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Research Article

# Antioxidant and Antihypertensive Effect of *Azadirachta Excelsa* Leaf Extract in Spontaneously Hypertensive Rat (SHR) Model

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## Abstract

A research was carried out to evaluate the antioxidant activities of *Azadirachta excelsa* and its antihypertensive effect in Spontaneously Hypertensive Rat (SHR). The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) was quantified and $IC_{50}$ level of *A. excelsa* was determined. For the antihypertensive effect, the rats were randomly assigned into four treatment groups as followed: Group I (normotensive control from Wistar-Kyoto rats), Group II (hypertensive control from SHR), Group III (SHR receiving 250 mg/kg of *A. excelsa* extract), and Group IV (SHR receiving 40 mg/kg of captopril). The Systolic Blood Pressure (SBP) of these animals was performed by tail-cuff method. The average of TPC and TFC was 202 ± 0.42 mg Gallic Acid Equivalent (GAE)/g extract and 198 ± 0.67 mg rutin equivalent/g extract, respectively. Meanwhile, the $IC_{50}$ value of free radical scavenging activity was about 308 µg/ml. The systolic blood pressure level of the SHR treated with *A.excelsa* significantly reduced (153 mmHg; $P$ <0.05) compared to the untreated SHR control (187 mm Hg; Group II). In conclusion, we found that *A. excelsa* extract at a dose of 250 mg/kg possesses phenolic properties that can be used as a potential treatment for hypertension due to its high antioxidant activities.

**Keywords:** Antihypertensive; Antioxidant; *Azadirachta excelsa*

## Introduction

Hypertension or high blood pressure, a metabolic disorder is characterized by the persistently elevated blood pressure in the arteries. For centuries, the pathogenesis and maintenance of blood pressure in hypertension was attributed by oxidative stress [1,2]. Oxidative stress served as an important mediator between vasoconstrictor and vasodilator mechanisms in both experimental and human [2-4]. Generally, oxidative stress occurs when the availability of oxidants such as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) overcome the availability of antioxidant level. An equilibrium state between oxidants and antioxidant are vital to assure the blood vessel function and maintaining normal cell signaling [5]. Under pathogenesis of hypertension, an excessive production of ROS in arterial wall may directly alter vascular function and reduce an endothelium-derived relaxing factor which is Nitric Oxide (NO), thus resulted to hypertension. In the meantime, epidemiological studies have demonstrated that naturally antioxidant in plants such as phenolic and flavonoid compounds possess a protective role against the development of pathological conditions such as hypertension and obesity which associated to oxidative stress [6]. The oxidation processes by oxidants can be delayed or inhibited by the antioxidant compounds [7].

*Azadirachta excelsa* or locally known as "sentang" from *Meliaceae* family, where it belongs to a plant family that is known to have various benefits associated to medical properties. Various parts of *A. excelsa* tree have been used for traditional medicine due to its biological activity such as antiseptic, anti-inflammatory, antimicrobial and antifeedant [8]. Subsequently, there was a finding reported that this plant had a great potential as medicinal plants due to the presence of phenolic and flavonoid as well as antioxidant compounds [9] although the compound have not been characterized yet. However, there were only a few findings about the effect of *A. excelsa* extract on mammals.

Therefore this study was conducted to evaluate the antioxidant activity by quantifying the total phenolic and flavonoid contents, followed by the determination of free radical scavenging activity of *A. excelsa* ethanolic extract. The quantitative determination of total antioxidant capacity was conducted to investigate the relationship between dietary antioxidants and pathologies induced by the oxidative stress [10]. In addition, the evaluation of blood pressure on experimental hypertension rats model was measured to investigate the potential antihypertensive effects of this plant.

## Materials and Methods

### Preparation of *A.excelsa* leaves extract

*A. excelsa* leaves were collected from Forest Research Institute Malaysia (FRIM) in Kepong, Selangor. Fresh leaves were collected and washed with water before shade dried for one to two weeks. By using a mechanical grinder, the leaves were ground into fine powders. The powdered leaves were soaked in 70% ethanol for two days at room temperature. The leaves extract was then filtered and concentrated by using a rotary evaporator at 40°C. The dark semi-solid material of the ethanolic extract was stored at 4°C for quantitative analysis and further use in animal study.

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### Determination of total phenolic, total flavonoid content and antioxidant activity by 2,2-Diphenyl-1-Picryl Hydrazyl (DPPH) assay

The total phenolic and flavonoid content in *A.excelsa* ethanolic extract were determined using Folin-Ciocalteu and aluminium chloride colorimetric assay respectively [11], The total phenolic content result was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram extract, whereas total flavonoid content was expressed as milligrams of rutin equivalents per gram extract. The DPPH assay was used to determine the free radical scavenging activities of the extracts with slight modification [12]. Lower absorbance of the reaction indicates higher free radical scavenging activity calculated using the formula below:

Free radical scavenging activity (%) = [(A0 - A1)/ A0] × 100

Where A0 was the absorbance of the blank and A1 was the absorbance of the sample. A curve of percent scavenging effect against samples concentrations was plotted and the concentration of the sample required from 50% inhibition was determined as $IC_{50}$. Ascorbic acid and α-tocopherol were used as the standard references antioxidants.

