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Chemical Constituents and Bioactivity Studies of *Ardisia Elliptica*

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Abstract: *Ardisia elliptica* is a medicinal plant traditionally used for alleviating chest pains, treatment of fever, diarrhea, liver poisoning and for parturition complications. The objectives of this study were to investigate the chemical constituents in fruits and leaves extracts of *A. elliptica* and to determine the biological activities of these extracts. The fruits and leaves of *A. elliptica* were soaked with methanol and the extracts were analysed using GCMS. Both extracts were then tested for antibacterial activity using disc diffusion method against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative). After incubation for 24 hours, the inhibition zones of the extracts were compared with gentamicin and ampicilin. Antioxidant activities were determined using DPPH and analysed by Spectronic-20 with wavelength of 517 nm. From GCMS data, it was found that both extracts contained similar major compound which were 5-hydroxymethyl-2-furancarboxaldehyde with percentage area of 58.42%, and 45.13%; 2,4-di-tert-butylphenol with 3.04% and 20.87% and clindamycin with 8.77% and 5.79% for fruits and leaves extracts respectively. Clindamycin is a known antibiotic therefore both extracts showed positive result for antibacterial tests. Antioxidant assay also showed that both fruits and leaves extracts decolorized the DPPH.

Keywords: Ardisia elliptica, Clindamycin, DPPH.

INTRODUCTION

The genus of *Ardisia* is the largest genus in the family of Myrsinaceae. The usage of *Ardisia* as a source of food and medicine is rather limited. It appears that berries of at least few species, such as *A. elliptica* Thunb. and *A. macrocarpa*, a Himalayan species, have been used for human consumption [1, 2]. Johnson (1998) listed 15 identified and one unidentified species of *Ardisia* along with their medicinal properties and some ailments and conditions including cancer (*A. cornudentata*), diarrhea (*A. crispa, A. odontophylla* and *A. villosa*), hepatosis (*A. villosa*), parturition (*A. ridleyi*) and rheumatism (*A. crassa, A. crispa, A. odontophylla and A. villosa*) [3].

Ardisia elliptica also known as shoebutton *Ardisia*. Local name of *A. elliptica* is Mata Pelanduk or Mata Ayam which is a native medicinal plant commonly found in Malay Peninsula [4]. It is an invasive shrub or small tree to 6m in height and 15cm in basal diameter. The plants produce strong stems with grey bark. The stem are usually single, but additional sprout may arise from the rootstalk, especially if the plant in injured. *A. elliptica* grows a strong taproot, much branched laterals, and fine roots with rhizomorphic tips. Leaves have a rubbery or leathery texture and are pink when young, turning dark green later. They are glabrous and alternate with petioles about 1cm long and blades 8 to 12cm by about 3nm [5].

A. elliptica is traditionally used for alleviating chest pains, treatment of fever, diarrhea, liver poisoning, and parturition complications. The decoction of the leaves of this species is used by the Malays for treatment of pain in the region of the heart or to alleviate chest pains [6, 7]. According to Jianhong *et al.* (2010), *A. elliptica* is more potent than aspirin in inhibiting collagen-induced platelet aggregation by α- and β-amyrin contain in this herbs. This compound is responsible to alleviate the chest pain and head disease [8].

Based on Hideka and Elvira (2004), *A. elliptica* has antibiotic and antiviral properties. The phytochemical compound contained in *A. elliptica* leaves are Bauerenol, α- and β-amyrin and Bergenin. Bergenin is an isocoumarin found in various plant species and exhibits a wide range of biological activites including hepatoprotective, antifungal, anti-HIV, antiarrhythmic and hypolipidemic [9].

Even though there are a number of reported studies of this species, the studies on chemical components of *A. elliptica* extracts are limited. Therefore, this study would contribute on the chemical constituents of this species and the antibacterial and antioxidant activities of the extract.

EXPERIMENTAL

Collection of Sample

Fresh leaves and fruits of *Ardisia elliptica* were collected from Lubuk Merbau, Kuala Kangsar in July 2012. The samples were packed in plastic bag and kept in temperature of ±4 °C until extraction.

Extraction

Fruits and leaves of *Ardisia elliptica* were weighed (10 g) and cut into small pieces. The samples were transferred into Erlenmeyer flask and were soaked with 200 ml of methanol

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Table 1. The Chemical Constituents of Leaves Extract of *A. elliptica*.

