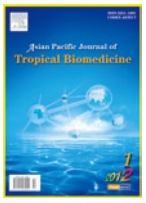
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

Antidiabetic activity and chemical characterization of aqueous/ethanol prop roots extracts of *Pandanus fascicularis* Lam in streptozotocin-induced diabetic rats

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ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form 19 January 2012
Accepted 29 March 2012
Available online 28 April 2012

Keywords:
Pandanus fascicularis Lam
Anti-diabetic
Aqueous extract
Ethanol extract
Streptozotocin

ABSTRACT

Objective: To evaluate antidiabetic activity and chemical characterization of aqueous and ethanolic extracts of prop roots of *Pandanus fascicularis* Lam (*P. fascicularis*) in streptozotocin (STZ) induced diabetic rats. **Methods:** Ethanol and aqueous extracts were prepared from *P. facicularis* by percolation and cold maturation, respectively. Anti diabetic activity of prop roots of *P. fascicularis* at the dose of 250 mg/kg in STZ (i.p / 60mg/kg body weight) induced diabetic rats. The fasting blood glucose levels were measured at 0, 1, 2 and 3 hrs after the treatment of ethanol and aqueous extracts. The ethanol fraction was then subjected to chromatographic analysis and a compound has been isolated and characterized by IR, ^{1}H-NMR and Mass Spectroscopy. **Results:** The reduced blood glucose level was significant ($P < 0.001$) in the dose of 250 mg/kg of ethanol and aqueous extracts of *P. fascicularis*, when compared with control. The blood glucose level of diabetic control animals after 3 h was (226.00 ± 1.78) mg/kg, whereas it were (102.00 ± 1.73) mg/kg and (131.00 ± 1.92) mg/kg for the groups treated with aqueous extract and ethanol extract, respectively. The ethanol fraction was then subjected to chromatographic analysis and a compound has been isolated. The structure of the isolated compound is may be characterized as Hepta deca-5-ene-1-ol by analysis it's IR, ^{1}H NMR and Mass spectroscopy data. **Conclusion:** These results demonstrated that aqueous/ethanol extracts of prop roots of *P. fascicularis* showed significant anti-diabetic activity, consequently this plant might be of value in diabetes treatment.

1. Introduction

Diabetes mellitus is a chronic and major endocrine disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is a growing health problem in most countries and its incidence is considered to be high all over the world[1]. It is also associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others [2]. Excessive oxidative stress has been implicated in the pathology and complications of diabetes mellitus[3].

According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetic patients worldwide. Recent estimates project that the number of patients diagnosed with Type II diabetes will more than double to 300 million before 2025. India has more than 30 million people with diabetics. It is estimated that by 2025, the number of diabetics will rise to 57 million in India, the highest number of diabetics in the world[4].

There are several drugs in clinical practice for the treatment of diabetes mellitus. Many of these oral anti-

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diabetic agents have been reported to show serious adverse effect such as liver problems, lactic acidosis and diarrhea[5]. In addition, they are not suitable for use during pregnancy. It is apparent that due to the side effects of the currently used drugs, there is a need for a potent drug with minimal adverse effects, which can be taken for long durations. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug[6]. Throughout the world many traditional plant treatments for diabetes exist. However, few have received scientific or medical scrutiny and the WHO has recommended that traditional plant treatments for diabetes warrant further evaluation[7-9].

Pandanus fascicularis Lam. (Synonyms - Pandanus odoratissimus, family - Pandanaceae) commonly referred to as screw pines are palm-like evergreen trees or shrubs belong to the genus *Pandanus*, order Pandanales, class Liliopsida, and division Mangoliophyta. Vernacular names of this plant are: Sanskrit-ketaki, Hindi-keura, Kewda, Ketki, Gagandhul, Tamil-Tazhai, Telugu-Mugali, Kannada-tale mara, English-screw pine. Pandanus comprises 500-600 species and is distributed mainly in subtropical and tropical regions. *P. fascicularis* is native to South Asia and has a significant presence particularly in mangrove swamps[10]. Although India has the tradition of alternative therapies there are no procedures to test the safety and efficacy of traditional remedies and to standardize their effective cure. For these reasons it is essential to increase our efforts in the area of medicinal plant research and exploit it efficiently for the benefit of humanity.

