

Full Length Research Paper

Hypoglycemic and anti-hyperglycemic study of *Phaleria macrocarpa* fruits pericarp

Rabyah B. Ali¹, Item J. Atangwho^{1,2*}, Navneet Kuar¹, Elsnoussi A. H. Mohamed¹,
Ali J. Mohamed¹, Mohd. Z. Asmawi¹ and Roziahanim Mahmud¹

¹School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia.

²Department of Biochemistry, College of Medical Sciences, University of Calabar, P. M. B. 1115, Calabar, Nigeria.

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Fruits of *Phaleria macrocarpa* are used in the traditional health system of the Indonesians and lower course of Malaysia, as effective remedy and management for diabetes mellitus. This study therefore investigated the hypoglycemic and anti-hyperglycemic effects of *P. macrocarpa* extracts in normal and diabetic rats, respectively. The fruits were sequentially extracted in petroleum ether, methanol and water to obtained 3 extracts. Blood glucose response to acute dose of the extracts (1g/kg b.w.) and intra-peritoneal glucose tolerance test (IPGTT) was performed in normal rats and a 12-day anti-hyperglycemic activity study was carried out in diabetic rats. The extracts exerted no effect in acute dose study. However, the methanol and water extracts showed a non significant blood glucose lowering effect between the 30 and 120 min in IPGTT, compared with the normal control. In diabetic rats, methanol extract only, lowered blood glucose by 56.25 and 58.33% ($P < 0.05$) after a 12-day treatment compared with diabetic control (DC) and pre-treatment values, respectively; an effect similar to metformin, glibenclamide and insulin treated groups. Plasma insulin measured in the methanol extract-treated group was found to decrease by 75 and 50% compared with DC and pre-treatment values, respectively ($P < 0.05$). This later effect only compared with the insulin-treated control with respective reductions of 65 and 30% ($P < 0.05$). Phytochemical thin layer chromatography (TLC) spray test of the methanol extract indicated the presence in abundance of flavonoids, terpenoids and tannins. The antidiabetic activity of *P. macrocarpa* was validated and this action which resides in the methanol fraction may be exerted by extra-pancreatic means.

Key words: *Phaleria macrocarpa*, plasma insulin, blood glucose, IPGTT, streptozotocin-induced diabetes.

INTRODUCTION

Phaleria macrocarpa (Scheff) Boerl is a shrub or small tree of the family Thymelaeaceae and usually grows up to 5 m or sometimes up to 18 m (Winarto, 2003). For centuries, the native Indonesians have used the fruits and leaves of the Mahkota Dewa (literally translated as God's Crown) tree, *P. macrocarpa* (Scheff.) Boerl to combat diabetes, liver diseases, vascular problems, cancer and high blood pressure (Tjandrawinata et al., 2010). Research has also shown that *P. macrocarpa* contains plant secondary metabolites that could combat not only cancers or infectious disease such as malaria, but also the so-called lifestyle diseases including

diabetes, hypertension, atherosclerosis and the likes (Harmanto, 2003). Parts of *P. macrocarpa* that are used for medicinal treatments are the stems, leaves and fruits. It is also used traditionally to control disorders in animals. The methanol extract of various parts of *P. macrocarpa* could be considered as a natural antimicrobial source due to the presence of flavonoid compounds (Hendra et al., 2011).

In that study, flavonoid compounds present in *P. macrocarpa* fruits pericarp were detected using Reversed Phase - High Performance Liquid Chromatography (RP-HPLC) including, kaempferol, myricetin, naringin and rutin. With respect to anti-diabetic studies, Triastuti et al. (2009) have reported that the ethyl acetate extract of *P. macrocarpa* had anti-diabetic activity against alloxan - induced diabetic rats, which according to the authors is mediated either by preventing the decline of hepatic

*Corresponding author. E-mail: aijustyno@usm.my or aijustyno@yahoo.com. Tel: +2348051684035, +60164802608.



Figure 1. *P. macrocarpa* (Mahkota Dewa, 'God's Crown') plant from the family of Thymelaeaceae showing the fruits.

antioxidant status or due to its indirect radical scavenging capacity. In another study by the same authors, the methanol extract of *P. macrocarpa* demonstrated anti-nephropathic action, which they argued may correlate the increase of renal antioxidant enzyme activity in alloxan-induced diabetic rats. Sugiwati et al. (2006) have also reported that the n-butanol of young and ripe fruit extracts of *P. macrocarpa* contain the highest inhibitory activity followed by ethyl acetate and methanol extracts on *in vitro* α -glucosidase enzyme. Also, these authors in a related study reported that the ethyl acetate extract from the old leaves of *P. macrocarpa* had the highest inhibition activity based on α -glucosidase inhibition test compared to the young leaves.

