Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.05.010

Phytochemical constituents, antioxidant and antibacterial activities of methanolic extract of *Ardisia elliptica*

Nazeh M. Al-Abd[1,2*], Zurainee Mohamed Nor[1], Marzida Mansor[3], Asdren Zajmi[4], Mohd Shahnaz Hasan[3], Fadzly Azhar[5], Mustafa Kassim[3]

[1]*Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

[2]*Faculty of Medicine and Health Sciences, University of Aden, Khormaksar, Aden, Yemen*

[3]*Department of Anesthesiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

[4]*Department of Diagnostic and Allied Health Sciences, Faculty of Health and Life Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia*

[5]*Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*

ARTICLE INFO

Article history:
Received 18 Jul 2016
Received in revised form 22 Aug, 2nd revised form 5 Sep 2016
Accepted 29 Dec 2016
Available online 3 Jun 2017

Keywords:
Ardisia elliptica
Antioxidant capacity
Total phenol content
Gas chromatography/mass spectrometry
Liquid chromatography/mass spectrometry

ABSTRACT

Objective: To investigate the antioxidant, antibacterial, and chemical ingredients of *Ardisia elliptica* (*A. elliptica*) methanolic extracts.

Methods: The plant was extracted using methanol. Antibacterial and antioxidant activities were evaluated.

Results: The results showed that both fruit and leaf extract of *A. elliptica* have significant antibacterial activities against Gram-positive and Gram-negative bacteria. Fruit extracts showed higher content of phenolic (71 ± 0.03 GAE/mg extract dry weight), in comparison to the leaf extracts (37 ± 0.05 GAE/mg extract dry weight). Flavonoid content, and Fe^{2+} chelating activity of fruit extracts were higher than leaf extract. The percentage radical inhibition of fruit extract is found to be higher (70%) than that of leaf extract (60%). LCMS results indicated that the major compounds in the fruit extract were Gingerol, Aspidin, Kampherol, and Stercuresin, while the leaf extract contained Gingerol, Aspidin, Triangularin, and Salicyl acyl glucuronide. Furthermore, the results of GCMS indicated that fruit extract contained these major compounds: Vitamin E Tocopherol, 5-hepylresornicol, 2-Nonylmalonic acid, 5-pentadecylresornicol, and Stigmasta-7-22-dien-3-ol. However, leaf extract of *A. elliptica* contained these major compounds: Alpha Amyrenol, 4,4, 6, 6a, 6b, 8, 8a, 9,10, 11,12,12a, 14, 14a, 14b octadehydro-2H-picen-3-one, and Lonasterol, 4-t-Butyl-2-[4-nitrophenyl] phenol.

Conclusions: The results provide evidence that fruit and leaf of *A. elliptica* extracts might indeed be used as a potential source of effective natural antimicrobial and antioxidant agents in pharmaceutical and food industries.

1. Introduction

Herbal medicine is considered as an essential source of natural products and useful for eliminating serious diseases in developing countries [1]. About 60% of the world's population rely on traditional medicine to treat several diseases [2]. In the recent years, plants have attracted a great deal of scientific interest, and thus have become an attractive alternative in complementary medicine [3]. Malaysia is rich in its traditional knowledge about the use of medicinal plants in combating diseases [4]. Several types of plant extracts or plant-derived molecules have been investigated for their potential as antioxidants and antibacterial sources against several diseases [4].

Free-radicals are composed in living organisms through biochemical and pathophysiological processes that may occur due to toxins, environmental pollutants, chemicals, radiation, *etc.* These could lead to an inequity between the creation and nullification of pro-oxidants pursuing constancy via electron

*Corresponding author: Nazeh M. Al-Abd, Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
Tel: +60 183943415
Fax: +60 379674754
E-mail: Nazehali78@yahoo.com (N.M. Al-Abd).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

2221-1691/Copyright © 2017 Hainan Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

pairing with large molecules necessary for life including lipids, DNA, and proteins, incurring oxidative stress in specific physiological conditions [5]. This results in damage to protein, lipids, enzymes, and DNA in healthy human cells, which consequently causes numerous chronic diseases like ageing, diabetes, cardiovascular diseases, atherosclerosis, inflammation, cancer, and other degenerative diseases in humans [5].

In this context, these plants have been given much attention due to their abilities to supress or to delay the oxidation process by the means of other molecules hindering the instigation of oxidizing chain reactions; these characteristics have made them an attractive alternative in complementary medicine. The plants are rich in naturally occurring antioxidant chemicals such as ascorbic acid, nitrogen compounds, phenolic compounds and carotenoids [6]. Indeed, many studies have been demonstrated that the phytochemical extracts have exhibited antioxidant activities, among which are those belonging to the constituents in plants such as alkaloids, flavonoids, vitamins, terpenoids, carotenoids, phenolic compounds and polyphenols [7].

This warrants a search for new drugs to manage health problems via natural sources. In all human civilizations around the world, ancient traditional medicine has proven plants to be one of the promising sources of curative agents. Hence, scientific research is focused on antimicrobial and antioxidant activities of a number of plant extracts in order to explore an alternative therapy against different types of microorganisms and oxidative reactions [8].

