# Phytochemical Constituents and Antihyperglycemic Activity of *Lygodium microphyllum* Against Alloxan Induced Diabetic Rats

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**ABSTRACT** Diabetes mellitus (DM) is a complex, chronic illness which requires continuous medical care as well as with multifactorial risk-reduction strategies beyond glycaemic control. Approximately 70% of the world's population use traditional medicines derived from medicinal plants. The present study was designed to evaluate the phytochemical constituents and antihyperglycemic activity of *Lygodium microphyllum*. The phytochemical screening of the plant showed the presence of flavonoids, alkaloids, tannins, and steroids. Total phenolic content of leaves was found to be 966.7 ± 0.03 mg/g (expressed as milligram gallic acid equivalent per gram of plant extract). Total flavonoid content of leaves was found to be 42.9 ± 0.01 mg/g (expressed as milligram catechin equivalent/g). Diabetes was induced by intravenous injection of alloxan monohydrate at a dose of 100 mg/kg body weight in rats. Blood glucose along with oxidative stress markers in pancreatic homogenate was assayed. Pancreas was also examined by haematoxylin/eosin staining. Treatment of diabetic rats with *L. microphyllum* at the dose level of 300, 400, and 500 mg/kg body weight once a day for 14 days prevented induction in blood glucose and attenuated alloxan induced oxidative stress. The protection was further evident through decreased histopathological alteration in pancreas. As a conclusion, optimistically this study will contribute towards validation of the traditional use of *L. microphyllum* in the treatment of diabetes. This study may be helpful in the prevention of diabetes complication associated with oxidative stress.

**KEYWORDS:** Diabetes; *Lygodium microphyllum*; Alloxan; phytochemical constituents; antihyperglycemic activity

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## INTRODUCTION

DM is found all over the world and becoming a serious threat to mankind health. More than 5.1 million people death every year while about a million people experienced lower limb amputations, kidney failures, and blindness caused by DM (Eid & Haddad, 2014). According to International Diabetes Federation (IDF), 415 million people have diabetes in the world and almost 153 million people in Western Pacific Region. This number will rise to 215 million people by 2040 (International Diabetes Federation, 2015). In Malaysia, 3.3 million cases of DM were reported in 2015. There are some factors involved in influencing the prevalence of DM including socioeconomic status, age, sex, genetic susceptibility, lifestyle, and other environmental factors (Takrouri, 2006).

DM is a chronic endocrine disorder involving most common metabolic disorders of carbohydrate, fat, and protein which are grouped under Non-Communicable Disease (NCD) (Ashraduzzaman *et al.*, 2011; Mohan *et al.*, 2013). The illness is requiring continuous medical care. Long term complications such as dysfunction and organs failure especially eyes, kidneys, nerves, heart, and blood vessels are the result of chronic hyperglycemia of diabetes (Olatunde *et al.*, 2014). DM also represented by lipidaemia and oxidative stress (Khalid *et al.*, 2014).

Malaysia is blessed with a rich diversity of plant kingdom and a number of indigenous plants have been described for antihyperglycemic. *Lygodium microphyllum* is a long-lived perennial vining fern and it is a serious invasive plant that grows in Africa, Australia, and Asia (Hasanah *et al.*, 2015). This plant has been used locally in folk medicine to treats skin diseases (Herin Sheeba Gracelin *et al.*, 2012), swelling, dysentery (Benniamin, 2011), anti-diuretic, anti-inflammatory agent, breast pain (Baltrushes, 2010), sore throat, dysuria, urinary tract stone, cutaneous and subcutaneous parasitic infection, oedema, gout, paralysis, epilepsy, convulsion, and spasm. This plant as well can treat a fever by leaf decoction, while leaves chewed to prevent fits (Hanum & Hamzah, 1999).

## METHODOLOGY

*Sample Collection, Preparation, and Storage of Plant Extract*

*L. microphyllum* was freshly collected at Universiti Malaysia Sabah (UMS). Only the leaves of the plant were used for the extraction. It was dried in an ambient temperature for up to 14 days until no moisture left. The dried samples grind to a coarse smaller sample or homogenous samples. Procedure described by Azalina Farina and Iqbal (2013) was used to prepare an aqueous extraction, where the ratio used is 1:10 of powdered sample and distilled water. The mixture boiled for 10 minutes and then filtered to obtain clear solution. The plant extracts then stored at -80$^{o}$C for 3 days. Lyophilisation of plant extracts done by using freeze-dryer and kept at -20$^{o}$C for further analysis.

*Phytochemical Screening*

The aqueous extract of *L. microphyllum* was screened for some phytochemical constituents (Table 1) using standard procedures (Azalina Farina & Iqbal, 2013; Soni & Sosa, 2013; Yadav *et al.*, 2014).