The inhibition activity from the methanol and boiled water extracts of old leaves too were found to be greater and higher than that of young leaves. These reports, though seem to indicate antidiabetic activity of parts of this plant, are indiscriminate, disjointed and non systematic. A research on medicinal plant should go beyond screening for biological activity, be tailored towards systematic standardization and development of a natural product or herbal medicine which would complement or supplement existing conventional measures (Bauer and Tittel, 1996; Ong, 2004) as well as follow quality assessment and evaluation guidelines (WHO, 2000). The present study was therefore a preliminary bio-guided testing of antidiabetic properties of *P. macrocarpa* fruits, the actual plant part used in traditional management of metabolic disease, such as

diabetes and hypertension. Initial screening such as this, is a fundamental requirement to natural product development and sourcing of therapeutic agents from medicinal plants.

MATERIALS AND METHODS

Chemicals

Standard antidiabetic drugs Glibenclimide 10 mg, metformin HCl 500 mg and insulin were obtained from Novo Nordisk (Copenhagen, Denmark). Streptozotocin (STZ) was procured from Sigma-Aldrich Chemical Company US. Extraction solvents including petroleum ether and methanol were of analytical grade.

Plant material collection and preparation of extracts

Ripe fruits of *P. macrocarpa* Benth were collected from Kepala Batas, Seberang Perai, Pulau Pinang, Malaysia (Figure 1). The pericarp of the fruits were sliced, dried and ground into powder using a milling machine. About 2400 g of the grounded material was sequentially extracted with petroleum ether (40 to 60°C), then methanol using soxhlet apparatus (40°C) for 48 h each. The residue from methanol extraction after complete drying was re-extracted with water by maceration at 60°C for 24 h. The extraction procedure with each solvent was repeated three times and the different extracts obtained were filtered with Whatman No. 1 filter paper and concentrated *in vacuo* by rotary evaporation (Buchi, Switzerland) at reduced pressure. The concentrated extracts were frozen at -70°C for 48 h then freeze-dried under vacuum for 24 h to yield dried extracts as shown in Table 1. The extracts were kept in the fridge (4°C) from where aliquots were withdrawn for the test procedures.

Animals

Healthy male Sprague Dawley (SD) rats weighing between 200 to 250 g obtained from the animal research and service centre (ARSC), Universiti Sains Malaysia (USM) was used in the study. The animals were housed and kept at 25 to 30°C in the animal transit room, School of Pharmaceutical Sciences, USM. They were allowed access to food (standard laboratory chow, Gold Coin Sdn. Bhd., Malaysia) and tap water *ad libitum*. The experimental procedures were approved by the Animal Ethics Committee of Universiti Sains Malaysia (AECUSM) Penang, Malaysia.

Acute dose hypoglycemic test in normal rats

Glucose response to a single acute dose of the extracts (1g/kg body weight) compared to standard antidiabetic drugs was carried out in 25 overnight fasted normal rats (200 to 250 g) divided equally into five groups of six rats each and thus treated: Group I served as a negative control and received normal saline, 10 ml/kg b.w, Group II served as a positive control and treated with glibenclimide, 10 mg/kg b.w, Group III received 1g/kg b.w of petroleum ether extract of *P. macrocarpa*, Group IV received 1g/kg b.w. of methanol extract of *P. macrocarpa* and Group V received 1g/kg b.w. of water extract of *P. macrocarpa*.

The extracts/drug/saline was administered orally via intra-gastric tube. Blood samples were collected from tail vein prior to dosing and then at 1, 2, 3, 5 and 7 h after, for glucose level estimation using a clinical glucose meter (Accu-check Advantage II, Roche diagnostics Company USA).

Table 1. Percentage yield of extracts obtained with various solvents starting with 2400g of *P. macrocarpa* fruit powder.

Material	Amount (gm)	Percentage yield
Fruits powder	2400	100
Petroleum ether extract	73.6	3.06
Methanol extract	445.36	18.55
Water extract	146.08	6.08

IPGTT in normal rats

The procedure, dosage of drugs/extracts and animal grouping was as in acute dose hypoglycemic test in normal rats. But in this procedure, an hour after drug/extract treatment the rats were administered glucose (1g/kg b.w.) intra-peritoneal and blood samples withdrawn via tail puncture at times 0 (prior to drug/extract treatment) 15, 30, 45, 60, 90 and 120 min after the glucose loading for glucose level estimation using Accu-check Advantage II (Roche diagnostics Company USA).

Short term (12-days) anti-hyperglycemic test in STZ -induced diabetic rats (SDRs)