### Preparation of animal model and experimental design on the antihypertensive effect of *A.excelsa* extract in SHR

All testing related to animal care and handling was approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia, Malaysia [UPM/IACUC/AUP-R075/2014]. Then, 12-14 weeks old of SHRs and WKYs male were purchased from Animal Facility Unit of Monash University Sunway Campus, Malaysia and were housed in animal house at Agro-Biotechnology Institute (ABI), National Institutes of Biotechnology Malaysia (NIBM). The rats were acclimatized for 1 week, at 24˚C with 60-70% relative humidity, and 12-h light/ 12-h dark cycle with free access to standard rat diet and water *ad libitum*. Prior to the experiment day, all rats were familiarized for 1 week with oral force feeding and blood pressure measurements procedure. Then, they were randomly assigned into four treatment groups consisting of six rats and were treated via oral gavage daily for 4 weeks as followed; Group I: WKY received distilled water, Group II: SHR received distilled water, Group III: SHR received 250 mg/kg of *Azadirachta excelsa* extract, Group IV: SHR received 40 mg/kg of captopril.

### Measurement of Systolic Blood Pressure (SBP)

The SBP of the rats were performed by tail-cuff method using non-invasive blood pressure monitoring CODA system (Kent Scientific, USA) once a week during treatment period. One rat a time was placed into a restrainer with the tail placed inside a tail cuff and then put on to the warmer pad for 10-15 minutes. The blood pressure reading is measured by CODA system when the cuff was inflated and deflated simultaneously for 15 cycles in one session. Six consecutive measurements were taken and the lowest and highest values were discarded.

## Statistical Analysis

All data obtained were analyzed statistically using GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego California, USA). The measurement of SBP data were analyzed using repeated measures of two-way analysis of variance (ANOVA) over the time course. P-values of less than 0.05 were considered to be significant. All data are expressed as mean ± SEM.

## Results

### Phenolic, flavonoid content and antioxidant activity

Results on the phenolic, flavonoids and $IC_{5O}$ value of *A.excelsa* ethanolic extract are presented in table 1. Total phenolic content of *A.excelsa* was 202 ± 0.42 mg gallic acid/g extract and total flavonoid content was 198 ± 0.67 mg rutin /g extract. Figure 1 shows the percentage of DPPH scavenging against samples concentration (ascorbic acid, α-tocopherol and *A.excelsa* ethanolic extract) were plotted. From the graph, the $IC_{50}$ value of *A. excelsa* was only 308 µg/ml needed to inhibit 50% of DPPH free radical. At 500 µg/ml concentration, the percentage of DPPH scavenging for ascorbic acid, α-tocopherol and *A.excelsa* samples were 91%, 89% and 76% respectively (Figure 2).

| | **Ethanolic leaf extract of *A.excelsa*** |
|---|---|
| Total Phenolic Content | 202 ± 0.42 mg gallic acid/g extract |
| Total Flavonoid Content | 198 ± 0.67 mg rutin /g extract |
| $IC_{50}$ | 308 µg/ml |

**Table 1:** Total phenolic, flavonoid content and $IC_{50}$ value of *A.excelsa* extract.

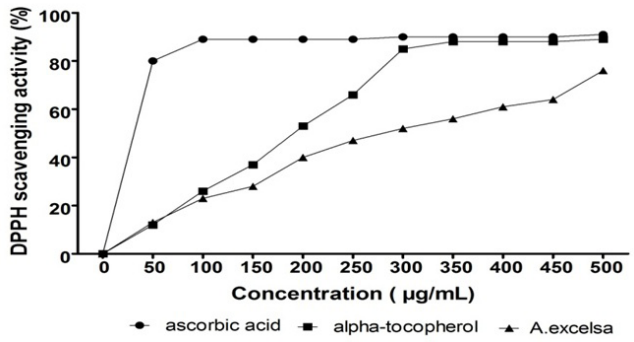


**Figure 1:** DPPH free radical scavenging activity of *A. excelsa* ethanolic extract and reference antioxidants as concentration increase.