In this regard, several plant extracts have been evaluated. Among the recently reported literature are those on *Chenopodium murale* [9], *Woodfordia fruticosa* [10], and *Melaleuca cajuputi* [11]. *Ardisia elliptica* (*A. elliptica*) (Marlberry), a member of the Primulaceae family is known for its remarkable biological activity and is widely distributed throughout Asia [12]. In Malaysia, *A. elliptica* is used to assuage retrosternal pains as well as to treat herpes and measles, whereas in Thai traditional medicine, it is used to cure diarrhoea with fever. Generally, in the Southeast Asian region *A. elliptica* is used to treat scabies and intestinal worms [13]. *A. elliptica* is reported in several literature for having anti-HIV [14], anti-salmonella [15], anti-viral [16], and anti-cancer potential activities [17]. The aims of the current study are to evaluate the potential antioxidant and antibacterial activities, as well as to determine the chemical constituents of the methanolic extract of fruits and leaves of *A. elliptica*.

2. Materials and methods

2.1. Chemical and reagents

Methanol, chloroform, NaOH, HCL, $FeSO_4$, $FeCl_3$, $NaNO_3$, $FeCl_2$, B-carotene, quercetin, chloragenic acid, TPTZ, Tween 20, Proprylgallate, 2,2-diphenyl-1-Picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), acetate buffer, Folin–ciocalteu reagent, EDTA, ferrozine, ascorbic acid, and linoleic acid have been used in the study. These chemicals are of analytical grade and were purchased from Merck (Germany).

2.2. Plant materials and preparation of methanolic extracts

The plant was collected in September 2013 from the state of Kedah, Malaysia. The plant material was identified and confirmed by specialists in botany and the voucher specimen was deposited at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya, Kuala Lumpur (voucher number KLU048232). The fruits and leaves of *A. elliptica* were washed using distilled water, ground into powder and dried for several days. For sample preparation, 100 g parts each of the leaves or fruits were added to 1 000 mL of 95% methanol at 25 °C and kept for 72 h. Then the extracts were filtered out of the mixture using Whatman filter paper (No: 1); this procedure was repeated three times. The extracts were then concentrated using a rotary evaporator in vacuo at 40°C, and dried to remove the remaining residue, thus leaving only the *A. elliptica* fruit and leave extracts, respectively.

2.3. GCMS methodology

The extraction process of the AF or AL crude extract (10 mg) was performed assisted by 15 min of sonication in a sealed vial with 2.5 mL of dichloromethane at 40 °C. Following the extraction, 1 mL of the extract samples was analysed by filtering it with a 0.20-μm Nylon filter into an auto-sampler vial. Separation of hydrocarbons and other volatile compounds were determined with a GC (Shimadzu, Japan) equipped with a QP2010 mass detector. GC–MS analyses were done with an ionization energy of 70 eV. The initial oven temperature was programmed to 60 °C for 2 min, and was gradually increased at a rate of 7 °C/min to 150 °C until reaching the final temperature of 310 °C for 15 min. The temperature for the injector and detector was kept at 300 °C (split), and 310 °C, respectively. The Helium (He) was the carries gas used at a linear flow-rate of 40 cm/s. The operation of MS detector was done at 200 °C. The scan range was at a rate of 0.50 scan/s from 50 to 1 000 m/z. Purity of each GC peak was checked by taking MS at various parts of each peak. All compounds were identified via mass spectral database search (NIST/EPA/NIH) followed by the matching of MS data. The MS match factors $\geq$ 90% were shown as "positive".

2.4. LCMS

AL and AS extracts were diluted with Acetone to the concentration of 1 mg/mL. Following the dilution, 1 mL of both extracts were filtered through a 0.22 mm hydrophobic PTFE filter into an auto-sampler vial for LCMS analysis. An Agilent 1290 Liquid Chromatography system (Agilent Technologies, Santa Clara, CA, USA) which was adjoined to a 6520 Q-TOF tandem mass spectrometer was used to separate the compounds from the samples. The mass detector was an electrospray ionization (ESI) interface equipped with a Q-TOF accurate mass spectrometer controlled by the MassHunter software; 4 μl of the crude sample comprising a mixture of phenolic compounds were loaded on a 2.1 mm (i.d) Narrow-BoreSB-C18 (length 150 mm) analytical column (particle size 3 mM) used at a flow rate of 0.4 mL/min in a solution A (0.1% formic acid in water) and solution B (100% Acetonitrile with 0.1% formic acid). The gradient was run as follows: 3% B for 1 min, 3–100% B for 20 min, and 100% for 4 min. Total gradient time for the LCMS was 28 min. The ionization conditions were adjusted at 300 °C and 4000 V for capillary temperature and voltage, respectively. The nebulizer pressure was 45 psi and the nitrogen flow rate was 10 L/min. All mass spectrometry data were recorded in both

positive and negative ion modes. The acquisition rate was 1.03 spectra/s across the range of 115m/z–3200m/z in negative mode. Finally, the MS data were analysed by using Agilent MassHunter Workstation Qualitative Analysis Software and the compounds were identified using MassHunter Workstation METLIN Metabolite PCD/PCDL Software.

2.5. Total flavonoid assay

Total flavonoid content of the extracts was evaluated using the aluminium chloride method [18] (Ghasemzadeh *et al.*, 2011). In brief, in either AL or AF (10 mL) test tube, an aliquot (1 mL) of extracts (1 mg/mL) or standard solution of quercetin (31.5, 62.5, 125, 250, 500 and 1 mg/L) was added. The sample was then diluted with 4 mL of double distilled water at zero time, followed by an addition of 0.3 mL of 5% (W/v) $NaNO_2$ to the tube; 5 min later 0.6 mL of $AlCl_3$ (10%) was added. Next, 2 mL of NaOH (1 M) was added to the mixture at the sixth minute. Finally, double distilled water was added to the mixture to bring the total volume to 10 mL. The mixture was mixed well and the absorbance was taken at 430 nm against reagent blank. This experiment was carried out in triplicate and total flavonoid content was expressed as quercetin equivalents in mg/100 g of dry weight.