Forty two (42) diabetic and 6 non diabetic rats were assigned into 8 groups of 6 rats each and treated as shown thus. Group 1, 6 normal rats were treated with normal saline, 10 ml/kg b.w, Group 2, 6 Diabetic rats were treated with normal saline, 10 ml/kg b.w, Group 3, 6 Diabetic rats were treated with glibenclamide, 10 mg/kg b.w, Group 4, 6 Diabetic rats were treated with metformin, 250 mg/kg b.w, Group 5, 6 Diabetic rats were treated with insulin, 5IU/kg b.w, Group 6, 6 Diabetic rats were treated with petroleum ether extract, 1g/kg b.w, Group 7, 6 Diabetic rats were treated with methanol extract, 1g/kg b.w, Group 8, 6 Diabetic rats were treated with water extract, 1g/kg b.w. Diabetes was induced in rats by intra-peritoneal injection of 65 mg/kg b.w. of STZ after an overnight fast (Abdul-Razak et al., 2002). 72 h after STZ administration, blood glucose level was measured. Rats with fasting blood glucose levels ≥ 15 mmol/l were considered diabetic and used for the study. Treatment was once a day, and lasted for 12 days. Whereas the drug/extracts were administered via intra-gastric oral tube, insulin was given subcutaneous. Blood glucose during and at end of study was monitored using Accu-check Advantage II with tail vein blood. Blood samples collected at onset and at end of study in the methanol extract treated group was used for insulin assay. These samples were collected into hematocrit-capillary tubes (Hirschmann Laborgerate GmbH and Company KG, Eberstadt, Germany) and centrifuged at 12,000 rpm for 3 min. The plasma samples obtained were stored at -20°C until measurement of insulin concentration. Insulin in samples was assayed by enzyme-linked immunosorbent assay (ELISA) using the Rat Insulin ELISA Kit (Crystal Chem).

Phytochemical screening

The active methanol extract was tested for the presence of selected phytochemicals by running the extract and mangiferin a reference compound through TLC using ethyl acetate: methanol: formic acid: water (8:2:1:1) as the solvent system (mobile phase). The chromatogram was thereafter developed by spraying specific spray reagents as follows:

Flavonoids

10 ml of natural product - polyethylene glycol reagent (NP/PEG) was used (by spraying method) to develop the TLC plate and

examined under ultraviolet (UV) at 356 nm. The presence of dark yellow, blue and green fluorescence indicated the presence of flavonoids (Wagner et al., 1984). *Terpenoids*: Anisaldehyde-sulphuric acid was used as spray to develop TLC plate and then heated for 5 min on a hot plate. The presence of blue-violet fluorescence to red violet spots under visible light and UV 365 nm indicates the presence of terpenoids (Wagner et al., 1984).

Alkaloids

Dragendroff reagent was sprayed to develop TLC plate followed by spraying 5% sodium nitrate, then examined under visible light and UV light. The presence of orange-brown spots/zones (which may be unstable) indicates the presence of alkaloids (Wagner et al., 1984).

Detection of tannins

Tannins were detected by test tube method. An aqueous solution of the extract that contains hydrolysable tannins is precipitated by 1 ml (10% w/v) of lead acetate on addition of 1ml (10% v/v) of acetic acid, while non-hydrolysable tannins (condensed) are soluble in addition of 1 ml (10%v/v) of acetic acid (Wagner et al., 1984).

Statistical analysis

All data were expressed as the mean \pm SEM. Statistical analysis of data was performed using one-way analysis of variance (ANOVA) followed by Dunnett test for post hoc analysis. The SPSS statistical software (version 15.0) was used. $P < 0.05$ was considered significant.

RESULTS

Acute dose hypoglycemic test in normal rats

Figure 2 shows the effect of an acute dose (1g/kg) of petroleum ether, methanol and water extracts of *P. macrocarpa* fruit in normal rats. The extracts had no significant effect on measured blood glucose during the seven-hour test when compared to the normal control group which received saline treatment only. On the other hand, the standard antidiabetic drug, glibenclamide (10 mg/kg b.w.) significantly lower blood glucose level from 1 to the 7 h, compared to normal control.

Intra-peritoneal glucose tolerance test with *P. macrocarpa* fruit extracts in normal rats

Result of the effect of extracts of *P. macrocarpa* on

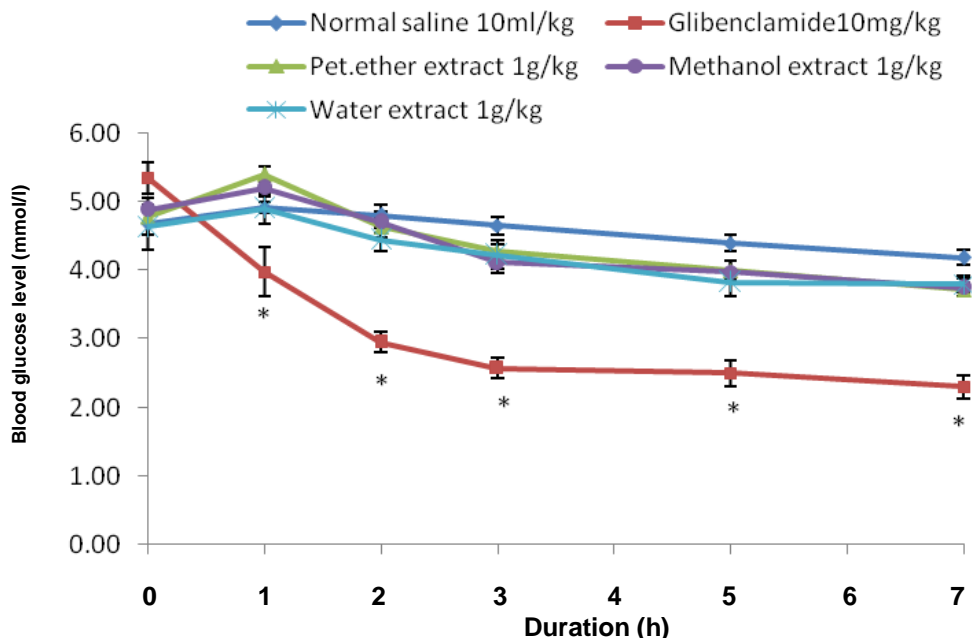


Figure 2. The effect of oral administration of single dose (1g/kg b.w.) of petroleum ether, methanol and water extracts of *P. macrocarpa* fruit on blood glucose levels of normal rats. Values are the mean±SEM, n = 6, * = P<0.05 vs. normal control.

