The Effectiveness of Hot Water and Aqueous-Methanol Extractions of Malaysian *Swietenia Macrophylla* King Seeds and Endocarps Combined on Diabetic Rats

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Abstract

Practically, it's thought by the traditional use of Swietenia macrophylla in Malaysia that it has a rational means for the treatment of diabetic patients, but with regard to alternative medicine, the ethno medicinal literature does not mention any adverse effect or reaction associated with Swietenia macrophylla seeds. This study has designed to evaluate the hypoglycemic effectiveness exhibited by the combination of seeds and endocarps extraction performed using water as solvent and compares it with an aqueous-methanol solvent extraction in vivo. We found that aqueous-methanol extract is active for alkaloids, flavonoids, cardiac glycosides, reducing sugar, saponins, tannins and terpenoids and it is also more efficient than the hot water extract in improving altered biological parameters impacted by hyperglycemia. Therefore, the aqueous-methanol appears ideal for extracting the necessary potent hypoglycemic compounds of the plant parts studied.

Keywords: *S. macrophylla* King, Hot Water Extract, Aqueous-Methanol Extract, Hypoglycemic, Diabetes

Introduction

Diabetes mellitus is a wide spread disorder which has long been in the history of medicine. Despite continuous introduction of the modern drugs, diabetes and its related complication is still a global medical issue. Before the advent of synthetic insulin and oral hypoglycemic drugs, the major form of treatment involved the use of plants (Wadkar et al. 2008). Some of them are being used in traditional systems of medicines from hundreds of years in many countries across the world. The indigenous medicinal practices as an alternative to modern drugs have been uprising worldwide apparently due to their merits of efficacy, better patient tolerance, relatively less expensive and less frequent side-effects.

With regards to alternative medicine, the ethno medicinal literature does not mention any adverse effect or reaction associated with *Swietenia macrophylla* seeds (Dewanjee & Maiti, 2011). The endocarp which holds the seed has also been documented to possess a significant hypoglycemic activity (Radhamani et al. 2009). Practically, consumption of this plant is known in general in exerting their rational means for the treatment of diabetes. However, the various modes of action depend on the phytochemicals and bioactive components endowed in such plant parts (Atangwho et al. 2010).

The traditional consumption often involved the low-heat process and water as solvent in the preparation. The laboratory extractions on the other hand involves highly scientific method of standardization, which involves measuring and extracting specific compounds believed to be responsible for the plants' medicinal effects.

Nevertheless, successful determination of the biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Tiwari et al. 2011). It is a fact that different extraction methods illustrate the contrasting philosophies of the compound pulling at the ends of processes.

The phytomedicine properties gained at the end of the extraction processes shall determine the hypoglycemic effects upon the animal studied. Hence, the present investigation was undertaken to authenticate the fasting blood glucose (FBG) lowering activity between the extractions employing different solvent.

Materials and Methods

Plant material: *Swietenia macrophylla* King's seeds together with its endocarps were collected from the northern part of Malaysia where they are presumably in abundance. Subsequently, the plant parts were forwarded for taxonomical identification.

Chemicals: The solvents and other reagents used in this experiment were of analytical grade. Streptozotocin (STZ) for diabetic induction (including injectable anaesthetics) procured from Sigma-Aldrich Co., USA. Glibenclamide tablets (Daonil™ Aventis) were purchased from a local pharmacy.

Extracts Preparation: The selected plant parts were dried, crushed in an electric grinder and pulverized into a coarse powder form. Out of this powder, 80 g weighed and placed into a cheese cloth to form like a tea-bag. The bag was suspended into a beaker (Pyrex Iwaki, Thailand) which filled with 1L of distilled water to obtained hot water extract by decoction method (Koffi et al. 2009). The prepared extraction was filtered with filter papers (Whatman, no. 1) and evaporated to dryness using a rotary evaporator at 60 ºC. The final crude extract (16.05 g) was used in the biological assay.