2.6. Total phenolic content

Total phenolic content of extracts was evaluated using the Folin–Ciocalteu methods as described [11]. The 200 μL of Folin–Ciocalteu phenol reagent (Sigma-aldrich) was mixed with 100 μL of extract and 16–1000 μg/mL in 80% methanol of standard solutions of Gallic acid. Then, 15% Na_2CO_3 (1 mL) and deionized water (2 mL) were added and the mixture was blended thoroughly. Thereafter, the mixture was kept in the dark for 120 min at 25 °C; following that, the absorbance was taken at 765 nm using a spectrophotometer (UV–Visible, GBC, Cintra 40). Determination of total phenolic content was conducted using the extrapolation of the calibration curve from the standard Gallic acid concentrations measurement. The tests were carried out three times (triplicate) and presented as mg Gallic acid equivalent per mg of extract weight.

2.7. DPPH radical scavenging method

In vitro measurement of the free radical scavenging activity of the extracts was conducted using the stable radical, 2,20-diphenyl-1-picrylhydrazyl (DPPH) assay, following the method described previously [11]. Various concentrations of 30 μL extract (31.3, 62.5, 125, 250, 500 and 1000 μg/mL) were actively mixed with 120 μL of 0.25 mM DPPH, shaken well and kept in the dark for 30 min at 25 °C. The absorbance for different concentrations of methanol extracts as blank was measured at 518 nm, and DPPH in methanol without extract was used as negative control. On the other hand, butylhydroxytoluene was used for positive control. The scavenging activity percentage (AA % – antiradical activity) of the extract was evaluated using the equation as reported previously [18]:

$$AA\% = \left(100 - \left(\frac{Abs_{sample} - Abs_{blank}}{Abs_{control}}\right)\right) \times 100$$

2.8. Fe^{2+} chelating activity assay

The ICA of leaf and fruit extracts were measured following the previously reported method [11]. Each extract (0.05 mL in DMSO) was added into a solution of 0.05 mL and 2 mM $FeCl_2$ and vortexed for 30 s. Afterwards, ferrozine (5 mM), was added, the mixture was mixed and shaken vigorously. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. The mixture was incubated for 10 min at 25 °C, and the absorbance of the Fe^{2+} Ferrozine was then determined at 562 nm. The ability of the extract to chelate ferrous ion was calculated using the following formula:

$$\%\text{Chelating rate} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 (blank) represents the absorbance of the control (without extract) and A_1 represents the absorbance in the presence of the extract.

2.9. Ferric reducing antioxidant power (FRAP) assay

The extract reducing power was evaluated based on the reduction of ferric to ferrous ion and the developed blue colouration as described previously [19]. FRAP reagent was freshly prepared from the following: 2.5 mL of 20 mM $FeCl_3.6H_2O$ in 25 mL of 0.3 M acetate buffer (pH 3.6), and 2.5 mL of 10 mM tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl. Then, 0.2 mL of the extract (0.1 μg/mL) was mixed with 1.8 mL of FRAP reagent at 37 °C for 5 min. The absorbance was measured at 593 nm. Finally, the reducing power was measured by drawing a comparison between the absorbance of each extract against a standard curve generated from iron (II) sulphate (Fe_2SO_4).

2.10. Antimicrobial activity

2.10.1. Test organisms

The reference organisms were comprised of four Gram-positive bacteria such as *Staphylococcus aureus* (RF 122), *Staphylococcus epidermidis* (MTCC 3615), *Streptococcus pneumoniae* (ATCC 10015), *Bacillus cereus* (ATCC11778), and four Gram-negative bacteria such as *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (UT181), *Pasteurella multocida* (UMMC clinically isolated strain) and *Klebsiella pneumonia* (*K. pneumonia*) (ATCC13883).

Antimicrobial activities of the extracts were evaluated by the Kirby-bauer disc diffusion method as described by Carbonnelle [11]. For all bacterial strains, cultures grown overnight in broth were adjusted to an inoculum size of 10^6 CFU/mL for the inoculation of agar plates. In brief, the Nutrient Agar (NA) was autoclaved at 121 °C for 20 min; each extract (0.1 g/mL) was prepared by dissolving it in 100% dimethyl sulphoxide (DMSO, Merck, Germany). Then, the extract was filtered through a 0.20 mm millipore disposable filter (Minisart, Sartorius Biotech, Germany). Sterile filter paper discs (6 mm in diameter) were soaked with 50 μL of each extract and each disc was placed onto the surface of the agar plate (nutrient agar), that previously were inoculated with bacteria. A disc soaked with only 50 μL of DMSO served as negative control. Meanwhile, another disc loaded with 20 mg

streptomycin (reference antibiotic) was used as positive control. The samples were then inverted and incubated for 18 h at 37 °C. For each bacterial strain, the test was conducted in triplets and the diameter of the inhibition zones was measured and analysed.