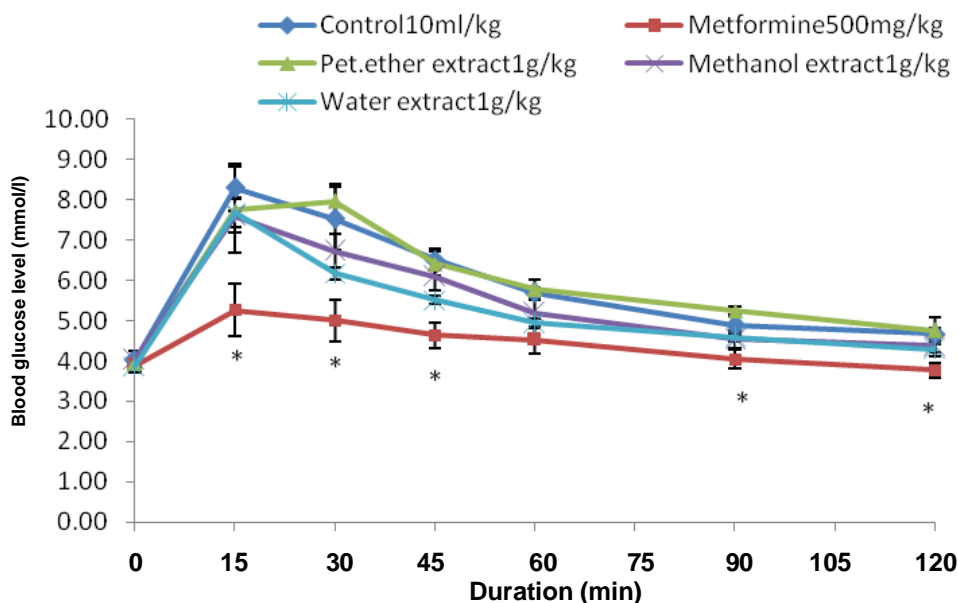


Figure 3. Effect of extracts of *P. macrocarpa* (1g/kg b.w.) on blood glucose following an intra-peritoneal glucose challenge (1g/kg b.w.) compared to metformin. Values are the mean±SEM, n = 6, * = P<0.05 vs. normal control.

blood glucose following a parenteral glucose challenge compared to metformin is shown on Figure 3. Whereas, petroleum ether extract exerted a null effect on the glucose level, methanol and water extracts showed a

mild glucose lowering activity between the 30 and 120 min compared to the saline treated normal control group (P>0.05). Metformin an anti-hyperglycemic agent significantly lowered blood glucose from the very first

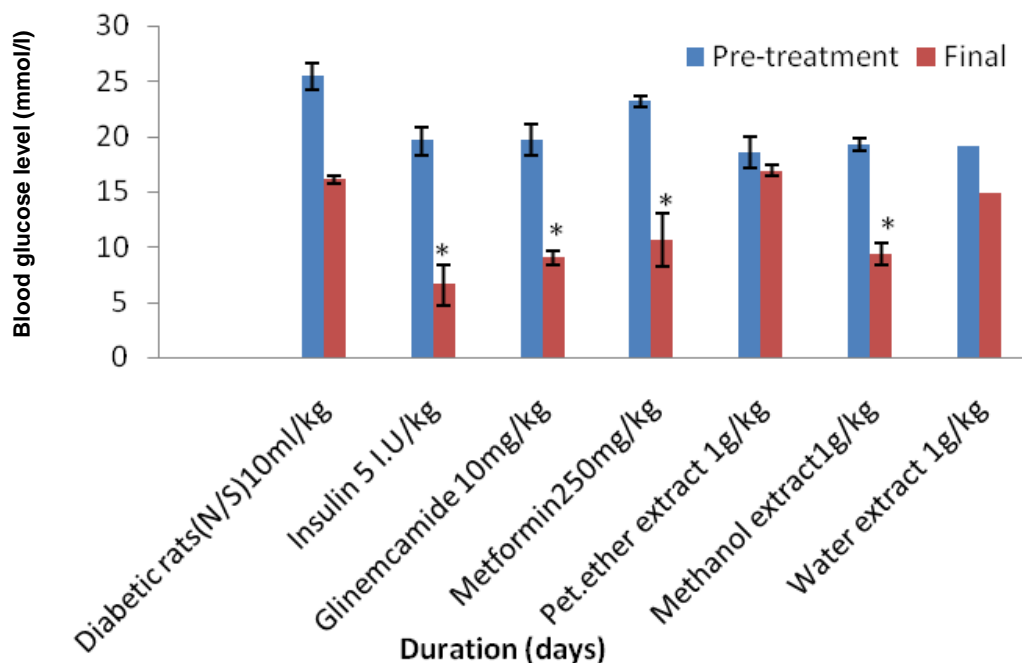


Figure 4. The effect of 12 day administration of *P. macrocarpa* fruit extracts on blood glucose level of streptozotocin-induced diabetic rats. Values are the mean \pm SEM, n = 6, * = $P < 0.05$ vs. diabetic control.

hour and sustained it throughout the duration of the experiment ($P < 0.05$).

