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Evaluation of the invitro antidiabetic and anti-cholesterols activity of selected plant extract

S Agustini^{1*}, Asmaliyah², EEW Hadi³, A Imanullah⁴

¹ Research Center for Agroindustry, National Research and Innovation Agency, BRIN, Jl. Raya Bogor km. 46 Cibinong 16911 Indonesia

² Research Center for Plants Conservation, Botanic Garden, and Forestry, National Research and Innovation Agency, BRIN, Jl. Raya Bogor km. 46 Cibinong 16911, Indonesia.

³ Research Center for Ecology and Ethnobiology, National Research and Innovation Agency (BRIN), Jalan Raya Jakarta-Bogor Km.46, Cibinong 16911, Indonesia

⁴ Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Jl. Raya Bogor km. 46 Cibinong, Bogor 16911, Indonesia

*Email: sria014@brin.go.id.ac.id

Abstract. Diabetic and hypercholesterolemia are the most common metabolic disorders in the world. The purpose of the study was to assess the anti-diabetic and anti-cholesterol effect of water leaf extract of *Lagerstroemia speciosa*, *Cinnamomum parthenoxylon*, and *Oroxylum indicum* in vitro. Those three plants are often used by the local community to treat diabetes and hypercholesterol. The water extract's active ingredients are steroids and flavonoids. The flavonoid content was reported to be 51.89, 110.92, and 63.41 mg QE/g respectively. In vitro studies on hemoglobin glycosylation, showed that 30 ppm aqueous leaf extract of *L. speciosa*, *C. parthenoxylon*, and *O. indicum* showed considerable inhibition of hemoglobin glycosylation by 12.06 %, 33.25%, and 73.96%, compared to a positive control (metformin) was 30.6 %. The in vitro anti-cholesterol properties of *O. indicum* and *C. parthenoxylon* could lower cholesterol by 33% compared to simvastatin by 33.104%. According to the study's findings, the water leaf extracts of *O. indicum* and *C. parthenoxylon* have excellent pharmaceutical potential and might be used as herbal remedy to manage diabetes and cholesterol. To verify this work, more in vitro and in vivo research is required.

1. Introduction

Traditional medicine has long employed plants to treat a wide range of illnesses and wounds. Numerous primary and secondary metabolites are found in plants, that possess medical applications and therapeutic purposes [1-4]. Plants are essential to the growth and development of modern herbal medicines as many diseases including chemotherapeutic, anti-diabetic, anti-inflammatory, and arthritic where no adequate treatment available is in modern medicines [5].

The scientific literature has described several plant species that may have anti-diabetic and anti-cholesterol effects. Due to their high effectiveness, low cost, and lack of clinically significant adverse effects, herbal medications are frequently given [2,5,6]. *Cinnamomum parthenoxylon*, *Oroxylum indicum*, and *Lagerstroemia speciosa* are among the plants those often applied as an alternative medicine to cure diabetes and hypercholesterols by the local community in South Sumatra Indonesia. Generally,



the application is in the form of water infusion, or decoction. Several studies reported that *L. speciosa*, *C. parthenoxylon*, and *O. indicum* have the potential to be antiulcer, antidiabetic, anti-inflammatory, and antilipidemic potentials but they don't lower liver enzyme levels [7-9]. The purpose of the study was to evaluate the flavonoids, tannins, steroids, and alkaloids present in the aqueous extracts of *C. parthenoxylon*, *O. indicum*, and *L. speciosa*, as well as their efficacy in reducing cholesterol and hemoglobin glycosylation.

2. Materials and methods

2.1. Extraction

C. parthenoxylon, *O. indicum*, and *L. speciosa* leaves were collected from the forest area in the district Baturaja South Sumatera Indonesia. The sample was ground into a dry powder that was prepared for extraction after being allowed to dry and kept out of direct sunlight until its moisture content reached 8%. Using the maceration method, two 24-hour extractions of leaf powder were made in a volume equal to five times the dry weight using demineralized water. Filtration was used to collect the resultant extracts (Whatman paper No.1). The extract was concentrated at 100 rpm, at 40–50°C, in an IKA RV-10 rotary evaporator vacuum. The concentrated extracts were dried, and stored in an airtight container at -20°C for subsequent examination.

