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Preliminary Phytochemical Screening and Antidiabetic Activity of *Pterocarpus santalinus* Leaf in Streptozotocin Induced Diabetic Rats

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Abstract: *Pterocarpus santalinus* has been widely used in traditional system of medicine for many ailments. The present study was aimed at determining the changes in blood glucose levels after administration of *Pterocarpus santalinus* leaf powder extract by using ethyl acetate, acetone and water as solvents. The solvent extracts are given at a dose of 500mg/kg bodyweight to diabetic rats. Ethylacetate extract showed significant hypoglycemic activity ($p < 0.01$). Phytochemical screening and FTIR analysis revealed the presence of alkaloids, steroids, flavonoids, glycosides, tannins, carbohydrates and sterols.

Keywords: *Pterocarpus santalinus*, solvent extract, hypoglycemic activity, Phytochemical screening, FTIR analysis

I. INTRODUCTION

Diabetes mellitus commonly referred as diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period(1). Diabetes is due to either the pancreas producing less insulin than required by body or the body not responding properly to insulin(2). Diabetes can be classified into three main categories (3).Type1Diabetismellitus which is due to failure of pancreas to secrete sufficient insulin. This is also called IDDM/juvenile diabetes.Type2diabetes is majorly due to insulin resistance where body cannot respond insulin properly. This is also called as NIDDM/Adult onset diabetes. Gestational diabetes is seen in pregnant women and resolves after baby birth. Of the three types type2 is most common and makes 90% of cases (4).Symptoms of high blood sugar includes frequent urination, increased thirst and increased hunger. Acute complications are diabetic ketoacidosis, nonketotic hyperosmolar coma and death (5). Long term complications include heart disease, stroke, kidney failure, foot ulcers and retinopathy(6).As of 2015,around 415 million people had diabetes worldwide(7).This indicates 83% of adult population with equal rates in both women and men(8). Various types of oral hypoglycemic drugs are available including insulin which is expensive. So there is a need for herbal drug to treat diabetes without any side effects, low costs and safer for long term use (9). Medicinal activities of plants is due to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins and terpenoids present in these plants (10).World Health Organization reveals that upto 90% of population in developing countries use plants and its products as traditional medicine for primary healthcare (11). The WHO has listed 21,000 plants which are used for medicinal purposes around the world. Among these 2500 species are in India (12). There are about 800 plants which have been reported to have antidiabetic potential (13).Pterocarpus santalinus is one of such plants used for treatment of diabetes (14). Pterocarpus santalinus belongs to the family Fabaceae. Ethno botanical reports shows that P.santalinus is being used to treat diabetes mellitus and related symptoms along with use of other diseases like skin infections, anthelmintic, aphrodisiac, alexiteric, vomiting,thirst,eye diseases, foot ulcers and blood diseases(15).Fruit decoction is used as an astringent tonic to treat chronic dysentery. Wooden cups made of P.santalinus were used to drink water twice a day as treatment of diabetes (16). The ethanolic extract of bark of P.santalinus is proved to have Hypoglycemic activity (17).The ethno medical use of leaves of p. santalinoides in the treatment of diarrhoea and other gastrointestinal disorders has been scientifically proven (18).Ethanol leaf extract of P. santalinoides and P.santalinus is proved to have hepato protective activity in albino rats (19, 20).The leaf extract of P. santalinus has also antibacterial activity(21).In this study an attempt has been made with leaf extract of P.santalinus to check for hypoglycemic activity.

II. MATERIALS AND METHODS

A. Collection of plant material

Leaves of *P.santalinus* were collected from the surroundings of Tirupati and Tirumala, A.P. India. The leaves were dried in shade and made into powder using electrical grinder. This leaf powder was stored in airtight container and is used for extraction process.

B. FTIR Analysis

The infrared (IR) spectrum of the leaf powder was determined on Perkin Elmer Spectrum One FTIR. The spectrum was obtained using potassium bromide (KBr) pellet technique in the range of 400 to 3500 cm^{-1} at a resolution of 1.0 cm^{-1} . Potassium bromide (AR grade) was dried under vacuum at 100°C and 100mg of KBr with 1mg of leaf powder was used to prepare KBr pellet. The spectrum was plotted as intensity versus wavenumber (23). The ethyl acetate extract of the leaf powder was also subjected to FTIR analysis.

