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PROVING BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF *AJUGA REMOTA* BENTH LEAVES EXTRACT: IMPACT OF ULTRASONIC POWER, SONICATION TIME, AND CONVENTIONAL EXTRACTION PARAMETERS

– Research paper –

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Abstract: *Ajuga remota* Benth plant contains a variety of phytochemicals including phenolic compounds with potent antioxidant activities. However, in order to improve the extraction efficiency, green technologies need to be used while extracting bioactive compounds. Therefore, this study was aimed to determine the impact of ultrasonic power and extraction time on the extract yield, bioactive compounds and antioxidant activities of *A. remota* leaves extract. Ultrasonic assisted extraction (UAE) was employed at amplitude of 50, 60 and 70% with the sonication time of 10 and 20 min. The highest total phenolic content (44.14 mg GAE/ g) and flavonoids content (126 mg QE/g), and antioxidant activities 100.11 mg TE/g, 263.97, mg TE/g and 286.19 mg TE/g for DPPH, FRAP and ABTS, respectively, were found at 70 % ultrasonic power with the extraction time of 10 min. Thus, UAE showed a significant ($p<0.05$) improvement of the extract yield, bioactive compounds, and antioxidant activities.

Keywords: *Ajuta remota*, ultrasonic power, extraction time, bioactive compounds, antioxidant activities, LC/MS.

INTRODUCTION

Ajuga remota Benth *(synonyms: Ajuga integrifolia* Buch. *Ham, Ajuga bracteosa wall ex* Benth) is *a Lamiaceae* shrub that grows at elevations ranging from 1500 to 3400 m above sea level in Ethiopia, Saudi Arabia, Yemen, and Afghanistan (Bekeri et al., 2018). *A. remota* is used as a traditional medicine to treat diabetes, blood pressure, toothache, skin disease, stomach pain, malaria, pneumonia and liver problems (Tafesse et al., 2017). This plant contains a variety of phytochemicals including phenolic compounds that have high antioxidant activities: phenols, flavonoids, tannins, terpenoides (Alene et al., 2020; Bekele et al., 2008), all of which are biologically active compounds that help to prevent oxidative stresses (Alene et al., 2020).

Bioactive compounds extraction depends on the nature of the sample cell matrix and the physicochemical properties of the target compounds. Thus, concentration of the extraction solvents, solid-to-liquid ratio and extraction time have effects on the extraction efficiency of targeted compounds (Tagrida & Benjakul, 2021). Hence, the success of extraction most importantly depends on the method and technology used.

Ultrasonic assisted extraction (UAE) has been employed widely in the food sector to overcome the limitations of conventional extraction, such as low extraction efficiency, use of high amounts of solvents and concentration, energy dissipation, time consuming (Dadi et al., 2019; Gulzar & Benjakul, 2018; Tagrida & Benjakul, 2021).

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UAE can be used to improve extraction efficiency when combined with appropriate solvents to enhance cell membrane permeability (Chotphruethipong et al., 2019; Petcharat et al., 2021). Cellular walls of plants could be disintegrated by ultrasonic waves which induces pressure and cavitation, allowing target components to be released (Dadi et al., 2019). UAE time, temperature, solute-to-solvent ratio and frequency all influence the bioactive compounds and antioxidant activities of the extracts (Chemat et al., 2017). It has been noted that the temperature and duration of ultrasonic extraction influence the content of flavonoid in pumpkin extract (Altemimi et al., 2016). As stated by Ahmed et al (2020), the ultrasonic power and frequency may also have an adverse effect on the flavonoid content of the extract. The selection of solvent-to-solid ratio is critical in UAE, owing to its significant impact on cavitation effectiveness, as well as in acoustic energy transference to reactants (Da Porto et al., 2013; Dadi et al., 2019; Yen & Quoc, 2020). Furthermore, sonication power and time are among the factors which determine the effectiveness of cavitation. Therefore, it is important to evaluate the effects of UAE parameters on the bioactive compounds and antioxidant activities of the plant extracts.