The leaf extract of *Pandanus amaryfollius* (*P. amaryfollius*) showed hypoglycemic effects in the STZ-diabetic rats[11]. However, there are no scientific studies available on the anti diabetic effects of prop roots of *P. fascicularis* extract although this plant is widely used as a folk remedy for the treatment of diabetes in India. Therefore, the anti-diabetic effects of prop roots of *P. fascicularis* extracts were investigated by experimentally STZ- induced diabetic in rats.

2. Materials and methods

2.1 Chemicals

Streptozotocin was purchased from Sigma-Aldrich Pvt. Ltd (New Delhi, India). Tolbutamide was obtained from Sun Pharmaceuticals Ltd., (Baroda, India). All chemicals used including the solvents, were of analytical grade.

2.2 Collection of plant material

The prop roots of *P. fascicularis* were collected from Theni District in Tamil Nadu, India during the month of June 2005. The plant was identified and authenticated by Mr. G.V.S. Murthy, Joint Di-rector, Scientist, C-I/C, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore bearing the reference number BSI/SC/5/23/05-06/TECH/447. A voucher specimen (Herbarium No: RTL-54) has been deposited at the Vinayaka mission's college of pharmacy herbarium for future reference.

2.3 Preparation of extracts

2.3.1 Aqueous extract

Prop roots of *P. fascic*ularis were dried in shade and powdered. The aqueous extract was prepared by cold maceration. The powder was soaked in equal amount of distilled water and stirred intermittently and then left overnight. The macerated pulps were then filtered through a coarse sieve and the filtrate was dried at reduced pressure in the rotor evaporator (Buchi Rotavapor R-114) and finally freeze dried. These extracts were used for further studies. The yield of the aqueous extract was 4.5 % w/w.

2.3.2 Ethanolic extract

Ethanolic extract was prepared from a powder of the prop roots of *P. fascicularis* prepared in an electric grinder. The 500 g powder was extracted with ethanol (95% v/v) in a soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in a vacuum desiccator (5.5% w/w).

2.4 Preliminary phytochemical screening

The presence of various phytochemical constituents in the extract was determined using standard screening tests[12].

2.5 Animals

Male Albino rats (150-175 g) of Wistar strain were used in the pharmacological studies. Before and during the experiment the animals were maintained in well-ventilated room at room temperature with natural day-night cycle in polypropylene cages lined with husk in standard environmental conditions (temperature (25±2) ℃, relative humidity (55±10) % and 12:12 light:dark cycle). The rats were fed on a standard pellet diet adlibium and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals.

2.6 Acute toxicity studies

The animals were randomly divided into three groups (n = 6). A control group having carboxymethylcellulose 10 ml/kg by oral route was compared with single dose (5 g/kg; p.o.) of aqueous and ethanolic extracts of *P. fascicularis*. Access to food and water, toxic symptoms and the general behavior of

mice were observed continuously for 1 h after the treatment, intermittently for 4 h, and thereafter over a period of 24 h. The mice were further observed for up to 14 days following treatment for any signs of toxicity and mortality[13].

2.7 Induction of diabetes

Diabetes was induced in rats by intra peritoneal (i.p) injection of STZ at a dose of 60mg/kg body weight, dissolved in 0.1 M cold citrate buffer (pH= 4.5) [14].

2.8 Experimental design

Different groups of rats were used to study the effect of aqueous and ethanol extracts of *P. fascicularis*. The rats were divided into five groups each consisting of 6 rats. Animals were allowed to fast 24 h and were injected with freshly prepared STZ (60 mg/kg, i.p.) dissolved in citrate buffer pH 4.5 except group 1.

Group 1 – Normal untreated rats.

Group 2 – Diabetic control

Group 3 – Diabetic rats treated with 250 mg/kg of aqueous extract.

Group 4 – Diabetic rats treated with 250 mg/kg of ethanol extract.

Group 5 – Diabetic rats treated with 100 mg/kg of tolbutamide.

After an overnight fast the plant extracts suspended in carboxymethylcellulose were fed by gastric incubations with a syringe. Group I and Group II were fed on distilled water alone. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2 and 3 h after feeding the plant extracts. The blood glucose levels were determined by using electronic glucometer. The values were compared with that standard which was received tolbutamide (100 mg/kg).

