotine, Strychnine, Digoxin, Atropine, Morphine are some common examples [4-6]. The efficacy of some herbal products is beyond doubt, the most recent examples being Silybum marianum (silymarin), Artemisia annua (artemesinin) and Taxusbaccata (taxol). On the other hand, randomized, controlled trials have proved the efficacy of some established remedies, for instance, Ginkgo biloba for tinnitus, Hypericum perforatum is a reputed remedy for depression [7-12. In Hypericum some researchers are of the view that hypericin is the active principle of the herb and some believe that hyperforin is responsible for antidepressant action of the herb [13-18]. Recently research has supported biological activities of some medicinal herbs. Diabetes is such a segment where researchers are expecting new molecules from herbs that can provide us with tools for fighting this dreaded disease.

2. MATERIALS AND METHODS

Plant material

From the previous literature studies *Pterocarpus santalinus Linn* (Red Sandalwood) Heartwood for studying anti-diabetic activity in C2C12 Cell lines. Thus material was dried and powdered and used for a antimicrobial and antidiabetic studies.

Methanolic extract of Heartwood of *Pterocarpus santalinus* Linn

The methanol extract (5g) of Heartwood was washed with acetone and allowed to settle. After 2 hrs the acetone soluble fraction was separated and the insoluble fraction was resuspended and washed again with fresh acetone two to three times. The acetone soluble fraction was chromatographed on a silica gel column using chloroform and acetone in the ratio of 6:4, The eluted fractions were collected at an interval of 5 ml each and were monitored by thin layer chromatography. The fraction one recovered in higher concentration was recrystallized from acetone to get a whitish compound. Dark brown residue (l0g) of methanol extract of stem was separated into a major fraction by chromatography on Silica gel with methanol/water (9.5:0.5). The elution was collected and profiled by TLC showed single spot. Then the fraction was subjected to HPLC showed single peak. The recovered compound was washed with cold methanol and filtered. This compound was labeled [19].

In vitro antidiabetic activity of Leucas aspera leaves extracts in C2C12 cell line

Chemicals

MTT, fetal bovine serum (FBS), phosphate-buffered saline (PBS), bovine serum albumin (BSA), D-glucose, Dulbecco's Modified Eagle's Medium (DMEM), metformin, trypsin (Sigma-Aldrich Co.,

St. Louis, USA), ethylenediaminetetraacetic acid (EDTA), antibiotics, insulin, dimethyl sulfoxide, NaOH, and propanol.

Cell lines and culture medium

Stock cells of C2C12 (Rat skeletal muscle, ATCC, USA) were cultured in DMEM supplemented with 10% inactivated FBS, penicillin (100 IU/mL), streptomycin (100 µg/mL), and amphotericin B (5 µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with trypsin phosphate versene glucose solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks, and all experiments were carried out in 96 microtiter plates (Tarsons India Pvt. Ltd., Kolkata, West Bengal, India).

Test solution

For *in vitro* antidiabetic studies, test substance dissolved in DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1.0 mg/mL. This solution was sterilized by filtration and two-fold serial dilutions are prepared to carryout cytotoxic studies.

Determination of cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The cytotoxic capacity of Methanol extract of *Pterocarpus santalinus* L Heartwood was determined by MTT assay. This method was adopted from the procedure explained by Denizot and Lang [20-22]. Monolayer cell culture was trypsinized, and cell count was adjusted to 1.0×10^5 cells/mL using DMEM containing 10% of FVS. To each well, 0.1 mL of diluted cell suspension was added. Supernatant was flicked off after 24 h and monolayer was washed once with medium. Different concentrations of extracts (100 µL) were added on to the partial monolayer in microtiter plates. The plates were incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations noted at 24-h interval. After 24 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well and gently shaken. This mixture is again incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µL of propanol was added to the plates, gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\%\,\text{Growth inhibition} = 100 - \left(\frac{\text{Mean optical density of test group}}{\text{Mean optical density of control group}} \right) \times 100$$