Though several investigations on the traditional applications of the *A. remota* Benth plant have been undertaken, including phytochemical screening and antidiabetic activity (Bekele et al., 2008), few researches have been focused on enhancing the efficiency of *A. remota* Benth using conventional extraction. In addition, no published information on effective and cutting-edge extraction methods. Hence, the aim of this study was to evaluate the effect of ultrasonic power and extraction time on the improvement of extract yield, bioactive compounds and antioxidant activities.

MATERIALS AND METHODS

Sample collection and preparation

A. remota Benth plant leaves sample was brought from Gubure province, Wolkite, Ethiopia. The leaves sample was then washed thoroughly using distilled water. The sample was kept under the shade until was dried (MC~ 10% db). Then, the dried sample was ground and packed in a sealed plastic bag. The sample was transported to International Center of Excellence in Seafood Science and Innovation, Faculty Agro-Industry, Prince of Sonkal University, Hat Yai Songkhla, Thailand for laboratory analysis.

Chemicals and reagents

All chemicals and reagents were analytical grade. Folin-Ciocalteu phenol reagent, Gallic acid, quercetin, DPPH, ABTS, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Trolox, EDTA were purchased from Sigma-Aldrich (Bangkok, Thailand). Ethanol, sodium nitrite, aluminum chloride, ferric sulfate, acetic acid, ferric chloride, sodium acetate, ethyl acetate and hydrochloric acid, sodium hydroxide, ferrozine, potassium persulfate were purchased from Guangzhou Huangpu Chemical Co., Ltd. (Guangzhou, China).

Conventional extraction of *A. remota* Benth

The extraction was done following the method of Tagrida and Benjakul (2021) with some modifications. About 10 g of *A. remota* leaves powder was put into 500 mL beakers and mixed with 70 % and 90 % ethanol at the ratios of 1:20 and 1:40 (w/v). The extraction was done by continuous stirring using a magnetic stirrer for 12 h at room temperature. Then, the extracts were centrifuged at 5000 ×g at 4 °C for 30 min using a high speed refrigerated centrifuge (Hitachi, Japan). Then, the supernatant was collected and evaporated under vacuum by a rotary evaporator (EYELA rotary evaporator N-1000, Japan) at 40 °C. The residual ethanol was removed by nitrogen purging. Then, the concentrate was diluted with distilled water at 1:2 (w/v) ratio and kept at 4 °C for 12 h to remove the chlorophyll by the process of sedimentation. Dechlorophyll extract was collected after centrifuged at 10000 ×g and 4 °C for 30 min before being lyophilized. Then after, the dried extract was placed in an amble bottle and stored at -20 °C until it was analyzed.

Ultrasonic assisted extraction (UAE)

Ultrasonic assisted extraction was done according to Dadi et al. (2019) with some modifications. About 10 g of the leaves powder and 200 mL of 90% ethanol were mixed in the 500 mL beaker with solid-to-solvent ratio of 1:20. The mixture was put in ultrasonicator (Sonics, Model VC750, CT, USA) at amplitudes of 50 %, 60 % and 70 % with a sonication time of 10 and 20 min. The temperature of UAE was kept below 35 °C using an iced bath. Then, the extract was centrifuged at 5000×g and 4 °C for 30 min. The supernatant was collected and the solvent was evaporated followed by dechlorophyllization and lyophilization as described above. Then, the sample was kept in an amble bottle and stored at -20 °C until analysis.

Extraction yield determination

The extract yield was calculated by subtracting the mass of the extract from the dried mass of leaves powder and expressed in percent.

Total phenolic content (TPC) determination

The total phenolic content (TPC) of the extract was determined according to the method described by Sinlapapanya et al. (2022). The absorbance was read at 760 nm. The TPC was calculated using the standard curve of gallic acid (0-0.05 mg/mL) and presented as mg gallic acid equivalent (GAE)/g dried sample.