**Table 1.** Phytochemical constituents of *L. microphyllum*

| Phytochemical Constituent | Test | Observations |
|---|---|---|
| **Flavonoids** | 5 mL of dilute ammonia ($NH_3$) solution were added to a portion of the plant extract and then concentrated $H_2SO_4$ added into the mixture | Yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing |
| **Alkaloids** | Plant extract was mixed with 2 mL of Wagner reagent | Reddish brown colored precipitate indicates the presence of alkaloids |
| **Tannins** | 2 mL of plant extracts was taken in test tube and 2 mL of $dH_2O$ added. Then, 3 drops of 5% $FeCl_3$ added to the tube | Green precipitate indicates the presence of tannins |
| **Terpenoids** | 5 mL of plant extract was mixed with 2 mL of chloroform and 3 mL concentrated $H_2SO_4$ was carefully added to form a layer | A reddish brown coloration of the interface was formed to show positive result as indicated the presence of terpenoids |
| **Saponins** | Plant extract was mixed with 5 mL of $dH_2O$ in a test tube and it was shaken vigorously. Then, a few drops of olive oil added into it | Formation of stable foam indicated the presence of saponins |
| **Steroids** | 2 mL of acetic acid anhydride was added to 0.5 mL plant extract with 2 mL $H_2SO_4$ | The color changed from violet to blue or green in samples indicates the presence of steroids |

*Determination of Total Phenolic Content (TPC)*

TPC of the plant extract was determined by Folin-Ciocalteu protocol according to Gnaraj, Haque, and Iqbal (2012) with slight modification. Solutions of 1 mg/mL of plant extract and various concentrations of gallic acid in methanol were prepared in triplicates. The solutions of plant extract and gallic acid for each concentration were made to 200 µL by adding $dH_2O$. 1.5 mL of 10% Folin-Ciocalteu reagent was added. The mixtures incubated at room temperature for 5 minutes in the dark after vortex. Then, 1.5 mL of $Na_2CO_3$ (Sodium carbonate) was added. The mixtures vortex, and leave at room temperature for another 90 minutes in the dark. The absorbance was measured at 725 nm against blank. Calibration curve plotted based on the average absorbance values obtained. TPC of the extract was expressed as milligram of gallic acid equivalents (GAE) per gram plant extract (mg/g).

*Determination of Total Flavonoids Content (TFC)*

A modified protocol of TPC described by Bhandari and Rajbhandari (2014) was employed. The TFC was determined by $AlCl_3$ (aluminium chloride). Solutions of 1 mg/mL of plant extract and various concentrations of standard catechin were prepared in triplicates. The plant extract and the aliquot of catechin of each concentration were made to 250 µL in $dH_2O$. At the zero time, 75 µL of 5% $NaNO_3$ (sodium nitrite) was added and the mixture was vortex. It was leaved for 6 minutes in the dark. After that, it was vortex again and 150 µL of 10% $AlCl_3$ added. At 5 minutes, 500 µL of 1 M of NaOH (sodium hydroxide) was added to the mixtures. Immediately, the total volume of the mixtures were made up to 2500 µL $dH_2O$ and mixed thoroughly. The mixtures wait for 15 minutes before determined at 510 nm versus a blank containing all reagents except catechin. Calibration curve of catechin was plotted according to the average absorbance values obtained for different concentrations. TFC of the extract was expressed as milligram of catechin equivalents per gram of plant extract (mg of CE/g of extract).

*Determination of Free Radical Scavenging Activity (DPPH)*

The antioxidant activity was determined on the basis of their scavenging activity of the stable DPPH free radical rendering to the method described by Ahmad, AbdEl-Salam, and Ullah (2016) with slightly modification. Solutions of 1 mg/mL of plant extract and various concentration of ascorbic acid was used as control for comparison. The volumes were made up to 300 µL by adding dH2O. DPPH solution was prepared at concentration of 0.00236 g per 100 mL of ethanol. DPPH solution was prepared freshly in ethanol 6 × 10-5 M. The standard solutions were then treated with 2.7 mL of DPPH solution. The mixture vortex and left in dark for 60 minutes. Spectrophotometer was used for absorbance determination at 517nm. The antioxidant activity determination of plant extract solution was following the procedure as described for standard. The percent antioxidant activity was calculated by the given formula:

$$\% \text{ Inhibition} = \left[\frac{Abs\,of\,Control - Abs\,of\,Sample}{Abs\,of\,Control}\right] \times 100$$

## RESULT AND DISCUSSION

Available literature indicated that medicinal plants are the backbone of traditional medicine. As reported by Abdulrahman Hussain & Hasan Marouf (2013), over 400 traditional plants treatments was used as an antidiabetic agent, though only a small number of these have received a scientific and medical evaluation. Based on the study, *L. microphyllum* extract was found to contain flavonoids, alkaloids, tannins, and steroids but, terpenoids and saponins were found to be absent (Table 2). The phytochemical constituents demonstrated different type of compounds which observed present in the plant extract could be responsible for the antihyperglycemic activities.