An aqueous-methanolic extraction was prepared by soaking 100 g of the coarse powder in a conical flask with mixture solvent, consisting of 240 ml distilled water and 320 ml absolute methanol. The mixture, that was prepared according to De et al. (2011), kept in an incubator at 37 ºC for 36 hours and stirred intermittently at 4 hours intervals. Then, it filtered and the filtrate dried under a low pressure and a low temperature using rotary evaporator fitted with vacuum pump. A final 23.75 g of crude extract was collected at the end of the process.

Qualitative Phytochemical Analyses:

Alkaloid Test. 0.5 ml of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath for 10 minutes. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and a second portion with Wagner's reagent. Turbidity or precipitation with either of these reagents was observed.

Cardiac Glycoside Test. 5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was layered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

Flavonoid Test. 1 ml of ammonia was diluted with 4 ml of distilled water. 1 ml of the extract poured in a test tube and the diluted ammonia was poured slowly into the test tube, then, 1ml of sulphuric acid was added into the test tube and the yellow coloration that disappears on standing indicates the presence of flavonoids.

Reducing Sugar Test. 2 ml of the extract was placed in a test tube. 2.5 ml of Fehling's solution IA and Fehling's solution IIB were added to the tube. The mixture boiled for 5 minutes, the brick red precipitate was observed for free reducing sugar.

Saponin Test: 0.5 ml of diluted extract solution was shaken vigorously with 3 ml of distilled water in a test tube until frothing appears. The frothing that persist for 15 minutes indicates the presence of saponin.

Tannin Test: 0.5 ml the extract diluted with 3.5 ml of distilled water, then, 0.5 ml of the diluted extract was placed in a test tube. 1 ml of distilled water added to the tube, followed by a drop of ferric chloride solution. A Blue coloration was observed for gallic tannin and green black for catecholic tannin.

Terpenoids Test: 0.5 ml the extract mixed in 2 ml chloroform. 3 ml of concentrated sulphuric acid was added slowly at the side of the test tube to form a layer. A reddish brown coloration of the interface formed, which shows the positive results for the presence of terpenoids.

Selection of Animals and Animal Care:

Thirty matured male and normoglycemic rats of Sprague Dawley strain (250-300 g) were selected. Animals acclimated for a period of 7 days under controlled laboratory conditions.

They were kept under a standard of 12:12 hours ratio for light and dark cycle, fed on a standard laboratory pellets and water *ad libitum*. The principles of Laboratory Animal throughout the study were supervised by an internal Ethical Committee.

Induction of Diabetes: Animals were made diabetic by induction a combination of STZ and nicotinamide (NA). Overnight fasted rats were administered intraperitoneally with nicotinamide (230 mg/kg) only 15 minutes prior to STZ (65 mg/kg bw, dissolved in normal saline) administration, by a single intravenous injection, in order to develop a moderate and a stable non-fasting hyperglycemia (Masiello et al. 1998). STZ-induced diabetic like state were validated by elevated glucose levels in plasma, which determined at 14 days after injection. STZ-rats with FBG>170 mg/dl for at least a week were forwarded for the proposed study.

Acute Oral Toxicity Studies: There were no deaths of rats at the dose of 2000 mg/kg bw of the extracts, both within the short and long outcome. Different doses (125, 250, 500, 1000 and 2000) of hot water and aqueous-methanolic extracts were given orally. The animals observed for the initial 4 hours, alternately in the next 6 hours, and once again at 24 hours following the extract administration. The LD_{50} was calculated to be greater than 2000 mg/kg bw.

Animal Treatment: The normoglycemic rats randomly divided into five groups of six animals each. The extract treatments were force-fed daily for the duration of 21 days at a fixed dose of 250 mg/kg bw. Diabetic control rats received 5 mg/kg bw of glibenclamide, while controlled rats received 0.5 ml of normal saline.

Data Collection: Body weight and fasting blood glucose (FBG) level were determined right from the day after the diabetic state was confirmed (which was on day 14 following the induction of STZ) and subsequently at days 0, 7, 14 and 21 after being treated with the extractions and treatment. Animal's body weight was measured using a laboratory electrical balance and FBG level was made possible via AccuCheck Advantage blood glucometer (US, Roche Diagnostics).