2.2. Quantification of concentration of tannin, alkaloids, steroids, saponin, and flavonoids

The amount of flavonoid was determined by the aluminum chloride (AlCl_3) colorimetric method according to [10] with slight modification. Quercetin was used to create a standard curve (0–0.1 mg/mL). At 420 nm, the absorbance was measured. The results are given as mg QE/100g. Based on [11], the alkaloid content of plant fractions was determined using Berberine chloride (0–500 $\mu\text{g/mL}$) as the standard. At 416 nm, the chloroform layer's absorbance was measured. The amount of alkaloid concentration was presented as mg BCE/g. The saponin content was calculated using [12]. The tannin concentration was calculated using [12]. As a standard, Tannic acid (0–2000 $\mu\text{g/mL}$) was used. The tannin concentration was expressed as mg TAE/g.

2.3. In vitro antidiabetic assay

The antidiabetic activity was performed in-vitro using glycated hemoglobin methods as described by [5] with slight modification. Hemoglobin preparation was carried out by combining the red blood in a ratio of 1:1 with chloroform and centrifuging for 10 minutes at 150 rpm. The upper layer (red color/hemoglobin-rich fraction) was separated into a bottle for storage and refrigerated until analysis. Test sample: 1 ml hemoglobin-rich fraction + one milliliter of Glucose 2% (in 0,01 M Buffer phosphate pH 7.4) + five microliters gentamicin + 25 microliters extract (30 $\mu\text{g/mL}$). Control solution: 1 ml hemoglobin + five microliters gentamicin + 1 milliliter Glucose 2% (in 0,01 M Buffer phosphate pH 7.4) + 25 microliters PBS pH 7.4. Metformin was used as a positive control. All samples, control solutions, and standard solutions were incubated in the dark for 24 hours at room temperature. Ten microliters of the solution were diluted with two milliliters of 0.9% NaCl. A wavelength of 443 nm was used to measure the absorbance.

$$\text{Inhibition of glycosylation (\%)} = [(\text{Control} - \text{Test Abs}) / \text{Control Abs}] \times 100 \quad (1)$$

2.4. Anti-cholesterol test in vitro

Anti-cholesterol activity was performed in vitro as described elsewhere [13]. Five milliliters of extract (100 ppm in ethanol 96%) were added to 5 mL cholesterol standard (200 ppm cholesterol in 96% ethanol). Two milliliters of acetic acid anhydrate and 0.1 milliliters of sulfuric acid were added to the test tube after five milliliters of solution had been pipetted into it. The solution was then shaken vigorously and left for 15 minutes at ambient temperature until the color turned green. At the wavelength of 430 nm, the absorbance was assessed ($A_1/\text{test sample}$). The negative control comprised 5 mL buffer phosphate pH 7, and the positive control was standard simvastatin.

$$\text{Cholesterol reduction (\%)} = [(\text{Abs. of control} - \text{Abs. of test}) / \text{Abs. of control}] \times 100\% \quad (2)$$

2.5. Chemicals used and statistical analysis.

Analytical-grade substances were employed in the entire study. To obtain reproducibility findings, every analysis was done in triplicate, and the values reported in the results are the average of those triplicate assays, PCA analysis was carried out by using the the Unscumbler X.10.4.

3. Results and discussion

3.1. Screening phytochemistry

The phytochemical profile of *L. speciosa*, *C. parthenoxylon*, and *O. indicum* of aqueous leaf extracts is presented in Table 1. Test results showed that the majority of the phytochemicals that were extracted had medicinal use, and the most common type of phytochemical discovered in extracts is flavonoid, followed by steroids, tannins, and alkaloids. Among the three plants, the *C. parthenoxylon* has the highest flavonoids, and tannins content, while the *O. indicum* sample exhibits the highest steroid content.