2.10.2. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

MIC values were determined using the microdilution method [3], as the lowest extract concentration that totally supresses the growth of microorganisms after 48 h of incubation at 37 °C whereas MBC values were defined as the lowest concentration of an antibacterial agent needed to inhibit the growth of a certain bacteria followed subculture onto antibiotic free media. The MBC was evaluated by sub-culturing from the MIC assay tubes into the Nutrient Agar plates; each well's plates, exhibited no growth after incubation at 37 °C for 24 h. Positive and negative cultures were also prepared. In brief, extracts (100 mg/mL dissolved in DMSO), and two-fold serial dilution were prepared in a 96-well microplate. Streptomycin was used as positive control antibiotic. The solution without extract served as a blank control. Each microplate well included 40 µL of the growth medium, 50 µl of the diluted sample extracts, and 10 µL of the inoculum (10^6 CFU/mL). Wells showing no growth on the plate were taken as MBC. These experiments were carried out in triplicate.

2.11. Statistical analysis

The results were expressed as mean ± the standard deviation. The IC_{50} was measured from the plot of percentage inhibition against extract concentration using anon-linear regression algorithm. An independent *T*-test was used to evaluate the significance differences; a difference was considered significant when the value was <0.05.

3. Results

3.1. GC/MS and LC/MS analysis of the methanol extracts from A. elliptica

Results of the GC/MS analysis of *A. elliptica* fruit extract as shown in Table 1 reveal that essential oils are the major compounds present, characterized by the presence of fatty acids including n-tridecanoic acid methyl ester (1.43%), 2-nonylmalonic acid (8.81%), hexadecanoic acid isomers (both 0.71%), and 3,7,11,15-tetramethyl-2-hexadecen-1-ol butyl octadecanoate (0.71%), respectively. In addition, aromatic compounds such as 5-pentadecylresoricol isomers (8.10%, 4.05%, 3.81%, 4.29%, and 2.38%), 5 heptylresornicol (2.86%), and tocopherol (14.29%) were observed to be present. GC/MS analysis of the *A. elliptica* leaf extract affirms the presence of 15 bioactive compounds. As indicated in Table 2, the methanol extract largely contains aromatic compounds such as pentadecane (0.67%), 4-t-Butyl-2-[4-nitrophenyl] phenol isomers (2.01%, 16.78%, 2.01%, 2.01%), alpha tocopherol (3.36%), and alpha amyrenol (23.49%), and also terpenoids such as 4,4, 6, 6a, 6b, 8, 8a 9,10, 11,12,12a 14, 14a 14b octadehydro-2H-picen-3-one (16.78%), Longifolenaldehyde (8.72%), Lonasterol (16.78%), 1,6,10,14,18,22-Tetracosahexaen-3-ol 2,6,10,15,19, and 23-hexametyl (0.67%). Fatty acid 3, 7, 11, 15-Tetramethyl-2 -hexadecen-1-ol (2.01%) was also detected.

The LC/MS results as presented in Table 3 show that most of the detected compounds were typical phenolic acid derivatives. The *A. elliptica* fruit extract displayed the presence of Gingerol, aspidin, kampherol and stercuresin. However, the leaf extract was comprised of trigularin, gingerol, salicyl acyl glucuronide, and aspidin.

3.2. Total phenolic contents

Results for the total phenolic content (TPC) of the *A. elliptica* leaf and fruit extracts are shown in Table 1. When comparing the TPC content of the extracts, fruit extract was found to show a higher

Table 1
List of major compounds identified from *A. elliptica* seed extract.

ID	Possible compound name	Class of compounds	Molecular formula	Mol mass	Rt time	%
1	2,2,4, 4,6,6,6,8,8,10,10,12,12 Dodecamethylcyclohexasiloxane	None	$C_{12}H_{36}O_6Si_6$	444	6.844	3.81
2	2,2,4, 4,6,6,6,8,8,10,10,12,12,14,14 Tetradecamethylcyclohexasiloxane	None	$C_{14}H_{42}O_7Si_7$	518	8.697	4.05
3	3,5-Di-t-butylphenol	Phenolic	$C_{14}H_{22}O$	206	9.502	2.86
4	n-Tridecanoic acid methyl ester	Fatty acid	$C_{14}H_{28}O_2$	228	11.416	1.43
5	2-Nonylmalonic acid	Fatty acid	$C_{14}H_{26}O_4$	258	17.183	8.81
6	Hexadecanoic acid	Fatty acid	$C_{17}H_{34}O_2$	270	17.64	0.71
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Fatty acid	$C_{20}H_{40}O$	296	23.909	0.71
8	Butyl octadecanoate	Fatty acid	$C_{22}H_{44}O_2$	340	27.515	0.95
9	Hexadecanoic acid	Fatty acid	$C_{19}H_{38}O_4$	330	29.929	0.95
10	5-Pentadecylresornicol	Phenolic	$C_{21}H_{36}O_2$	320	34.896	8.10
11	5-Pentadecylresornicol	Phenolic	$C_{21}H_{36}O_2$	320	35.1	4.05
12	5-Pentadecylresornicol	Phenolic	$C_{21}H_{36}O_2$	320	35.25	3.81
13	Squalene	Alkene	$C_{30}H_{50}$	410	35.455	2.86
14	5-Hepylresornicol	Phenolic	$C_{13}H_{20}O_2$	208	38.309	13.33
15	5-Pentadecylresornicol	Phenolic	$C_{21}H_{36}O_2$	320	38.383	4.29
16	5-Pentadecylresornicol	Phenolic	$C_{21}H_{36}O_2$	320	38.529	2.38
17	Vitamin E Tocopherol	Phenolic	$C_{29}H_{50}O_2$	430	40.529	14.29
18	Stigmasta-7-22-dien-3-ol	Terpenoids	$C_{29}H_{48}O$	412	43.33	8.81
19	4,4, 6, 6a, 6b, 8, 8a 9,10, 11,12,12a 14, 14a 14b Octadehydro-2H-picen-3-one	Terpenoids	$C_{30}H_{48}O$	424	43.992	7.14
20	Alpha amyrin acetate	Terpenoids	$C_{32}H_{52}O_2$	468	44.699	2.86
21	Lonasterol	Terpenoids	$C_{30}H_{50}O$	426	45.914	3.81