Anti-hyperglycemic test in streptozotocin induced-diabetic rats

The effect of 12-day administration of *P. macrocarpa* fruit extracts on blood glucose levels of STZ-induced diabetic rats is shown in Figure 4. From the result, there was no significant effect on blood glucose levels of diabetic rats treated with petroleum ether and water extracts. However, methanol extract lowered blood glucose level by 56.25 and 58.33% compared to DC and pre-treatment values, respectively ($P < 0.05$). This glucose lowering effect compared well with the standard drugs, glibenclamide (10 mg/kg), metformin (250 mg/kg) and insulin (5 I.U/kg) which also significantly reduced blood glucose level ($P < 0.05$). Alongside blood glucose, plasma insulin was measured in blood samples collected pre- and post treatment in the active methanol extract-treated group (Figure 5). Result showed significant reduction in plasma insulin concentration of 75 and 50% by methanol extract treatment compared to the DC and the pre-treatment values respectively, ($P < 0.05$). Unlike blood glucose, this plasma insulin lowering effect only compared with the insulin treated positive control with respective reductions of 65 and 30% in plasma insulin level ($P < 0.05$). The effects of metformin and glibenclamide on plasma insulin were not significant.

Phytochemical screening of most active methanol extract

Selective phytochemical screening of the methanol extract of *P. macrocarpa* fruit using TLC and/or test tube procedures revealed the presence of flavonoids, tannins and terpenoids, whereas the extract tested negative for alkaloids (Figures 6, 7, 8 and 9).

DISCUSSION

Using a preliminary systematized ethno-medical drug discovery approach, the present study, evaluated the hypoglycemic and anti-hyperglycemic activity of sequential extracts from fruits of *P. macrocarpa* in normal and STZ diabetic rat models, respectively. In an acute dose (1g/kg) glucose response test in normal rats, there was no observed glucose lowering activity of the three extracts; unlike the reference oral hypoglycemic agent (glibenclamide) used which significantly lowered blood glucose level over the 7 h duration of the test. Glibenclamide, a typical sulphonylurea stimulates the β cells of the pancreas to secrete insulin which, in turn stimulates the uptake of glucose from plasma for conversion into glycogen, triglyceride and proteins in its major target tissues, liver, fat and muscle (Nolte and Karam, 2001). It is probable from this observation that *P. macrocarpa* exerts its glucose lowering activity via an extra pancreatic rather than a pancreatic effect.

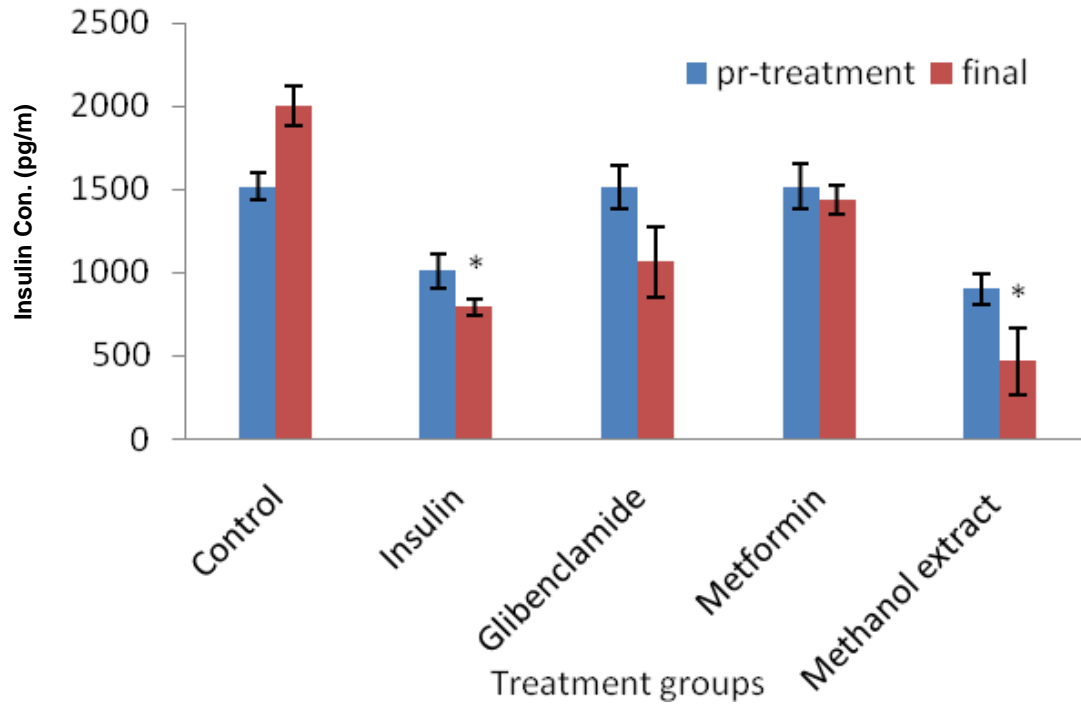


Figure 5. Effect of 12 day administration of methanol extract of *P. macrocarpa* fruit on plasma insulin level of streptozotocin-induced diabetic. Values are the mean±SEM, n = 6, * = P<0.05 vs. diabetic control.