Table 1. Phytochemical profile of aqueous leaf extract of *L. speciosa*, *C. parthenoxylon*, and *O. indicum*

Phytochemical	Concentration (mg/g)		
	<i>L. speciosa</i>	<i>C. parthenoxylon</i>	<i>O. indicum</i>
Flavonoid	51.89±1.293	110.915±2.501	64.39 ±0.998
Steroids	0.906±0.055	0.877±0.019	12.228±1.157
Alkaloids	0.556±0.024	1.755±0.068	2.187±0.241
Tannins	1.63±0.000	6.793±0.384	0.201±0.004
Saponin	0.314±0.011	0.668±0.011	0.103±0.004

Values are means ± SD (n = 3).

Almost all herbs, fruits, and vegetables contain flavonoids, which constitute the biggest family of phenolic metabolites [14]. Flavonoids can inhibit oxidative stress by seizing free radical superoxide, improving SOD production, blocking the production of ROS, and seizing ROS directly, safeguarding lipophilic antioxidants, and encouraging enzymatic antioxidant growth [15]. Phytosterols are phytochemicals that have a similar structure and comparative biological action of cholesterol. It can heal the changed lipid levels brought on by cancer and prevent chronic illnesses including diabetes, heart disease, and other cancers [16]. The alkaloids are also reported to have muscle relaxant properties, antioxidant activity, anticancer activity, antimicrobial and amoebicidal activity [17], analgesics, anesthetics, antimalarials, antihypertensive agents, stimulants, spasmolysis agents, antiarrhythmic agents, antiasthma therapeutics, vasodilators. Plant saponins are informed to slow cholesterol captivation in experimental animals and as a result lower plasma cholesterol concentration [18].

3.2. The in vitro biological activity

In most cases, free radicals are regularly involved in developing many human ailments, including cancer, diabetes, and neurological diseases. The aqueous leaf extracts of *L. speciosa*, *C. parthenoxylon*, and *O. indicum* were made, and their anti-diabetes and anti-cholesterol were examined.

Test on antidiabetic assay in vitro of extracts using glycated hemoglobin methods in the presence of 2% glucose over 24 hours were performed and compared with metformin as a positive control. Metformin, a guanidine derivative was chosen for its efficacy in lowering glycemia, and known as an agent to treat diabetes for a long. It has the capacity to reduce the generation of glucose in the liver, postpone or prevent the absorption of glucose from the gastrointestinal tract, and is a medicine used to treat type 2 diabetes and gestational diabetes [19]. The results show that all aqueous leaf extracts were able to inhibit hemoglobin glycosylation as indicated by the decrease in absorbance as an index for hemoglobin glycosylation. The *O. indicum* showed the highest inhibition (73.965 ± 0.335%) followed by *C. parthenoxylon* (33.025 ± 2.433%), and *L. speciosa* (12.001 ± 1.805%). Compared to a positive control metformin which has inhibition activities of 33.602 ± 1.466 %, the *O. indicum* showed higher

inhibitory activity. This result indicates the ability of *O. indicum* extracts at the concentration of 30 ppm to inhibit the formation of a glucose-hemoglobin compound more than twice as compared to metformin. The glucose-hemoglobin compound in the blood will trigger the formation of reactive oxygen. The ability of the plant extract to inhibit the formation of glucose-hemoglobin compounds in the blood is in order *O.indicum*> *C. Parthenoxylon*> *L. Speciosa* respectively.