Statistical Analysis: The results were expressed as mean ± SEM. The significant differences in the values were confirmed by one-way ANOVA test with multiple Dunnett t-tests. $P<0.05$ is considered to have significant differences and $P<0.001$ is considered to have highly significant differences.

Results

Table 1 shows the results of the phytochemicals assay of hot water and aqueous-methanolic extracts of *Swietenia macrophylla* King combined seeds and endocarps. The phytochemical analysis revealed the presence of alkaloid, cardiac glycosides, reduced sugar, tannins and terpenoids in both extractions, whereas, saponins were indicated in aqueous-methanol extract.

Table 1: Phytochemical constitutes of *Swietenia macrophylla* seeds and endocarps combined

Phytochemical Assay	Hot Water Extract	Aqueous-Methanol Extract
Alkaloid	+	+
Cardiac Glycosides	+	+
Flavonoids	+	+
Reducing sugar	+	+
Saponins	–	+
Tannins	+	+
Terpenoids	+	+

(+) Present (–) Absent

The initial body weight, at day 0, showed that rats body weight in all groups did not differ significantly (P>0.05) as demonstrated in Table 2 and Fig. 1. Rats' body weight continued to decrease in the diabetic group, throughout the experiment period, compared to control group.

The effect of aqueous-methanolic extract treatment showed to be significant at day 14, whereas, at the end of treatment (day 21), rats' body weights increased significantly ($P<0.05$) in all experiment groups compared to control group. Thus, there were an increment of body weight patterns in both, extracts and drug, treated diabetic rats, with the highest in aqueous-methanol extract group (10.1%) followed by glibenclamide (6.9%) and hot water extract groups (4.4%).

Table 2: Effect of *Swietenia macrophylla* seeds and endocarps combined extract treatment on body weight

Treatment Group	Body Weight (g)			
	Day 0	Day 7	Day 14	Day 21
Control	270.02 ± 7.06	290.29 ± 4.07	306.84 ± 2.88	335.80 ± 2.45
Glibenclamide	273.30 ± 7.82	282.50 ± 7.94	286.44 ± 8.88^{NS}	293.66 ± 8.95*
Hot Water Extract	278.42 ± 7.83	278.55 ± 8.00	282.32 ± 8.42^{NS}	291.07 ± 8.53*
Aqueous-Methanol Extract	271.48 ± 8.82	277.43 ± 9.85	293.79 ± 8.94*	301.82 ± 9.07**

Data expressed as mean ± SEM; n = 6.
* P<0.05 = significant; ** P<0.001 = highly significant; ^{NS} = non-significant.