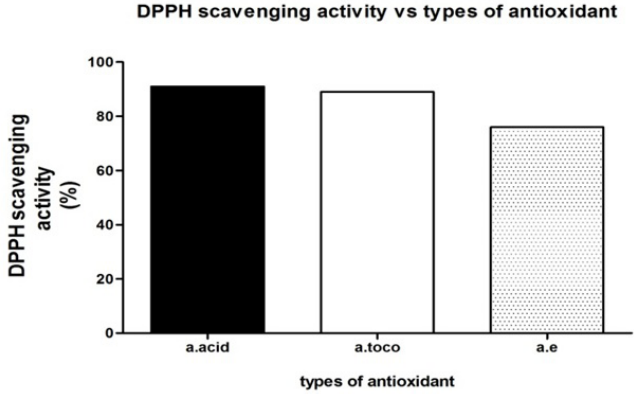


**Figure 2:** Scavenging activities (%) of different types of antioxidant at 500 µg/ml.

### Effect of *A.excelsa* extract on systolic blood pressure in SHR model

Figure 3 shows the changes of SBP in WKY control, SHR control and SHR treated with *A.excelsa* and captopril. During baseline, all

SHRs except for WKY experienced an elevation of SBP. However, all SHR treated groups showed a significant reduction of SBP compared to untreated SHR (187 ± 2.97 mmHg). Over the duration of 4 weeks treatment, SHR *A.excelsa* and SHR captopril showed a reduction of SBP by 19% (153 ± 2.78 mmHg) and 28% (136 ± 4.52 mmHg) respectively. However, the SBP in WKY and SHR groups remained constant (denoted by same alphabet).

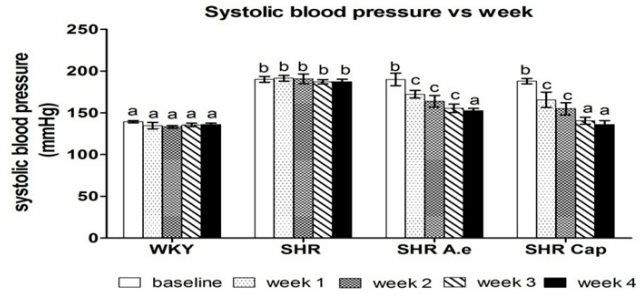


**Figure 3:** Values are expressed as mean ± SEM (n=6). [a,b,c] Values with different alphabets are significantly different at P<0.05 between group. WKY control: (WKY), SHR control: (SHR), SHR received 250 mg/kg of *Azadirachta excelsa*: (SHR A.e) and SHR received 40 mg/kg of captopril: (SHR cap).

## Discussion

Polyphenol compounds comprise a large group of secondary metabolites found in plant. The most important classes of polyphenols include phenolic acids and flavonoids compounds which served as an important bioactive constituent in plants due to their antioxidant properties. In our previous studies, it has been shown that the phenolics and flavonoids were strongly presence in *A. excelsa* leaf extract by qualitative phytochemical screening [9]. Meanwhile, our results on quantitative analysis in *A.excelsa* ethanolic extract showed high phenolic and flavonoid content. In fact, the highest total phenolic content was observed in *A. excelsa* leaves compared to the other trees of *Azadirachta* genus [13]. These results are might be due to high phenolics and flavonoids content found in this plant. Furthermore, there are plenty of reports suggesting the use of antioxidants as free radical scavengers for most type oxidizing molecules including ROS [7,14]. For instance, the smaller value of $IC_{50}$ corresponds to a higher antioxidant activity of the plant extract [14]. Our result revealed that *A. excelsa* ethanolic extract can serve as a good free radical scavenger due to the lower value of $IC_{50}$. This result was in line with previous study demonstrated the capability of *A. excelsa* as the best scavenger of Fremy's salt radicals [13]. Thus, this could suggest that the antioxidant activity of *A. excelsa* as a free radical scavenger might be attributed to the presence and synergistic effect of phenolics and flavonoids compound.

The result on SBP of animals showed that oral administration of *A.excelsa* prevented the prolonged of hypertension in SHR model. It was established that the persistent elevated blood pressure in these genetic hypertension animal model was due to an excessive production of ROS in arterial wall where the major source is NADPH oxidase [15]. Hence, any compounds that can ameliorate the oxidative stress by inhibiting an excessive ROS production seem to be the therapeutic interest in lowering the blood pressure. An antioxidant activity of *A.excelsa* might promoted its antihypertensive effect in animal model similar to the antihypertensive drug captopril. However, captopril served a good ability in normalizing blood pressure better due to its antioxidant and cardioprotective effect [16]. In blood pressure regulation, captopril acts as an Angiotensin Converting Enzyme Inhibitor (ACEI) that inhibits the production of Angiotensin II, a potent vasoconstrictor in Renin-Angiotensin System (RAS). While the protective effect of phenolics and flavonoids not only as an antioxidant, but also as an antithrombotic, antiischemic and vasorelaxant in heart disease [17]. Thus, this plant has a great potential as medicinal plants due to the presence of phenolics and flavonoids as well as antioxidant compounds.

The present study showed that the quantification on total phenolic and flavonoid content present in *A. excelsa* ethanolic leaf extract. In addition, this study suggested that the antioxidant activity of *A. excelsa* alleviate hypertension in SHR model. To the best of our knowledge, this is the first study reporting of *A. excelsa* on its antioxidant and antihypertensive activity. Hence, further isolation, characterization and purification of the active constituents and experimentation would be necessary to elucidate the exact mechanism of *A.excelsa* as a plant with antioxidant and antihypertensive effect.

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