Table 2
List of major compounds identified from *A. elliptica* leaf extract.

ID	Possible compound name	Class of compounds	Molecular formula	Mol mass	Rt time	%
1	Dodecamethylcyclohexasiloxane	None	$C_{12}H_{36}O_6Si_6$	444	6.847	0.67
2	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris(trimethylsiloxy)tetrasilone	None	$C_{19}H_{54}O_7Si_7$	590	8.705	0.67
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Fatty acid	$C_{20}H_{40}O$	296	15.708	2.01
4	Pentadecanal	Phenolic	$C_{15}H_{30}O$	226	16.636	0.67
5	4-t-Butyl-2-[4-nitrophenyl] phenol	Phenolic	$C_{16}H_{17}NO_3$	271	22.14	2.01
6	4-t-Butyl-2-[4-nitrophenyl] phenol	Phenolic	$C_{16}H_{17}NO_3$	271	24.831	16.78
7	4-t-Butyl-2-[4-nitrophenyl] phenol	Phenolic	$C_{16}H_{17}NO_3$	271	28.677	2.01
8	4-t-Butyl-2-[4-nitrophenyl] phenol	Phenolic	$C_{16}H_{17}NO_3$	271	31.635	2.01
9	Squalene		$C_{30}H_{50}$	410	35.469	3.36
10	Alpha tocopherol (Vitamin E)	Phenolic	$C_{29}H_{50}O_2$	430	40.618	3.36
11	Alpha Amyrenol	Phenolic	$C_{30}H_{50}O$	426	44.055	23.49
12	4,4, 6, 6a, 6b, 8, 8a 9,10, 11,12,12a 14, 14a 14b Octadehydro-2H-picen-3-one	Terpenoids	$C_{30}H_{48}O$	424	44.737	16.78
13	Longifolenaldehyde	Terpenoids	$C_{15}H_{24}O$	220	45.544	8.72
14	Lonasterol	Terpenoids	$C_{30}H_{50}O$	426	45.965	16.78
15	1,6,10,14,18,22-Tetracosahexaen-3-ol 2,6,10,15,19, 23-hexametyl		$C_{30}H_{50}O$	320	38.383	0.67

Table 3
Chemical composition comparison of methanol extract from seed and leaves of *A. elliptica*.

Phytochemical extract	AS extract		AL extract		RT	m/z
	Presence	% Abundance	Presence	% Abundance		
Triangularin	(−)	0	(+)	2.73	12.795	283.09762
Gingerol	(+)	8.04	(+)	0.61	12.625	293.17563
Salicyl acyl glucuronide	(−)	0	(+)	0.56	3.683	313.05629
Aspidin	(+)	0.61	(+)	0.84	13.815	459.20997
Kampherol	(+)	1.1	(−)	0	7.093	285.22914
Stercuresin	(+)	1.21	(−)	0	12.79	284.10455

TPC value (71 ± 0.03 GAE/mg extract dry weight) than the leaf extract (37 ± 0.02 GAE/mg extract dry weight). A similar trend was also noted in the flavonoid content between the extracts (Table 1); *i.e*. the *A. elliptica* fruit has higher flavonoids content compared to leaf extracts.

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

DPPH is a stable free radical which has been commonly utilized as a measure for assessing free radical-scavenging activities for antioxidant analysis. The free-radical scavenging activities of *A. elliptica* extracts (fruit &leaf) are shown in Figure 1 and Figure 2. Generally, the *A. elliptica* fruit extract exhibited DPPH percentage inhibition activity with (63.16 ± 0.4%) at a concentration of 500 μg/mL with IC_{50} = 45.0 ± 2.3, while the leaf extract was (58.0 ± 0.1%) at a concentration of 500 μg/mL and an IC_{50} of 95.0 ± 6.1. However, there was a significant difference in IC_{50} value of both extracts in comparison to standard BHT (65.0 ± 0.031) (Figure 1).

3.3.2. Metal chelating power

The metal chelating activities of *A. elliptica* extracts were also evaluated (Figure 2). As expected, the fruit extract was found to show higher chelating power [43.6 ± 0.13%] with IC_{50}

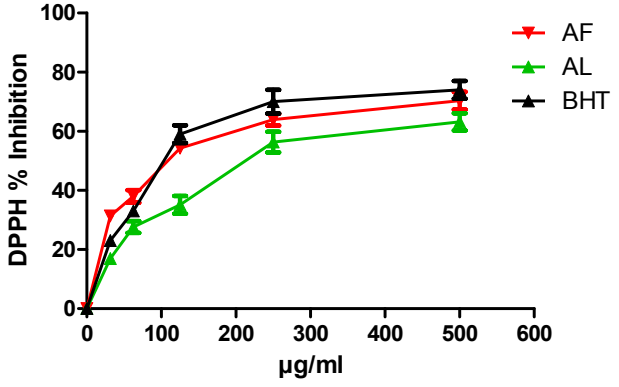

Figure 1. Free radical scavenging activity of *A. elliptica* extracts.
AF: *A. elliptica* fruit extract; AL: *A. elliptica* leaf extract; BHT: butylated hydroxytoluene included as a positive control. Activity was measure by the scavenging of DPPH radicals. Each value is expressed as the mean 6 standard deviation.