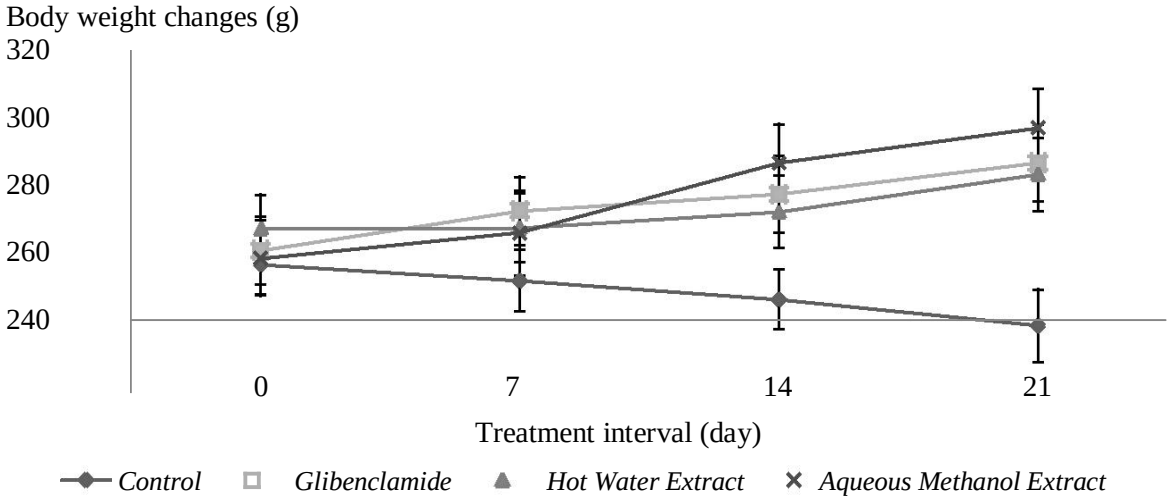

Fig. 1: Body weight profile of STZ-diabetic rats treated with *Swietenia macrophylla* seeds and endocarps combined extract

FBG results (tabulated and depicted in table 3 and fig.2) indicates that STZ-induced diabetic rats showed a significant elevation of FBG levels in all studied group. FBG levels on day 0 disclosed no significant inter-group variation (*P>0.05*). Meanwhile, the effectiveness of aqueous-methanol extracts and glibenclamide administration were substantiated by declension of the FBG levels measured on day 7, 14 and 21 (*P<0.001*). The hot water extract have reduced FBG beyond a significant level slightly later, which was after the interval of day 14 and 21. Though, the mean percentage of blood glucose drop down throughout the overall treatment were comparable between hot water (48.4%) and aqueous-methanol extracts group (46.6%).

Table 3: Effect of *Swietenia macrophylla* seeds and endocarps combined extracts treatment on FBG level.

Treatment Group	FBG level (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
Control	185.76 ± 4.78	189.00 ± 4.83	194.76 ± 7.94	205.92 ± 5.08
Glibenclamide	177.12 ± 5.74	93.60 ± 4.18**	82.08 ± 4.09**	68.40 ± 3.51**
Hot Water Extract	186.84 ± 4.35	180.0 ± 3.46 ^{NS}	135.76 ± 7.65**	96.48 ± 3.35**
Aqueous-Methanol E.	176.56 ± 7.07	142.04 ± 6.31**	101.52 ± 5.39**	94.32 ± 4.32**

Data expressed as mean ± SEM; n = 6
** P<0.001 = highly significant; ^{NS} = non-significant.

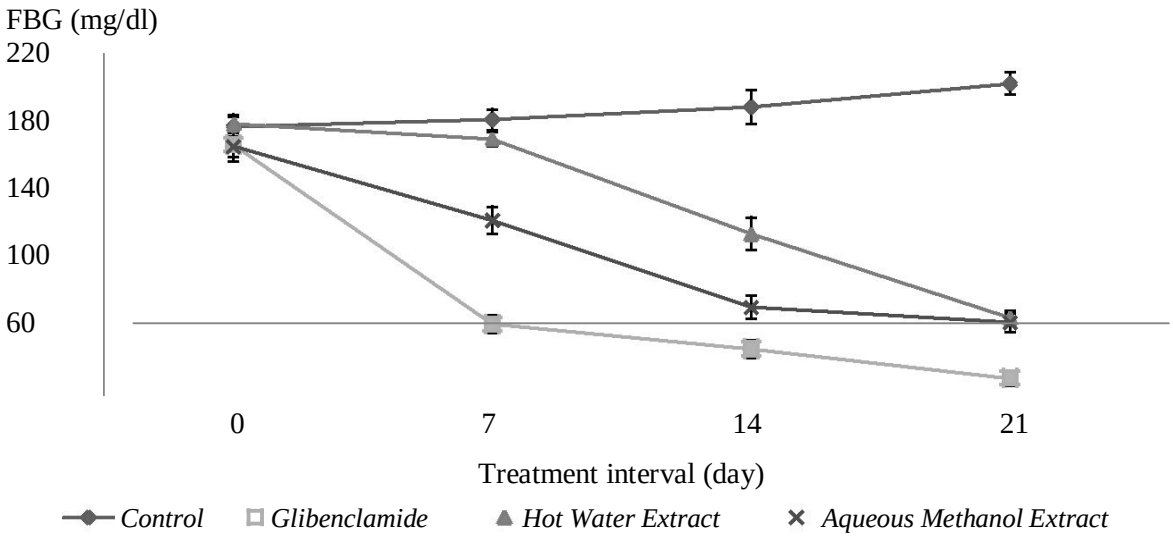

Fig. 2: FBG levels of STZ-diabetic rats treated with *Swietenia macrophylla* seeds and endocarps combined extract