2.9 Fractionation, isolation, purification and characterization of compounds from the ethanol extract

Chromatographic techniques were used for the isolation of compounds from the fractions. The column chromatographic technique most commonly used for the separation of compounds into several fractions according to the affinity or solvating capacity of the compounds to the solvent used. The study involves in fractionation and isolation of compounds from pharmacologically active ethanol extract. The structure of the compound were tried to establish by spectroscopic methods.

2.9.1 Study design

In order to carry out column chromatography, a solvent system was established by developing TLC technique. The silica gel (100–200 mesh size) slurry was made with the solvent system established earlier. The slurry was poured time to time into the column very carefully and the silica gel was allowed to settle down to from a uniform packing. Then the stop-cock of the column was opened and the excess of solvent over the column head was allowed to run. The dry crude ethanol extract was mixed with small amount of silica gel in a mortar to get a free flowing powder. The powdered sample was then applied carefully on the top of the prepared column and successfully eluted with solvent/solvent system. Elutes were collected in a number of conical flasks marked from fractions 1–100. Elutes were spotted successfully on TLC plate and the flasks having similar spots were combined together.

2.9.2 Analysis of fraction F2

The fraction F2 containing 20–36 conical flasks having similar spots on TLC plate were combined. Then the fractions were subjected to TLC by using petroleum ether:benzene (50:50) as a solvent system. The expected bands were separated off and eluted with petroleum ether 100%, petroleum ether:benzene (50:50), yield of 150 mg obtained.

The compound was obtained as dull white sticky amorphous waxy solid. The fraction was characterized by spectroscopy techniques like Perkin-Elmer Vector 22 model FT-IR Spectrophotometer (Nujol), ^{1}H NMR spectra were recorded in a BRUKER DPX-200 MHz using TMS as internal standard and GC-Mass spectrometer spectra was recorded in SHIMADZU QP 50000.

2.10 Statistical analysis

Data were presented as mean ± SEM. Statistical differences between the control and treated groups were tested by one-way ANOVA followed by Student's two-tailed unpaired *t*-test. The differences were considered to be significant at $P < 0.05$.

3. Results

3.1 Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of carbohydrates, proteins, aminoacids, saponins, tannins, phenolic compounds, alkaloids and flavonoids.

3.2 Acute toxicity study

The extracts of *P. fascicularis* were safe up to a dose of 5 g/kg (p.o.) body weight. Over the study duration of 14 days, there were no deaths recorded in the groups of mice given aqueous and ethanolic extracts of *P. fascicularis* during the observation period, *P. fascicularis* administration did not induce any variations in the general appearance or toxic

Table 1
Effect of ethanol and aqueous extracts of *P. fascicularis* Lam, on STZ induced diabetic rats (n=6).

S.No	Design of treatment	Dose (mg/kg)	Blood glucose level (mg/kg)			
			0h	1h	2h	3h
1	Normal control	–	82.00 ± 1.60	79.00 ± 1.10	85.00 ± 1.24	83.00 ± 1.32
2	Diabetic control	–	237.00 ± 2.45	243.00 ± 1.64	234.00 ± 2.10	226.00 ± 1.78*
3	Ethanolic extract	250	215.00 ± 9.16	190.00 ± 1.14	160.00 ± 1.18	131.00 ± 1.92*
4	Aqueous extract	250	228.00 ± 2.70	183.00 ± 1.24	134.00 ± 13.32	102.00 ± 1.73*
5	Tolbutamide	100	84.75 ± 2.17	85.75 ± 0.97	76.00 ± 1.82	73.00 ± 0.95**

*P < 0.05, **P < 0.01 *vs* control values are expressed as mean ± SEM.

signs in the animals.

3.3 Antidiabetic activity

The hypoglycemic effect of ethanol and aqueous extracts of *P. fascicularis* was investigated in STZ induced diabetic rats. The fasting blood glucose levels were measured at 0, 1, 2 and 3 hrs after the treatment of ethanol and aqueous extracts. The reduced blood glucose level was significant (P < 0.05) in the dose of 250 mg/kg of ethanol and aqueous extracts of *P. fascicularis*, when compared with diabetic control (Table 1). The blood glucose level of diabetic control animals after 3 h was (226.00 ± 1.78) mg/mL, whereas it was (102.00 ± 1.73) mg/mL and (131.00 ± 1.92) mg/mL for the groups treated with aqueous extract and ethanol extract 250 mg/kg, respectively. All these values were compared with that of standard group which was received tolbutamide 100 mg/kg.