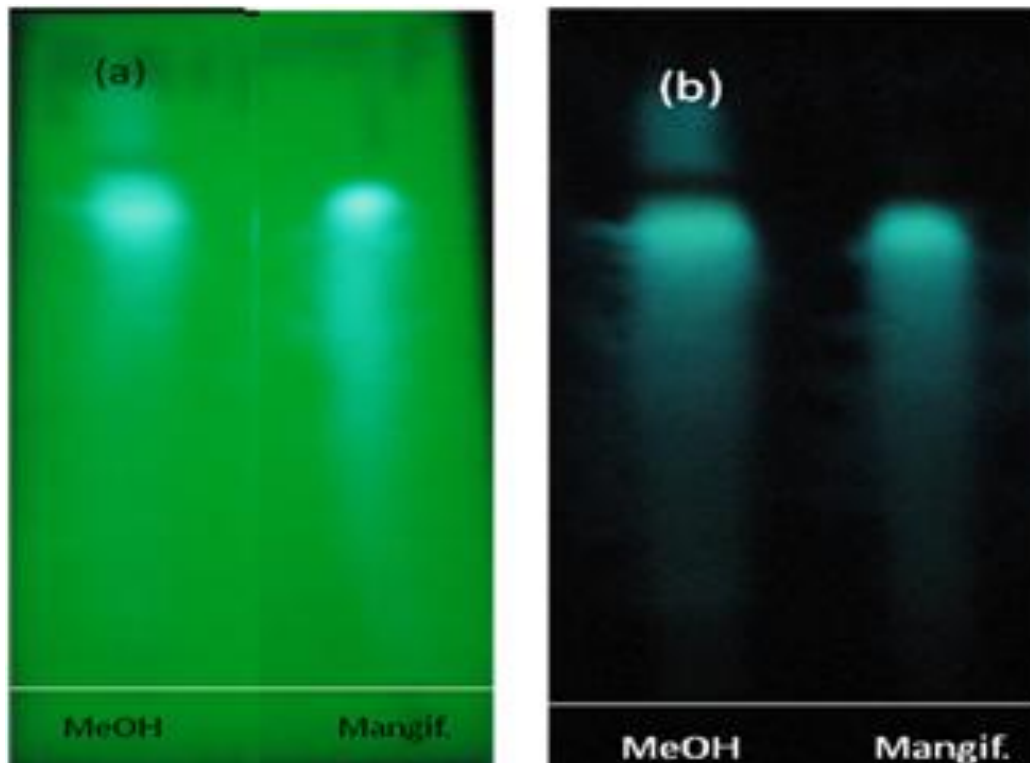


Figure 6. TLC profile indicating presence of flavonoids in methanol extract (MeOH) of *P. macrocarpa* compared to mangiferin (Mangif) a standard isolate from the fruit; a before and; b after spray with NP/PEG and detected under UV light at 365 nm.