Discussion

This study has been conducted to evaluate the hypoglycemic activity of *Swietenia macrophylla* King seeds and endocarps combined extracts, hot water and aqueous-methanol. We are the first team who use this combination of swietenia's seeds and endocarps to evaluate their effectiveness on diabetes-like state, even though few previous studies have led the way and mentioned to the hypoglycemic effect of swietenia's seeds (Maiti et al., 2008; Kalaivanan & Pugalendi, 2011) and endocarps (Radhamani et al., 2009) but none have tested the effect of the combination of these parts before. The rationale for performing extractions from polar to non-polar solvents is to validate the hypoglycemic activity of the extractions performed in decoction manner using water as a solvent and compares it with the aqueous-methanol solvent extraction. The preliminary philosophies were that each method pulls out different compounds, all of which were therapeutically important and more potent hypoglycemic compounds would be obtained through the extraction using organic solvents.

Basic phytochemicals screening of decoction extract showed the presence of alkaloids, flavonoids, cardiac glycosides, reducing sugar, tannins and terpenoids. The aqueous-methanol extraction has also revealed the presence of the above compounds with an addition of saponins (Table 1). This suggests that the combined plant parts were active for hypoglycemic potentials based on the literatures claiming that phytochemical compounds such as flavonoids, terpenoids, alkaloids, and phenolics are known to be bioactive hypoglycemic principles (Henningson et al. 2001; Sharma et al. 2003; Battu et al. 2007; Safithri & Fahma, 2008). Upon the two different extraction methods: hot water extract and aqueous-methanol extract, it has been observed that aqueous-methanol extract was more efficient in improving body weight of STZ-induced diabetic rats in comparison to the drug-glibenclamide. Besides, the same extract also reduced FBG levels steadily as it could be observed from day 7. In contrast to the hot water extract which did not manage to bring down blood glucose level significantly at the initial state. Nevertheless, the blood lowering effect of hot water extract was comparable to the aqueous-methanol extract at the end of the treatment on day 21. Conversely, the glibenclamide have demonstrated a marked reduction of FBG level in which it appeared to bring down glucose level towards the borderline of hypoglycemia. This was suspected since it has been clearly reported that the risk of hypoglycemia is highest with long-acting sulfonylureas such as chlorpropamide, glibenclamide and long-acting glipizide (Tattersall, 1999; Stahl & Berger, 1999; Rendell, 2004).

These results suggest that the aqueous-methanol extract is a better preference for the correction of altered biological parameters in diabetic rats. It supports the claimed made by Perva-Uzunalic et al (2006) that the use of an alcoholic solution provides satisfactory results for the extraction process. This is partly because they are more efficient in cell walls and seeds degradation. From our remarkable observation, there were also higher yields from the aqueous-methanol extract (23.75 g) as compared from the hot water extract (16.05 g). The use of alcohol and water mixture may have provide the advantage of modulating the polarity of alcohol solvents (Mohammedi & Atik, 2011). This mixture whose polarity was modified with water becomes ideal and selective to extract a great number of bioactive compounds, thus producing a higher total yield.

In contrast to the hot water extract, the use of water alone as a solvent may not provide adequate strength in pulling out subtle compounds, and when subjected to heat, the heat-labile compounds were flop. Therefore, the traditional preparation for consumption of this plant through boiling preparation (decoction method) was less effectual.

Conclusion

In this study, aqueous-methanol appears ideal for extracting the necessary potent hypoglycemic compounds within the seeds and endocarps of *Swietenia macrophylla* King. Hence, fractions from the substance expressing good biological capacity indicated by powerful biological effect exists in this extract shall be isolated and purified to verify its phytomedicinal usage.

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