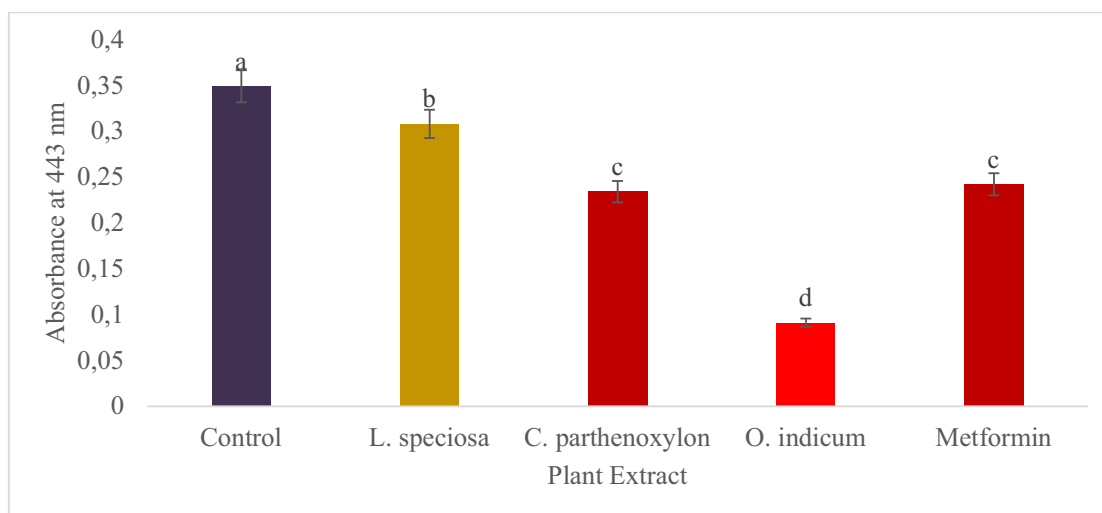


Figure 1. Estimation of hemoglobin glycosylation over 24 hours (values are mean of 3 replicates)

The anti-cholesterol properties of *L. speciosa*, *C. parthenoxylon*, and *O. indicum* extracts were tested in vitro in this study and compared to simvastatin. The entire incubation period of 15 minutes was taken into account, and as the activity increased over time, the maximal anti-cholesterol activity was seen after 10 minutes. An effective positive control for evaluating the possible anti-cholesterol action of plant extract is simvastatin, a medication that may be used to lower cholesterol levels. Based on the findings, *O. indicum* had significant activity in lowering cholesterol (33.047%) compared to simvastatin (33.14%), suggesting that *O. indicum* may have the ability to regulate cholesterol levels. On the contrary, the extract of *C. parthenoxylon* (16.238%) and *L. speciosa* (8.29%) showed lower activity. These results suggest that the cholesterol-lowering ability of *O. indicum* extract is comparable to that of simvastatin, which is a typical medical that is frequently used to regulate cholesterol.