Peak	Retention Time, t_R	Compound Name	Percent Abundance %
1	1.889	2-methoxy-1-(2-nitroethenyl)-3-benzene	1.06
2	3.608	Chlorfenapyr	6.74
3	**6.800**	**Clindamycin**	**5.79**
4	7.377	Decamethyl-cyclopentasiloxane	1.35
5	**7.710**	**1-naphthyl ester acetoxyacetic acid**	**12.41**
6	**8.578**	**5-hydroxymeth 2-furancarboxaldehyde**	**45.13**
7	10.404	Methyl 3-amino-2-thiophenecarboxyldehyde	0.56
8	11.30	4-cyanophenyl 2,6-difluorobenzoic acid	6.08
9	**11.501**	**2,4-bis(1,1-dimethylethyl)-phenol**	**20.87**
		Total	99.99

Table 2. The Chemical Constituents of fruits Extract of *A. elliptica*.

Peak	Retention Time, t_R	Compound Name	Percent Abundance%
1	1.908	2-methoxy-1-(2-nitroethenyl)-3-benzene	0.92
2	**6.785**	**Clindamycin**	**8.77**
3	7.363	Decamethyl-cyclopentasiloxane	4.03
4	**7.691**	**1-naphthyl ester acetoxyacetic acid**	**16.90**
5	**8.565**	**5-hydroxymeth 2-furancarboxaldehyde**	**58.42**
6	9.165	Dodecamethyl-cyclohexasiloxane	5.16
7	10.866	tetradecamethyl-cyclohexasiloxane	2.38
8	11.282	Tridec-2-ynyl 2.6-difluorobenzoic acid	0.39
9	**11.501**	**2,4-bis(1,1-dimethylethyl)-phenol**	**3.04**
		Total	100.01

for 4 days. The extracts were filtered and further concentrated and stored at ±4 °C.

Gas Chromatography Mass Spectrometry

The extracts were analyzed using Varians GC-MS. The oven temperature was programmed from 70 °C to 180 °C at 4 °C/min. The solvent used was hexane >95% analyses grade with helium as the carrier gas and split ratio was 1:50. The ionization voltage was 70eV with the ion source temperature of 280 ^{o}C and mass range of 30-300 mass units.

Antioxidant Test

From each sample, different concentrations of essential oils were prepared in ethanol: 20, 40, 60 and 80 ppm. The antioxidant activity of essential oils was carried out using free radical scavenging activity using DPPH. 3 ml of DPPH solution was added to 3 ml of extract solution of different concentrations. The absorbance was measured at 517nm in a spectrophotometer Spectronic-20. The DPPH radical concentration was calculated using the following equation: Scavenging effect % = $[(A_0 - A_1) / A_0] * 100$ where A_0 was the absorbance of the control sample (without essential oil) and A_1 was the absorbance in the presence of the sample.

Antibacterial Test

Antibacterial activity of fruits and leaves extracts was determined by disc diffusion method on nutrient agar medium. *Staphylococcus aureus* (gram positive bacteria) and *Escherichia coli* (gram negative bacteria) were used. The bacteria (400 µL) were transferred into the petri dish using micropipette (1000 µL) and were spread equally on the medium using stirrer. The stirrer was soaked in alcohol, heated and then cools in the laminar flow before applied to the medium containing the bacteria. Then 10 µL of each solvent extract was placed in the paper disc. The treatments also included 10 µL of solvent (methanol) served as control and the standard control were ampicilin and gentamicin. Then, the plates were sealed using parafilm and were incubated at 37°C (room temperature) for 24 hours. The inhibition zone around the paper disc was measured in mm (milimeter).

RESULTS AND DISCUSSION

Chemical constituents of leaves and fruits extracts were determined using GCMS. Percent yield of leaves and fruits extracts were 40.56 and 54.30% respectively. The identified chemical constituents were listed in Tables **1** and **2** and were

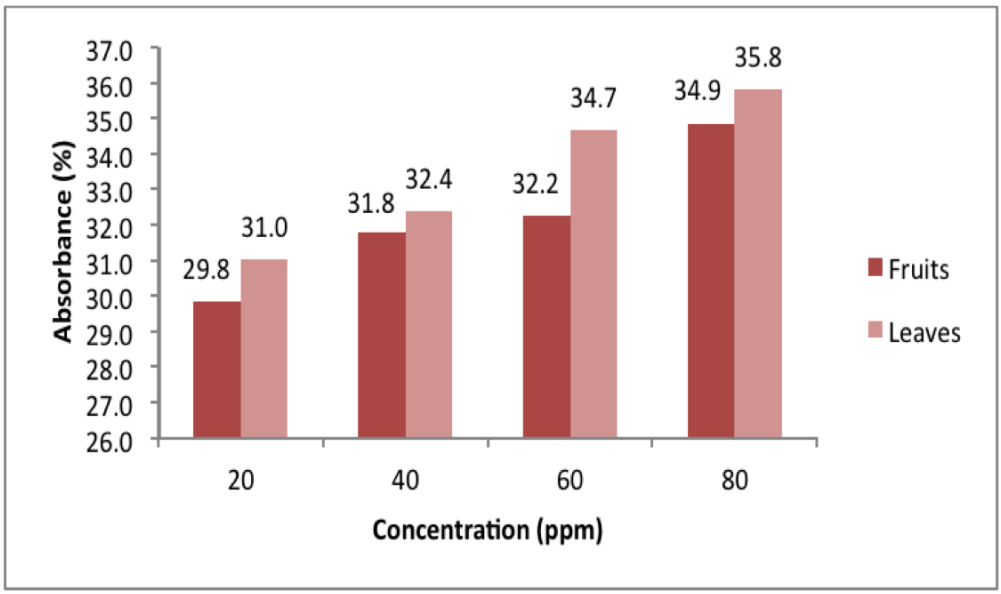
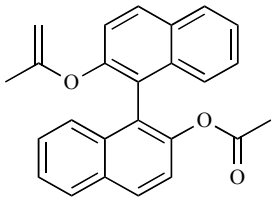