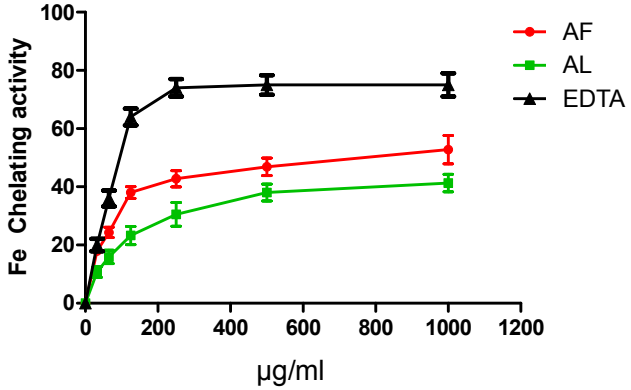

Figure 2. Chelation power on ferrous ions of *A. elliptica* extracts and its fractions.
AF: *A. elliptica* fruit extract; AL: *A. elliptica* leaf extract; EDTA: included as a positive control.

Table 4
Crude extracts activity of investigated plants.

Plant extract and standard	DPPH IC_{50} (μg/ml)	FRAP (μmol Fe (II)/g)	Iron chelating IC_{50} (μM)	TPC value mg Gallic acid/g of extracts	Flavonoid mg Quercetin/g of extract
A. elliptica fruit	120 ± 2.3a	0.12 ± 0.3b	17.5 ± 1.1c	71 ± 1.3g	5.6 ± 0.4f
A. elliptica leave	150 ± 6.1a	0.16 ± 0.1b	29.3 ± 0.05c	37 ± 2.2h	3.4 ± 0.2f
BHT	21.1 ± 0.031b	188.8 ± 24.83c	–		
EDTA	–	–	6.3 ± 0.53d		

Results are mean ± SD of triplicate experiments. GAE: garlic acid equivalent; QE: quercetin equivalent. Means followed by the same letters are not statistically significant.

Table 5
Antibacterial activity of *A. elliptica* seed and leaf crude extracts.

Inhibition diameter (mm ± SD)								
Extract samples	*S. epidermidis*	*S. aureus*	*B. cereus*	*P. multocida*	*K. pneumonia*	*S. pneumonia*	*E. coli*	*S. typhi*
A. elliptica leave	–	–	–	–	9.66 ± 0.31	–	–	–
A. elliptica fruit	–	–	–	9.66 ± 0.35	12.66 ± 0.26	7.66 ± 0.45	–	–
Streptomycin sulfate*	20.33 ± 0.38	18.0 ± 0.2	21.0 ± 0.25	21.0 ± 0.05	20.0 ± 0.1	22.0 ± 0.08	15.0 ± 0.1	10.0 ± 0.2

Results are mean ± SD of triplicate experiments. "–" No inhibition was observed. Doses of the samples were 1 mg/mL per disc. *Positive control – 10 μg per disc.

Table 6
MIC and MBC values of *A. elliptic* seed and leaf extracts against *P. multicida* and *K. pneumoniae*.

Sample	MIC (mg/mL)		MBC (mg/mL)	
	Pasteurella multicida	*Klebsiella pneumoniae*	*Pasteurella multicida*	*Klebsiella pneumoniae*
A. elliptica leaf	NA**	12.5	NA**	50.0
A. elliptica fruit	25.0	12.5	25.0	25.0
Streptomycin*	1.95	1.95	1.95	1.95

* Doses of Streptomycin was 1 mg/ml; ** No activity observed.

of [90.0 ± 1.1] compared to the leaf extract that exhibited a lower chelating activity [27.4 ± 0.13%] with IC_{50} of [90.3 ± 0.05], and both extracts showed significant difference in comparison with EDTA (Table 4). Furthermore, the chelating activity was observed to be concentration dependent.

3.3.3. Ferric reducing antioxidant power (FRAP) assay

The transformation of Fe (III) to Fe (II)-reducing activity in the methanol extracts of *A. elliptica* is presented in Table 4. Both leaf and fruit extracts of *A. elliptica* demonstrated some level of reducing power, achieving FRAP values of 0.12 and 0.16 μM Fe(II)/g, respectively.

3.4. Antibacterial activity of the extract

In vitro antibacterial activity of the extracts was studied against clinically-important Gram-negative and Gram-positive strains of bacterial pathogens as presented in Table 5. The leaf and fruit extracts of *A. elliptica* both displayed promising antimicrobial activities which could be useful against representative strains (Table 5). *A. elliptica* fruit extract in particular displayed an activity comparable to that of streptomycin, and *A. elliptica* leaf extract followed closely. The antibacterial activities of both extracts of *A. elliptica* are reported here for the first time. Generally, both extracts showed better antibacterial activities against Gram-negative than Gram-positive strains. The most susceptible strains toward *A. elliptica* extracts were shown to be *P. multicida*, *K. pneumonia*, and *S. pneumonia* (Table 5).