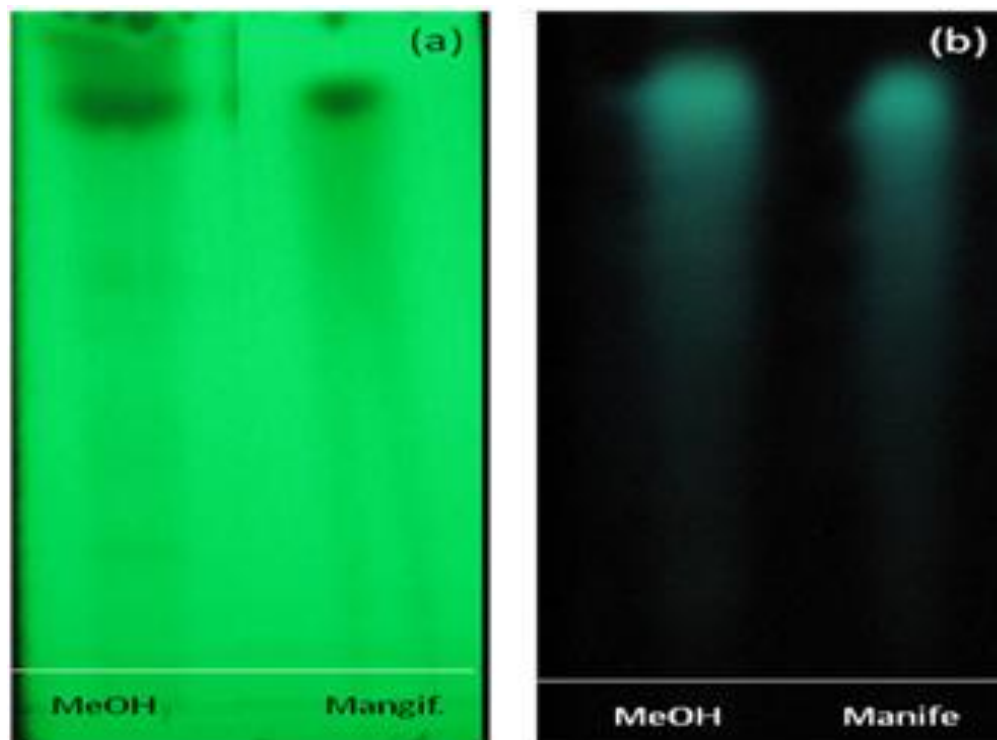


Figure 7. TLC profile indicating presence of terpinoids in methanol extract (MeOH) of *P. macrocarpa* fruit compared with and mangiferin (Mangif.) a standard; spray with natural product NP/PEG reagent and detected under; a 254 and; b 365 nm.

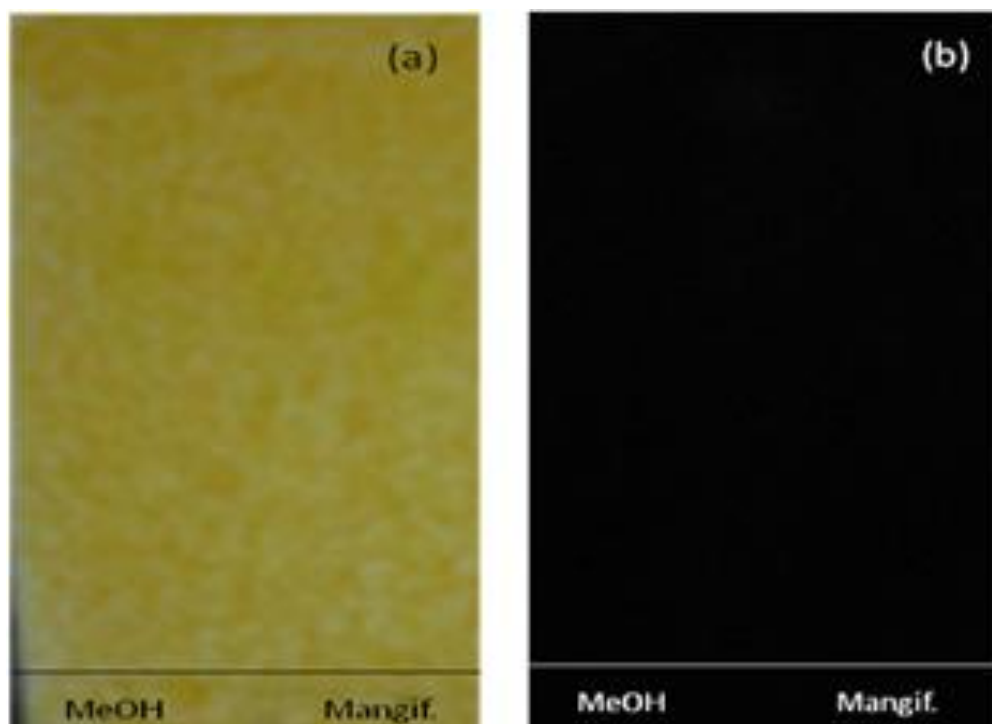


Figure 8. TLC profiles indicating absence of alkaloids in methanol extract (MeOH) of *P. macrocarpa* fruit compared mangiferin as a standard sprayed with Dragendroff's reagent and detected under; a visible light and; b UV 254 nm.