***In vitro* glucose uptake assay**

Glucose uptake activity of *Pterocarpus santalinus* L Heartwood was measured in differentiated C2C12 cells. The 24-h cell culture with 70%–80% confluency in 40 mm petri dish plates was allowed to differentiate by maintaining in DMEM with 2% FBS for 4–6 days. The differentiated cells were serum starved over a night, and at the time of experiment, cells were washed with HEPES-buffered Krebs-Ringer-Phosphate solution (KRP buffer). The mixture is incubated with KRP buffer with 0.1% BSA for 30 min at 37°C. Cells were treated with different nontoxic concentrations of standard drug and extracts for 30 min along with negative controls at 37°C. D-glucose solution (20 µL) was added simultaneously to each well and incubated at 37°C for 30 min. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washed thrice with ice-cold KRP buffer solution. Cells were lysed with 0.1 M NaOH solution, and an aliquot of cell lysates was used to measure the cell-associated glucose. The glucose levels in cell lysates were measured using glucose assay kit (ERBA). Two independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls [23-26].

Antibacterial Activity

Test Microorganisms and Growth Media

Staphylococcus aureus (MTCC 3160), *Bacillus cereus* (MTCC 1305) *E.Coli* (MTCC 443) and *Pseudomonas aureoginosa* (MTCC 2453) were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Department of Microbiology, Sri Yuva Biotech PVT.LTD, were used for evaluating antibacterial activity. The bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar. The bacteria were grown on Mueller-Hinton agar plates at 37°C.

Determination of zone of inhibition method

Preparation of Discs:

Whatman No.1 filter paper discs of 5mm diameter were autoclaved by keeping in a clean and dry Petri plate. The discs were soaked in compound solutions for 5 hours were taken as test material. After 5 hours the discs were shade dried. The concentrations of compound solutions per disc are accounted for 0.1 grams/1ml. Subsequently they were carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, Hexane, benzene and distilled water are prepared and used as control.

Testing of antibacterial activity:

To test the antibacterial activity, LB agar medium was prepared and the medium was sterilized at 121°c for 30 mins. The agar plates were prepared by pouring about 10ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium. 1ml of inocculum (containing suspension) of *Staphylococcus aureus*, *Bacillus subtilis*, *E.Coli* and *Klebsiella pneumoniae* was poured on to the plates separately containing solidified agar media. The

prepared sterile filter paper discs were impregnated with the compound solutions and shaken thoroughly and these test plates incubated for a period of 48 hrs in BOD at 37ºc for the development of inhibitory zones and the average of 2 independent readings for each organism in different compound solutions were recorded [27-32].

Measuring the diameter of inhibition zone:

The inhibition zones were measured after 1 day at 37ºc for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler. Five paper discs placed in one Petri plate.

3. RESULTS AND DISCUSSION

In vitro antidiabetic studies

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

MTT cytotoxic capacity of *Pterocarpus santalinus* L Heartwood methanolic extract was found to be 34.7%, 54.8%, 65.5%, 76.5%, and 90.5% at 62.5, 125, 250, 500, and 1000 µg/mL concentrations, respectively. The CTC_{50} values of *Pterocarpus santalinus* L Heartwood methanolic extract was were found to be 353.75 ± 4.33 µg/mL (Table 1; Fig 1).

Table 1: Cytotoxic properties of *Pterocarpus santalinus* L Heartwood against C2C12 cell line

Name of Sample	Test concentration (µg/ml)	Cytotoxicity (%)	CTC_{50} (µg/ml)
Methanolic Extract	1000	65.3±0.5	353.75±4.0
	500	55.2±1.5	
	250	35.5±1.2	
	125	22.5±1.3	
	62.5	10.5±1.1	

Fig 1: In vitro antidiabetic activity of *Pterocarpus santalinus* L Heartwood in C2C12 cell line

In vitro glucose uptake assay

Glucose uptake activity of *Pterocarpus santalinus* L Heartwood methanolic extract was determined in differentiated C2C12 cells. The percentage of glucose uptake activity against rosiglitazone standard showed *Pterocarpus santalinus* L Heartwood methanolic extract exhibited better activity. The glucose uptake capacity of *Pterocarpus santalinus* L Heartwood methanolic extract was 20.2% and 50.19% at 250 µg/mL and 500 µg/mL concentrations, respectively (Fig 2).