The observed MIC value for *A. elliptica* leaf extract was 12.5 μg/mL for *K. pneumonia*. MIC values for *A. elliptica* fruit extract were found to be 25 μg/mL and 12.5 μg/mL for *Pasteuralla multicida* and *K. pneumonia*, respectively. The MIC values obtained values suggested that the leaf extracts was less effective compared to the fruit extracts of *A. elliptica* (Table 6).

4. Discussions

Total phenolic and flavonoid contents of the methanolic extracts of the leaf and fruit of *A. elliptica* were evaluated. Phenolic and flavonoid compounds are commonly reported in plants and they are known to exert various biological activities, including antioxidant activity [20,21] as well as possess antibacterial properties [22,23]. Thus, the antioxidant and antibacterial activities of both leaf and fruit extracts of *A. elliptica* may be attributed to their high phenolic and flavonoid contents. Earlier studies have confirmed that some of the identified compounds in *A. elliptica* fruit extract indeed possess antioxidant activities [24]. Other reports have shown that alpha tocopherol, hexadecanoic acid and kaempferol exert antioxidant and antibacterial effects against a broad spectrum of microorganisms [25–28].

Several studies have reported a strong and significant correlation between the scavenging activity and total phenolic compound, as well as the flavonoid content and its significant contribution toward the total antioxidant activity [21]. The ability to reduce Fe (III) may be ascribed to the hydrogen donation of the phenolic compound [29], which is somehow associated to the presence of a reducing agent [30].

In addition, the antioxidant activity is governed by the position as well as the number of hydroxyl group of phenolic compounds [31,32]. The minimal bactericidal concentration (MBC) was also determined in comparison with the corresponding MIC values.

These results are of enormous value mainly in the case of bacteria well-known for their resistance to various antibiotics such as in the case of *S. aureus* and *K. pneumonia*. Moreover, this organism has the ability to secrete different types of enterotoxins that might lead to septicaemia. In a nutshell, the antibacterial activity of the extracts could be attributed to the presence of phenolic and flavonoid content [33,34].

Our study clearly supports the view that medicinal plants are a great source of potential antioxidants and may be used as safe and potent natural antibacterial and antioxidant agents in the treatment of Gram-negative bacteria as well as in the pharmaceutical and food industries. Total phenolic content in the fruit part extract was found to be higher in quantity compared to that of the leaf extract. Further research is needed on the use of *A. elliptica* extract as a preservative agent in various foods. The results are promising and strongly encourage the use of *A. elliptica* leaf and fruit as medicinal, nutraceutical application and functional food due to their antioxidant and antibacterial properties. To the best of our knowledge and understanding, this is the first report on the antibacterial and antioxidant activities of both extracts of *A. elliptica*.

Conflict of interest statement

The authors declare that they have no competing interests.

Acknowledgements

The authors are very grateful to University of Malaya for research grants PG085-2012B, RG546-14HTM and RG313-14FR.

References

[1] Valiakos E, Marselos M, Sakellaridis N, Constantinidis T, Skaltsa H. Ethnopharmacological approach to the herbal medicines of the “Antidotes” in Nikolaos Myrepsos' Dynameron. *J Ethnopharmacol* 2015; **163**: 68-82.

[2] Aniah P. The contribution of indigenous health care providers to health care delivery in Rural Ghana: an exploratory study of Bongo District. *Science* 2015; **3**(1): 20-8.

[3] Zajmi A, Hashim NM, Noordin MI, Khalifa SA, Ramli F, Ali HM, et al. Ultrastructural study on the antibacterial activity of artonin E versus streptomycin against *Staphylococcus aureus* strains. *PLoS One* 2015; **10**: e0128157.

[4] Madaleno IM. Traditional medicinal knowledge in India and Malaysia. *Pharmacogn Commun* 2015; **5**(2): 116-29.

[5] Martin-Puzon JJ, Rivera WL. Free-radical scavenging activity and bioactive secondary metabolites from various extracts of *Glinus oppositifolius* (L.) Aug. DC. (Molluginaceae) roots, stems and leaves. *Asian Pac J Trop Dis* 2015; **5**(9): 711-5.

[6] Baba Shoib A, Malik Shahid A. Evaluation of antioxidant and antibacterial activity of methanolic extracts of *Gentiana kurroo* royle. *Saudi J Biol Sci* 2014; **21**(5): 493-8.

[7] Mathew S, Abraham TE, Zakaria ZA. Reactivity of phenolic compounds towards free radicals under in vitro conditions. *J Food Sci Tech* 2015; **52**(9): 5790-8.

[8] Marasini BP, Baral P, Aryal P, Ghimire KR, Neupane S, Dahal N, et al. Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. *Biomed Res Int* 2015; **2015**: 265425.

[9] Abdel-Aziz MS, Shaheen MS, El-Nekeety AA, Abdel-Wahhab MA. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. *J Saudi Chem Soc* 2014; **18**: 356-63.

[10] Dubey D, Patnaik R, Ghosh G, Padhy RN. In vitro antibacterial activity, gas chromatography–mass spectrometry analysis of *Woodfordia fruticosa* Kurz. leaf extract and host toxicity testing with in vitro cultured lymphocytes from human umbilical cord blood. *Osong Public Health Res Perspect* 2014; **5**: 298-312.