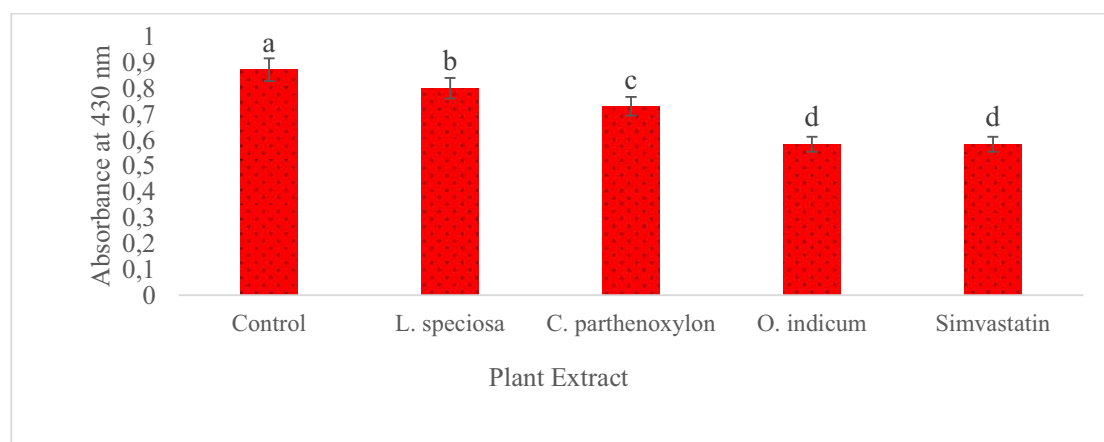


Figure 2. Effect of extract on cholesterol

3.3. Principal component analysis

All data were extracted was processed by principal component analysis. The PCA scores plot shows that the 3 samples are not arranged in a random way. It was clear distinctive groupings for plant extract according to plant species (Figure 3A). Overall, separation distances between clusters were greater for the extract from *L. speciosa* and *O. indicum* counterparts, implying that the bioactive compound and activity of the extract were distinctive to the plant's species. In this analysis, the score plot shows that PC-1 explains 55% and PC-2 explains 45% of the total variance in the data. Evaluation of the correlation loadings plots obtained after PCA analysis indicated that one variable (Flavonoids) has an extreme position at the top of the plot along the PC-2, and far from the center of variance. On the horizontal axis (PC-1) there are 3 variables with a negative value for this PC, namely hemoglobin glycosylation, anti-cholesterol, and steroids. These three variables correlate negatively with *L. speciosa*. The scores plot and correlation loading plot show that samples *O. indicum* have the highest hemoglobin glycosylation inhibition, anti-cholesterol activity, and steroid content, while samples *C. parthenoxylon* have the highest flavonoids and tannins. This implies that hemoglobin glycosylation and anti-cholesterol activity of the plant extract correlate to the steroid and flavonoid content. The alkaloids and saponin content do not have a contribution to the hemoglobin glycosylation and anti-cholesterol activity. This is seen by their position which is in the center of loading plots and far from hemoglobin glycosylation and anti-cholesterol activity. The *O. indicum* has both anti-glycosylation activity and cholesterol reduction activity, while *C. parthenoxylon* only has anti-glycosylation activity comparable to standard drugs. On the contrary *L. speciosa* doesn't have anti-glycosylation activity and cholesterol reduction activity.

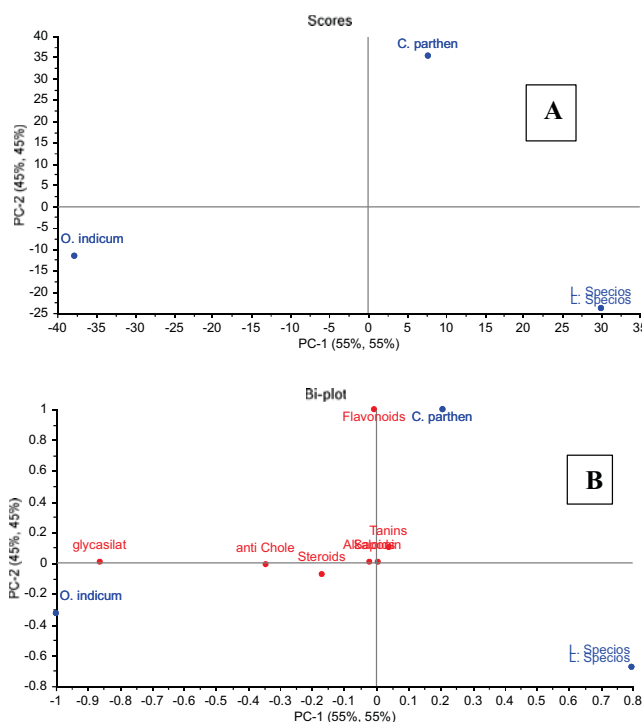


Figure 3. A. Score plot, B. Bi plot of Principal component analysis.

4. Conclusions

In summary, this study's findings indicate that water leaf extracts of *L. speciosa*, *C. parthenoxylon*, and *O. indicum* have anti-diabetes and anti-cholesterol properties. The highest cholesterol reduction

percentage and the highest inhibition hemoglobin glycosylation percentage in vitro were shown by *O. indicum* extract with the magnitude of 33.04%, and 73.966% respectively. To sum up, the water leaf extracts of *O. indicum* and *C. parthenoxylon* have the potential to be utilized as a natural remedy for hypercholesterol and diabetes. Further research both in vitro and in vivo is needed to determine any potential health impact to prevent degenerative diseases caused by cholesterol and oxidative damage.

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