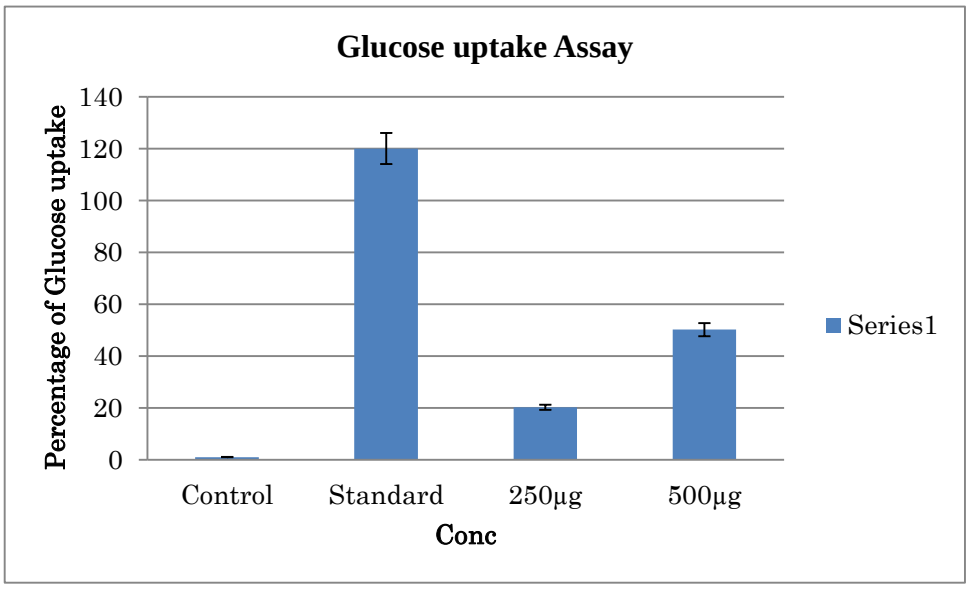

Fig 2: In vitro antidiabetic activity of *Pterocarpus santalinus* L Heartwood in C2C12 cell line: Glucose uptake assay

Antimicrobial Activity

By comparing all the zones of inhibition values it can be concluded that *Klebsiella pneumoniae* and *E.coli* were sensitive even in low concentration. Now in the present study the used plant extract was found as antimicrobial agents and inhibits the growth of *Staphylococcus aureus* and *E.coli* effectively at all concentrations.

Table-2: Antibacterial Bioactivite of Compounds

Compound No	Gram positive bacteria		Gram negative bacteria	
	Staphylococcus aureus	*Bacillus subtilis*	*E.Coli*	*Klebsiella pneumoniae*
	Zone of inhibition in mm [b]			
Pregnon-20- 0ne	3	3	5	4
CycloHexane, 1-ethyl- 1-methyl,2,4-bis(1-methyl,ethanyl)	4	3	3	5
4,5,7-trihydroxy isoflavone	5	3	6	3
Phytol	2	2	2	2
Caryophelleneoxide	1	1	1	1
Ciprofloxacin [a]	5	5	7	6

a. Concentration: 4 mg/mL^{-1} of DMSO; b. Values, including diameter of the well (8 mm), are means of three replicates; c. No activity

4. CONCLUSION

From our present study results, we concluded that *Pterocarpus santalinus* had shown better antidiabetic activity and antimicrobial activity under *in vitro* models. Methanol extract produced slightly higher activity and warrants further research and experiments on animal models to assess the potency and safety before the clinical use. Through the present work, we found that effectively the metabolites profiles of *Pterocarpus santalinus* in *m*ethanol extracts shown more Terpinoids constituents in *Pterocarpus santalinus Heartwood*. Results obtained in the present study confirm the difference of in vitro antimicrobial activity of *Pterocarpus santalinus* according to its geographical location. Thus, studies could be continued for the characterization of compounds responsible of antimicrobial activity.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of Interest.

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