C. Preparation of extracts and phytochemical analysis

The dried powder was used for extraction process using soxhlet extractor with ethyl acetate and acetone. Aqueous extract is also prepared by soaking leaf powder in distilled water in big glass jar for three days with occasional stirring and filtered. The process was repeated until the filtrate collected was colour less. The extracts were then concentrated using Rotary vacuum evaporator and stored at 0°C in airtight containers for further analysis. All the extracts were used for preliminary phytochemical analysis (24, 25).

D. Animal model

Male albino wistar rats of 3months age weighing 180-200g were purchased from venkateswaratraders, Bangalore. Before starting the experiment, the rats were acclimatized to animal house conditions at animal house, SPMVV. The rats were fed with standard pellet diet and provided water a dlibitum. The experimental protocol was approved by Institutional Ethical committee of SPMVV, Tirupati, AP, India.

E. Induction of diabetes

The animals prior to dosage with Streptozotocin were fasted overnight. STZ(45mg/kgbw) was prepared freshly in 0.1M ice cold sodium citrate buffer of pH 4.5. Group 2,3,4,5 and 6 rats were given single dose of intraperitoneal injection with streptozotocin. The blood glucose levels in the injected rats were determined after 48hours. The rats that showed more than 250mg/dl of glucose in their blood were selected for the study.

F. Experimental design

The rats were divided randomly into 5 groups containing 6 rats in each group.

Group 1-Normal rats

Group 2-Diabetic Rats

Group 3-Diabetic rats treated with *Ps* ethyl acetate extract (500mg/kgbw)

Group 4-Diabetic rats treated with *Ps* acetone extract (500mg/kgbw)

Group 5-Diabetic rats treated with *Ps* water extract (500mg/kgbw)

Group 6-Diabetic rats treated with Glibenclamide (20mg/kgbw)

The rats were allowed for fasting overnight and then administered with distilled water for group1 and group2. Group 3, 4, 5 diabetic rats received 500mg/kg BW of ethyl acetate, hexane and aqueous extracts respectively. Group 6 rats were given 20mg glibenclamide/kg bw by using a gavage. Blood samples were collected for measurement of blood from the tail vein at 0, 1, 2,3,4,5 and 6 hours after feeding the extract/glibenclamide. Blood glucose levels were measured by using glucose oxidase reactive strips and a glucometer.

III. RESULTS AND DISCUSSION

The FTIR spectrum of *Pterocarpus santalinus* leaf powder showed significant peaks at 3347, 2923, 1612, 1443, 1038,511,449,425 and 412 which corresponds to primary amines, alkanes, alkenes and benzoate compounds (Fig 1). The FTIR analysis of *Pterocarpus santalinus* ethyl acetate extract of leaf showed significant peaks at 3398, 2958, 2926, 2859, 1729, 1460, 1381,1279,1125,1072 and 1038 which corresponds to alkanes, aromatic compounds, amines, esters and polysaccharides (Fig 2).