3.4 Isolation and identification of the active compound

The ethanol extract which has the polarity in between the acetone and aqueous has been selected for isolation of the available active constituents. The purity of isolated compound was checked by TLC using different solvent system. R_f value of the isolated compound 0.62 [(benzene: methanol) (90:10)]. The isolated compound was characterized by its physical, chemical as well as spectrometric analysis. It is dull white sticky amorphous waxy crystalline compound soluble in pet ether, benzene and chloroform. The melting point is 58–60 ℃.

IR data of the isolated compound shows the intense peaks at the following frequency: 3 784 cm^{-1}, 3 340 cm^{-1}, 2 919 cm^{-1}, 2 852 cm^{-1}, 1 667 cm^{-1}, 1 621 cm^{-1}, 1 457cm^{-1}, 1 374 cm^{-1}, 1 052 cm^{-1}, 923 cm^{-1}, 852 cm^{-1} and 722 cm^{-1}. The peaks at 2 919 cm^{-1}, 282 cm^{-1} and 1 457 cm^{-1} show the paraffinic nature of the compound. Peak at 3 340 cm^{-1} indicates the presence of hydroxyl group and peaks at 1 667 cm^{-1} shows the presence of double bond. Band at 722 cm^{-1} shows its long chain nature.

^{1}H NMR Spectrum of the isolated compound shows the δ values at the following ppm: 0. 87, 1.25, 2.01, 2.52, 3.37, 3.75 and 4.44. The spectrum shows δ value 2.01 for terminal methyl group, δ: 3.3 for six protons due to three methylene groups directly attached of the OH group resonated at δ: 2.25 as a broad singlet. All other methylene protons resonated at δ: 1.25 and it also indicates the presence of hydroxyl protons. This spectrum also indicates the presence of long chain aliphatic nature of the compound.

Mass spectrum of the compound shows the following fragmentation pattern: 256 (M^+), 236, 201, 183, 167, 153, 137, 153, 97, 83, 69, 61 and 43. The mass spectrum showed it to be a straight chain compound (Base peak at m/z 43). The values indicate the presence of CnH_2n fragments which indicates the presence of methylene groups and long chain nature of the compound. Formation of fragments at m/z 83 and 167 confirm the position of double bond at C–5.

On the basis of the above data the isolated compound may be characterized as Hepta deca –5–ene–1–ol.

Structure: $H_3C-(CH_2)_{10}CH=CH(CH_2)_3CH_2OH$

Molecular formula: $C_{17}H_{34}O$

Molecular weight: 254

4. Discussion

Diabetes mellitus is possibly the world's largest growing metabolic disorder and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy[15]. The enormous costs of modern medicines indicate that alternative strategies are required for better management of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic complications. The study of such medicines might offer a natural key to unlock a diabetilogist's pharmacy for the future. In adult animals, STZ selectively destroys the pancreatic insulin – secreting beta cells leaving less active cells resulting in type 1 diabetic state[14]. Antihyperglycemic effects of *P. amaryfollius* leaf extract have been reported on STZ – diabetic rats[11].

The present manuscript discusses about the anti–diabetic effects of the aqueous and ethanolic extracts of prop roots of *P. fascicularis* on STZ–induced rats. Acute toxicity studies revealed that non toxic nature of the aqueous and ethanolic extracts of prop roots

of *P. fascicularis*. There was no lethality or any toxic reactions found with the selected dose until the end of the study period.

Aqueous and ethanolic extracts of *P. fascicularis* at the dose of 250 mg/kg produced a significant fall in the blood glucose level in diabetic rats. On the other hand, tolbutamide caused significantly more hypoglycemia in comparison with the plant extracts. Emphasize is laid on glucose homeostasis as a severe hypoglycemia can result in life threatening situation. Therefore, lesser hypoglycemia with plant extract in comparison with tolbutamide is a desirable feature. The mechanism of this hypoglycemic effect of the extracts is not elucidated in this study. Further investigation is expected to characterize the active hypoglycemic principle and to elucidate the mechanism of action. In this study the aqueous and ethanolic extracts of *P. fascicularis* is produced the maximum glucose lowering activity in diabetic rats after 3 h and aqueous extracts produced significant hypoglycemic activity than ethanolic extract. The compound was isolated from the fraction F2 using column chromatography.