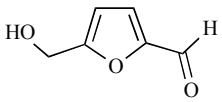
Fig. (1). DPPH scavenging activity of different concentrations of fruits and leaves extracts of *A. elliptica*.

constituted of 99.99% for leaves and 100.01% for fruits extracts.

The major compounds for leaves extracts were 1-naphthyl ester acetoxyacetic acid (1) (12.41%), 5-hydroxymeth-2-furancarboxaldehyde (2) (45.13%) and 2,4-bis(1,1-dimethylethyl)-phenol (3) (20.87%). In addition, clindamycin, an antibiotic used to treat anaerobic infections was found with percent abundance of 5.79%. Fruits extracts showed almost similar results with 1-naphthyl ester acetoxyacetic acid (16.90%), 5-hydroxymeth-2-furancarbo-xaldehyde (58.42%), and clindamycin (8.77%) as the major constituents. 2,4-bis(1,1-dimethylethyl)-phenol which is responsible for antioxidant activity was also found in fruits extracts. The major and active compound are higher in percentage in fruits extracts except for 2,4-bis(1,1-dimethylethyl)-phenol.



(1)



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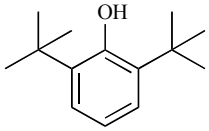

(3)

Antioxidant Assay

Fig. (**1**) showed the antioxidant activity of leaves and fruits extract against DPPH. Leaves extracts shows higher percent absorbance than fruits extracts. However, the values for both extract are in close proximity to each other.

The antioxidant capacity for both extracts was dependent on the concentration tested. For concentration of 80 ppm, the antioxidant capacity reached 34.9 and 35.8% while for 20 ppm, the percentages were 29.8 and 31.0% for fruits and leaves extracts respectively. The percentages of antioxidant activity ranged from 29.8% to 35.8% from 20 ppm to 80 ppm. It is possible to conclude that both fruits and leaves extracts have moderate antioxidant activity ranging from 20 ppm to 80 ppm. The phenolic compound which is 2,4-bis(1,1-dimethylethyl)phenol in both extracts was probably respo-nsible for the positive result of DPPH scavenging activity. This compound is one of the flavanoid group and was reported to have the potential to inhibit free radical activity.

Antibacterial Activity

The inhibition zone was measured by diameter (mm) and the inhibition strength were expressed by percent inhibition (%), where $\geq$ 70% is strong, 50 to 70% is moderate and < 50 is weak inhibition [10]. Table **3** showed the inhibition strength of extracts on tested bacteria.

Leaves extracts showed moderate inhibition against *S. aureus* and weak inhibition against *E. coli* with 56.52% and 43.58% respectively. On the contrary, fruits extracts have moderate inhibition capacity against *E. coli* (69.23%) and low inhibition capacity against *S. aureus* (31.87%).

CONCLUSION

Both extracts contained similar major compounds which is 5-hydroxymeth 2-furancarboxaldehyde, 1-naphthyl ester acetoxyacetic acid, 2,4-bis(1,1-dimethylethyl)phenol and clindamycin with different percentage. Antioxidant assay showed that both extracts have moderate antioxidant capacity with 29.8% to 35.8% with concentration ranging from 20 to 80 ppm. In addition, antibacterial test showed that leaves and fruits extracts of *A. elliptica* has moderate inhibition against *S. aureus* and *E. coli*.

Table 3. Inhibitory Activity of Fruits and Leaves Extracts of *A. elliptica*.

Compound	Mean (mm)		Percent Inhibition %	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
Methanol	7.00	8.00	-	-
Ampicilin	7.00	12.00	-	-
Gentamicin	23.00	26.00	-	-
Leaves extract	13.00	11.33	56.52 (++)	43.58 (+)
Fruits extract	7.33	18.00	31.87 (+)	69.23 (++)

Note: + : Weak Inhibition (<50)
++ : Moderate Inhibition (50-70%)
+++ : Strong Inhibition (≥70%)

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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