Fig-1 FTIR Spectrum of Pterocarpus santalinus leaf powder

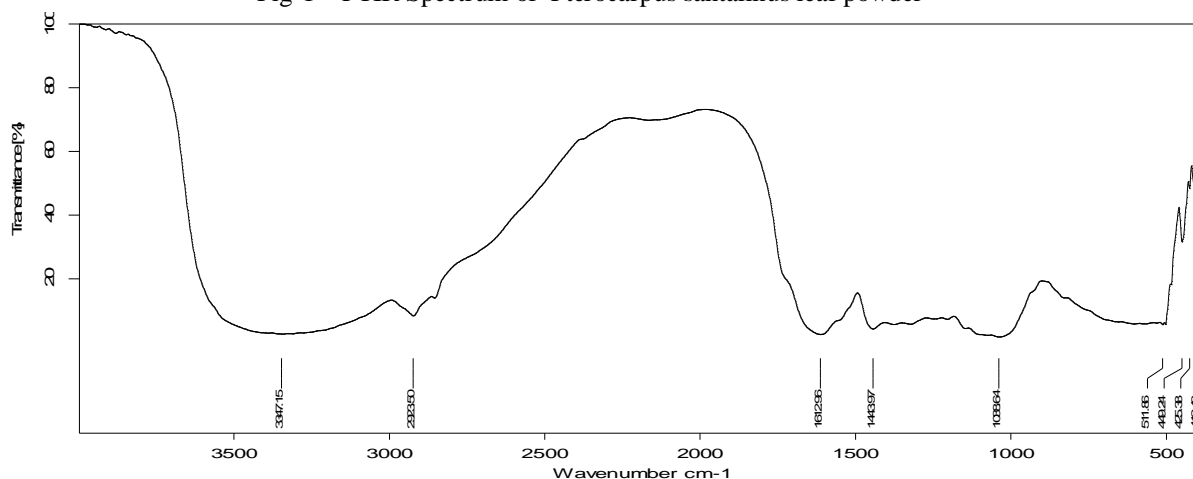
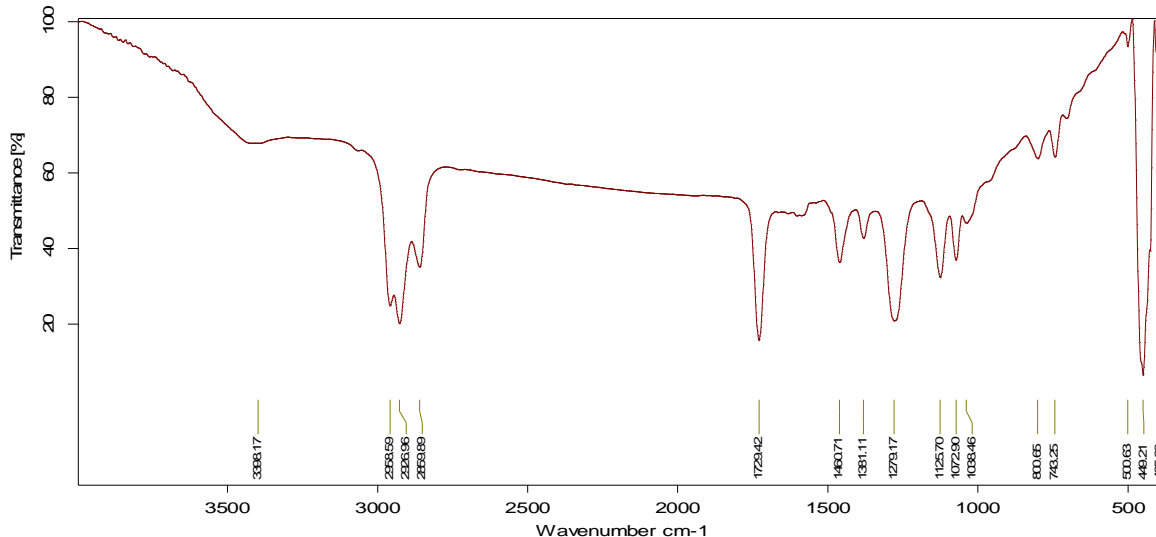


Fig-2 FTIR Spectrum of Pterocarpus santalinus ethyl acetate leaf extract



A. Phytochemical screening

Phytochemical analysis of the three extracts (Table 1) revealed the presence of different secondary metabolites like alkaloids, steroids, terpenes, saponins and tannins .Alkaloids were isolated from different plants and reported to have antidiabetic activity. Some of the alkaloids that were reported are Beberine, Casuarinegluco side, Cryptolepine, Harmane, Jambosine and Tecomine. Flavonoids also have various mechanisms by which they show hypoglycemic effect. Some flavonoids are prunin(26), kaempferitin(27), kaempferol(28) , kolaviron(29) , Marsupin(30) Similarly Terpenoids (31), steroids (32), polysaccharides (33) were also reported to have hypoglycemic effect.