The structure of the compound may be considered from IR, ^{1}H-NMR and Mass spectroscopy data as Hepta deca -5-ene-1-ol. From this study, it is concluded that aqueous extract of prop roots of *P. fascicularis* has beneficial effects on blood glucose level. It has the potential to impart therapeutic effect in diabetic.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors wish to acknowledge Mr. G.V.S. Murthy, Joint Di-rector, Scientist, C-I/C, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore for identification and authentication of *P. fascicularis*. The authors thank to management of Vinayaka Mission College of Pharmacy, Vinayaka Mission University, Salem for their kind support and facility provided for this project.

Reference

[1] Tharkar S, Devarajan A, Kumpatla S, Viswanathan V. The socioeconomics of diabetes from a developing country: a population based cost of illness study. *Diabetes Res Clin Pract* 2010; **89**: 334-340.

[2] Kristova V, Liskoya S, Sotnikova S, Vojtko R, Kurtansky A. Sulodexide improves endothelial dysfunction in streptozotocin-induced diabetes in rats. *Physiol Res* 2008; **57**: 491-494.

[3] Villeneuve LM, Natarajan R. The role of epigenetic in the pathology of diabetic complications. *Am J Phy Renal Physiol* 2010; **299**: 14-25.

[4] Sikarwar MS, Patil MB. Anti-diabetic activity of *Crateva nurvala* stems bark extract in alloxan-induced diabetes rats. *J Pharm Bioallied Sci* 2010; **2**: 18-21.

[5] Rajalaksmi M, Eliza J, Cecilia E, Nirmala A, Daisy P. Antidiabetic properties of *Tinospora cordifolia* stem extracts on streptozotocininduced diabetic rats. *Afr J Pharm Pharmacol* 2009; **3**: 171-180.

[6] Kumar BD, Mitra A, Manjunatha M. *In vitro* and *in vivo* studies of antidiabetic Indian medicinal plants: a review. *J Herbal Med Toxi* 2009; **3**: 9-14.

[7] Salahuddin M, Jalalpure SS. Antidiabetic activity of aqueous fruit extract of *Cucumis trigonus* Roxb. in streptozotocin-induced-diabetic rats. *J Ethnopharmacol* 2010; **127**: 565-567.

[8] Chika A, Bello SO. Antihyperglycaemic activity of aqueous leaf extract of *Combretum micranthum* (Combretaceae) in normal and alloxan-induced diabetic rats. *J Ethnopharmacol* 2010; **129**: 34-37.

[9] Aslan M, Orhan N, Orhan DD, Ergun F. Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in turkey for diabetes. *J Ethnopharmacol* 2010; **128**: 384-389.

[10] Vinod MS, Raghavan PS, George S, Parida A. Identification of a sex-specific SCAR marker in dioecious *Pandanus fascicularis* L. (Pandanaceae). *Genome* 2007; **50**: 834-839.

[11] Sasidharan S, Sumathi V, Jegathambigai NR, Latha LY. Antihyperglycaemic effects of ethanol extracts of *Carica papaya* and *Pandanus amaryfollius* leaf in streptozotocin-induced diabetic mice. *Nat Prod Res* 2011; DOI:10.1080/14786419.2010.523703.

[12] Pandikumar P, Babu NP, Ignacimuthu S. Hypoglycemic and antihyperglycemic effect of *Begonia malabarica* Lam. in normal and streptozotocin induced diabetic rats. *J Ethnopharmacol* 2009; **124**: 111-115.

[13] Rajeswari J, Kesavan K, Jayakar B. Phytochemical and pharmacological evaluation of prop roots of *Pandanus fascicularis* Lam. *Asian Pac J Trop Med* 2011; **4**: 649-653.

[14] Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and in vitro antioxidant potential of *Hybanthus enneaspermus* (Linn) F. Muell in streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed* 2011; **1**: 316-322.

[15] Tahrani AA, Bailey CJ, Del Prato S, Barnett AH. Management of type 2 diabetes: new and future developments in treatment. *Lancet* 2011; **378**: 182-197.