**Table 2.** Phytochemical constituent of *L. microphyllum*

| Phytochemical | Observation | Inferences |
|---|---|---|
| **Flavonoids** | Yellow coloration | Present |
| **Alkaloids** | Reddish brown colored precipitate | Present |
| **Tannins** | Green precipitate | Present |
| **Terpenoids** | No reddish brown coloration | Absent |
| **Saponins** | No formation of stable foam | Absent |
| **Steroids** | Color changed from violet to blue | Present |

Flavonoids are one of the bioactive compounds present in *L. microphyllum* which likely to have molecular roles in cell development. Mohan & Nandhakumar (2014) reported that flavonoids triggered improvement and stabilized the secretion of insulin from pancreatic beta cells. These activities of flavonoids can be term as "insulinomimetic" because of reduction of aldose reductase, pancreatic beta cells regeneration, and increment of insulin released. Insulin profile was derived as the result of hyperglycemia and hindrance in carbohydrate, protein, and fat metabolism in diabetes.

Tannins is another secondary metabolites that as stated by Uma Makeshwari & Sundarsanam (2011) in their research paper, alkaloids were the presence in over 20% of plants. Amino acids are the biological precursors of alkaloids. Ornithine, lysine, phenylalanine, tyrosine, tryptophan, histidine, aspartic acid, and anthranilic acid were the example of amino acids that contained in the most plants alkaloids. Uma Makeshwari & Sundarsanam (2011) also referring to the Li *et al*., (2004) journal, alkaloids such as aconitine, anisodamine, charantine, and leurosine, showed antidiabetic effects. The major roles of alkaloids are to inhibit alpha-glucosidase and decrease glucose transport through the intestinal epithelium (Patel *et al*., 2012) presence in *L. microphyllum*. It is a potential antioxidants and contained multifunctional properties beneficial to human health. Prior research by Morada *et al.* (2016) has demonstrated that tannins are hypoglycemic. The possible hypoglycemic mechanism of tannins in the pancreas is, by inhibition of tannins which caused a starch breakdown.

Since most antidiabetic effect of *L. microphyllum* revealed the presence of flavonoids, alkaloids, and tannins apart from steroids, it is very likely that steroids reported to reduce blood glucose level when supplemented to diabetic rats (Rizvi & Mishra, 2013). Instead of reducing blood glucose level, plant steroids also function to restore the insulin level (Revathi *et al*., 2015).

The total phenolic content of aqueous *L. microphyllum* extract calculated from calibration curve ($R^2$ = 0.9979), was 966.7 ± 0.03 mg of gallic acid equivalent per g (mg GAE/g) and the total flavonoid

content ($R^2$ = 0.9848) was 42.9 ± 0.01 mg catechin equivalent per g (mg CE/g). The $IC_{50}$ of DPPH was 5.77 µg/mL which is shown in Table 3.

**Table 3.** Total phenolic content, total flavonoids content, and DPPH of aqueous extract of *L. microphyllum*

| Plant Extract | Total Phenolics Content (mg of GAE/g of Extract) | Total Flavonoids Content (mg of CE/g of Extract) | $IC_{50}$ µg/mL |
|---|---|---|---|
| *Lygodium microphyllum* | 966.7 ± 0.03 | 42.9 ± 0.01 | 5.77 |

Development if many chronic disease can be prevent by consumption of plant with high phenolic content due to their powerful antioxidant and free radical scavenging properties. According to Sousa & Correia (2012), phenolic exert antidiabetic activity through inhibition of carbohydrate-hydrolysing enzyme. Common examples of the enzyme are alpha-amylase and alpha-glucosidase. Amylase inhibition by phenolic content contributed to the management of type 2 diabetes mellitus. Flavonoids present in food of plant origin are also potential antioxidants (Mitra & Uddin, 2014). It is from group poly phenolic compounds. As potential antioxidants, it is very important species which possess the ability to protecting organisms from damage caused by free radical-induced oxidative stress (Eghdami & Sadeghi, 2010). Matough *et al.,* (2012) were stated in their review paper, hyperglycemia induced free radical and it impairs the endogenous antioxidant defense system in patients with diabetes. Accordingly, plant phenolic and flavonoids used for the prevention and management of diabetes which is associated with the free radicals.

The free radical scavenging activity analysis was performed by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as sources of free radical (Widiyarti *et al.*, 2011). Based on the experiment done, colour change of reaction mixture determined the presence of free radical scavenging activity (Cijoy Jose *et al.*, 2014). The ranged of DPPH $IC_{50}$ in this study can be categorized as a very active antioxidant.

## CONCLUSION

The result of current study showed that the aqueous extract of *L. microphyllum* had the highest scavenging activity. This will leads to the development of alternative drugs and therapeutics strategies. It will also provide a starting point for programs leading to the development of indigenous botanical resources as inexpensive sources.

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