[11] Al-Abd NM, Nor ZM, Mansor M, Azhar F, Hasan M, Kassim M. Antioxidant, antibacterial activity, and phytochemical characterization of *Melaleuca cajuputi* extract. *BMC Complement Altern Med* 2015; **15**: 385.

[12] Kobayashi H, de Mejía E. The genus Ardisia: a novel source of health-promoting compounds and phytopharmaceuticals. *J Ethnopharmacol* 2005; **96**: 347-54.

[13] Koh HL. *A guide to medicinal plants: an illustrated scientific and medicinal approach*. Singapore: World Scientific; 2009.

[14] Dat NT, Bae K, Wamiru A, McMahon JB, Le Grice SF, Bona M. A dimeric lactone from *Ardisia japonica* with inhibitory activity for HIV-1 and HIV-2 ribonuclease H. *J Nat Prod* 2007; **70**: 839-41.

[15] Phadungkit M, Luanratana O. Anti-Salmonella activity of constituents of *Ardisia elliptica* Thunb. *Nat Prod Res* 2006; **20**: 693-6.

[16] Chiang LC, Cheng HY, Liu MC, Chiang W, Lin CC. In vitro antiherpes simplex viruses and anti-adenoviruses activity of twelve traditionally used medicinal plants in Taiwan. *Biol Pharm Bull* 2003; **26**: 1600-4.

[17] Newell AM, Yousef GG, Lila MA, Ramírez-Mares MV, de Mejia EG. Comparative in vitro bioactivities of tea extracts from six species of *Ardisia* and their effect on growth inhibition of HepG2 cells. *J Ethnopharmacol* 2010; **130**: 536-44.

[18] Ghasemzadeh A, Jaafar HZ, Rahmat A. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (Zingiber officinale Roscoe) extracts. *J Med Plant Res* 2011; **5**(7): 1147-54.

[19] Kim YS, Hwang JW, Sung SH, Jeon YJ, Jeong JH, Jeon BT, et al. Antioxidant activity and protective effect of extract of *Celosia cristata* L. flower on tert-butyl hydroperoxide-induced oxidative hepatotoxicity. *Food Chem* 2015; **1**(168): 572-9.

[20] Salem MZM, Ali HM, El-Shanhorey NA, Abdel Megeed A. Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: antibacterial and antioxidant activities, total phenolic and flavonoid contents. *Asian Pac J Trop Biomed* 2013; **6**(10): 785-91.

[21] Lim SM, Loh SP. In vitro antioxidant capacities and antidiabetic properties of phenolic extracts from selected citrus peels. *Int Food Res J* 2016; **23**(1): 211-9.

[22] Mahmoudi S, Khali M, Benkhaled A, Benamirouche K, Baiti I. Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian *Ficus carica* L. varieties. *Asian Pac J Trop Biomed* 2015; **6**(3): 239-45.

[23] Deng Y, Zhao Y, Padilla-Zakour O, Yang G. Polyphenols, antioxidant and antimicrobial activities of leaf and bark extracts of *Solidago canadensis* L. *Ind Crops Prod* 2015; **74**: 803-9.

[24] Dey SK, Hira A, Howlader MSI, Ahmed A, Hossain H, Jahan IA. Antioxidant and antidiarrheal activities of ethanol extract of *Ardisia elliptica* fruits. *Pharm Biol* 2014; **52**(2): 213-20.

[25] Cartron ML, England SR, Chiriac AI, Josten M, Turner R, Rauter Y. Bactericidal activity of the human skin fatty acid cis-6-hexadecanoic acid on *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2014; **58**: 3599-609.

[26] Tatsimo SJ, Tamokou JD, Havyarimana L, Csupor D, Forgo P, Hohmann J. Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from *Bryophyllum pinnatum*. *BMC Res Notes* 2012; **5**: 158.

[27] Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Sao Paulo Med J* 2015; **133**(2): 164-5.

[28] del Valle P, García-Armesto MR, de Arriaga D, González-Donquiles C, Rodríquez-Fernández P, Rúa J. Antimicrobial activity of kaempferol and resveratrol in binary combinations with parabens or propyl gallate against *Enterococcus faecalis*. *Food Control* 2016; **31**(61): 213-20.

[29] Edwards SE, da Costa Rocha I, Williamson EM, Heinrich M. *Phytopharmacy: an evidence-based guide to herbal medicinal products*. New York City: John Wiley & Sons; 2015.

[30] Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with antioxidant activity (part 1). *Int J Pharmacol Toxicol* 2015; **6**(3): 159-82.

[31] Tan JB, Lim YY. Critical analysis of current methods for assessing the in vitro antioxidant and antibacterial activity of plant extracts. *Food Chem* 2015; **1**(172): 814-22.

[32] Kuo CT, Liu TH, Hsu TH, Lin FY, Chen HY. Antioxidant and antiglycation properties of different solvent extracts from Chinese olive (*Canarium album* L.) fruit. *Asian Pac J Trop Biomed* 2015;31; **8**(12): 1013-21.

[33] Negi PS. Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. *Int J Food Microbiol* 2012; **156**: 7-17.

[34] Al-Saeedi AH, Hossain MA. Total phenols, total flavonoids contents and free radical scavenging activity of seeds crude extracts of pigeon pea traditionally used in Oman for the treatment of several chronic diseases. *Asian Pac J Trop Dis* 2015; 30; **5**(4): 316-21.