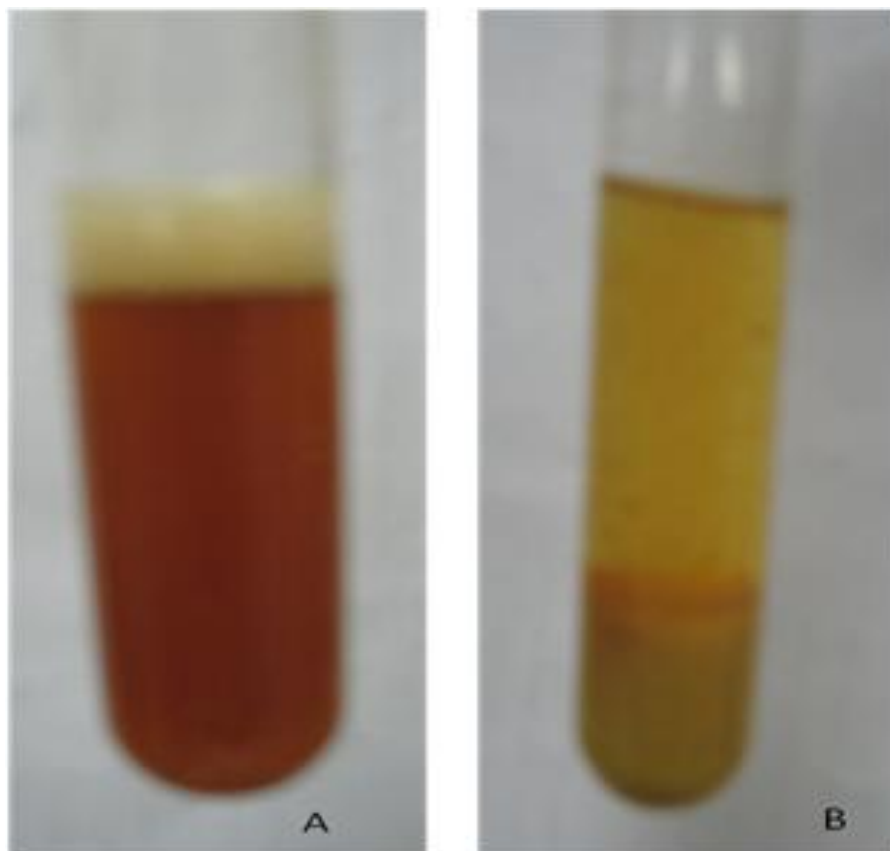


Figure 9. Brown ring (precipitate) indicating the presence of tannins in an aqueous solution of methanol extract of *P. macrocarpa* fruits before; A and after; B the addition of 1 mL (10% w/v) of lead acetate solution. On addition of 1 ml (10% v/v) of acetic acid the precipitate persisted, indicating the presence of hydrolysable tannins.

Evaluation of blood glucose response to parenteral glucose load (1 g/kg) in the presence of extracts of *P. macrocarpa* fruits showed mild lowering activity/response curve with methanol and water extracts but not petroleum ether extracts between the 15 and 120 min duration of the test.

To verify the perceived extra pancreatic action, metformin a typical biguanide which lowers blood glucose without increasing insulin secretion, was used for a reference this time around. Though the mechanisms of metformin action have remained obscure, multiple pathways of action have been proposed, including a decrease of hepatic glucose production, an increase of peripheral glucose disposal and a reduction of intestinal glucose absorption (Cheng et al., 2006). Its blood glucose lowering action does not depend on the presence of functioning pancreatic β cells. The similarity in trend of effect (not extent) of the methanol and water extracts to that of metformin was observed, and could strengthen our earlier submission on the manner in which the extract works. In a 12-day short term study with STZ diabetic rats, the antidiabetic effect of *P. macrocarpa* became clearer. Blood glucose level was restored by

over 50% within 12 days of daily treatment with 1g/kg body weight of methanol extract and a null effect with petroleum ether and water extracts. This action was quite similar to the standard drugs used for comparison: glibenclimide (10 mg/kg), metformin (250 mg/kg) and insulin (5I U/kg).

This potent glucose lowering action of methanol extract of *P. macrocarpa* not seen in acute condition is similar to the effect of most reported antidiabetic medicinal plants including *Vernonia amygdalina* Del. (Atangwho et al., 2007) and *Gongronema latifolium* (Akpasso et al., 2011). Obviously the active agents in a medicinal plant exist in very dilute concentrations, hence may require an initial duration of bioaccumulation for cumulative and concerted effect, hence explaining why in acute conditions the glucose lowering action of the methanol extract was not significant. Measurement of plasma insulin along with glucose is usually an efficient way of evaluating a diabetic therapy, as it also gives an insight into the antidiabetic mechanism of the therapeutic agent. We therefore measured insulin in blood sample of rats treated with active methanol extract before and at the end of treatment. It was observed that the active methanol

extract lowered plasma insulin in tandem with the blood glucose as well as the reference drugs. This observation is specifically in line with the report of Luo et al. (1999) on two novel antidiabetic compounds isolated from *Pycnanthus angolensis* namely, SP-18904 and SP-18905. Similar to our observation, these researchers observed a decline in plasma glucose concentration associated with lowered plasma insulin concentrations in diabetic animals treated with the novel plant compounds. The simultaneous lowering in plasma glucose and insulin may indicate that the active compounds *P. macrocarpa* are not insulin secretagogues but seem to be enhancing the ability of insulin to stimulate glucose disposal. A preliminary screening of this extract indicated the presence of tannins, terpenoids and flavonoids, corroborating our supposition on action of this active extract. For instance, the two novel compounds in the cited work were found to be terpenoids (Luo et al., 1999). Furthermore, Kumar et al. (2011) have in a review listed several examples of antidiabetic compounds belonging to the chemical groups tannins, flavonoids and terpenoids which exert their antidiabetic actions via α -glucosidase modulation, a typical extra pancreatic mechanism. On the grounds of this exciting findings, further studies are ongoing in our laboratory on bio-guided isolation and detailed antidiabetic mechanism of action of the methanol extract this plant with the aim of finding potent novel antidiabetic natural products with relative advantage(s).

Conclusions

Taken together, the results of the present study validate the antidiabetic activity of *P. macrocarpa* fruit pericarp, hence justifying its use in traditional management of diabetes. The work further suggests that the antidiabetic action which resides in the methanol fraction may be exerted by extra-pancreatic means.

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