Table 1: Phytochemical analysis of various solvent extracts of Pterocarpus santalinus

S. NO	Phytochemicals	Ethyl Acetate	Acetone	Water
1	Flavonoids	+	++	-
2	Terpenoids	+	+	+
3	Glycosides	+	+	+

4	Tannins		+		-		+
5	Saponins	+		+		-	
6	Alkaloids		++	+		+	
7	Carbohydrates	+		+		-	
8	Resins	+		+		-	
9	Sterols	-		+		-	

++ major; +minor; - nil

B. Variation of blood glucose levels with the oral doses of different extracts

Ethyl acetate extract treated diabetic rats (500mg/kgbw) showed significant reduction in blood glucose levels when compared with other extracts. Aqueous extract has very less effect on blood glucose levels in diabetic rats. Table 2 shows the effect of different solvent extracts on blood glucose levels at different intervals.

Table2: Effect of different solvent extracts of Pterocarpus santalinus on fasting blood glucose levels of STZ induced diabetic rats.

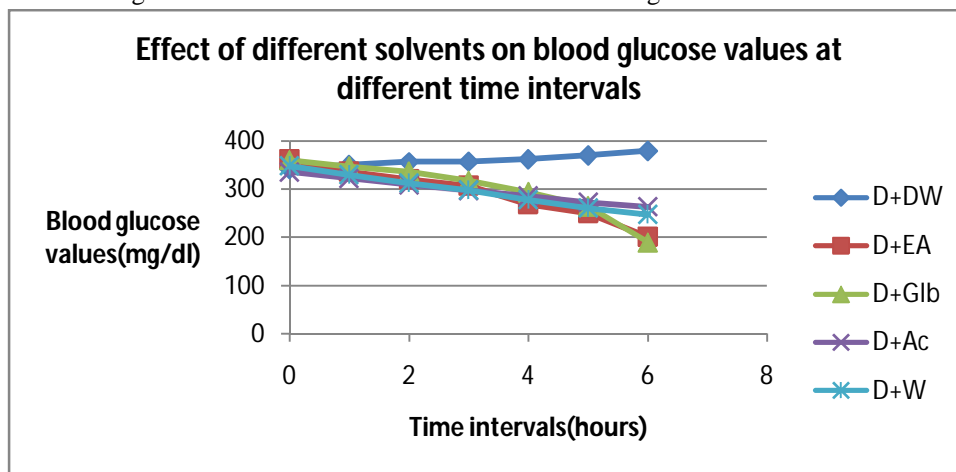
Groups	0h	1h	2h	3h	4h	5h	6h
1)	84.6±5.1	87.3±9.3	85.8±4.9	88.6±4.9	91.1±5.4	88.6±4.4	89.1±5.7
2)	343.5±20	351.5±18.3	357.6±17.9	358.8±22.5	363.3±20.4	371.8±18.4	380.8±19
3)	362.1±5.9	337.3±6.1	321.1±7.2	308.6±9.5	269±36.9	250±37.4	201.3±15.3*(44.4%)
4)	336±8.5	323±8.01	310.5±9.1**	298.6±14.2*	286.5±10.7	273±11.3	264.1±9.6 (21.39%)
5)	349.3±8.2	330.5±11.2**	313.5±9.1*	299.6±9.7*	279.8±7.1	261.1±9.8	248.1±9.5 (28.9%)
6)	361±17.7	348.8±18.7	337.8±19.1*	318±13.2*	294.6±7.5	265.8±11.7	190±11.4 (47.3%)

*p<0.05 in respective 0h value

**p<0.01 in respective 0h value

Numbers in parenthesis indicate the decrease in blood glucose value.

Fig 3-Effect of different solvent extracts on blood glucose levels in diabetic rats.



IV. CONCLUSIONS

From the results it can be concluded that the ethyl acetate extract of PS leaf showed significant reduction ($p < 0.01$) of blood glucose levels in diabetic rats. The underlying mechanism for this effect is might be due to the presence of various secondary metabolites like alkaloids, flavonoids, tannins, terpenoids, and saponins, glycosides that were observed during preliminary phytochemical analysis and FTIR analysis. Further experiments like bioassay guided fractionation, HPLC analysis are planned to observe the active compound from the group of compounds that is responsible for reducing blood glucose values in ethyl acetate extract of *Pterocarpus santalinus*.

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