Total flavonoids content (TFC) determination

The total flavonoid content (TFC) was determined using colorimetric assay as described by Tagrida and Benjakul (2021). The absorbance was read at 510 nm. Then, TFC was calculated from standard curve of quercetin (0-0.3 mg/ mL) and expressed as mg quercetin equivalent (QE)/ g dried sample.

Antioxidant activities

The antioxidant activities of the extract were determined in terms of DPPH, ABTS radical scavenging activities, ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and metal chelating activity (MCA) as described by Tagrida and Benjakul (2021). These antioxidant activities were calculated from the standard curve of Trolox (50-600μM) and expressed as mg Trolox equivalents (TE)/g dried sample, except for MCA, which was calculated from the standard curve of EDTA (10-60 μM) and expressed in μmol EDTA equivalents (EE)/g dried sample.

Bioactive compounds profile determination using LC/MS

Bioactive compounds profiling in the extract was done according to Chotphruethipong et al. (2019) using liquid chromatography/mass spectrometric (LC/MS). The extract was initially separated on the LiChro- CART Purospher STAR RP-18e column (Merck, Branchburg, USA) using an Agilent 1100 series (Agilent Technologies, Germany) (150-4.6 mm, i.d., 5 m). Mobile phases A and B were a 10 mM ammonium formate buffer (pH 4) and formic acid, respectively. The temperature was 40 °C and the flow rate was 1.0 mL/ min. The gradient program was composed of four steps: 100% B constant (0-5 min), 0-20% A (5-10 min), 20% A constant (10-20 min), and 20-40% A (20-60 min). The detection was carried out at the wavelengths of 270, 330, 350, and 370 nm. Nitrogen gas was used as a drying gas in an electrospray ionizing source, and MS detection method of t negative ionization was applied. The capillary voltage of 4000 V, the gas temperature of 320 °C, the drying gas flow rate of 13 L/ min, and the nebulizer pressure of 60 psi were used. For quantitative analysis, Mass Selective Detector (MSD) was employed in the Selected Ion Monitoring (SIM) mode.

Statistical analysis

All the experiments were conducted in triplicate and the results were presented as mean ± standard deviations. Analysis of variance (ANOVA) was used to analyze the data and Duncan's multiple range test was used for mean comparison using Statistical Package for Social Science (SPSS 22.0 for Windows, SPSS Inc., IL, USA) software. The significance test was performed by comparing mean values at level of $p < 0.05$

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RESULTS AND DISCUSSION

Effect of conventional extraction conditions on the yield, TPC and TFC

Table 1 shows the effect of ethanol concentration on yield, TPC and TFC. The extract yield was not favored with the increment of ethanol concentration and solid-to-solvent ratio. However, the extract yield was decreased significantly ($p<0.05$) when the solute-to-solvent ratio was changed from 1:20 to 1:40 using 70% ethanol as the extracting solvent. This showed that the addition of a higher volume of solvent may dilute the system, consequently it decreases the extract yield. This result is in agreement with the extract yield of polyphenols from crude pollen (Oroian et al., 2020), peppermint (Pavlić et al., 2019), and oleanolic acid from apple processing waste (Rubashvili et al., 2020).

The TPC and TFC were higher in the extract extracted with 90% ethanol at a solute-to-solvent ratio of 1:20 (Table 1). As the ethanol concentration increased from 70 % to 90 % while maintaining a constant solute-to-solvent ratio, the TPC and TFC increased by 18.65 % and 14.77 %, respectively. The same results were also reported for polyphenol extraction from *Fibraurea chloroleuca* (Marpaung & Handayani, 2018), cashew leaf (Sinlapapanya et al., 2022). This indicates that the increment of ethanol concentration leads to a significant ($p<0.05$) increment of TPC and TFC. This could be due to the presence of phenolics compounds in this plant, which are most likely extracted using weak polar solvent (Dhanani et al., 2017).

Effects of conventional extraction on antioxidant activities

The effects of conventional extraction conditions on antioxidant activities were shown on Table 2. When the ethanol concentration was increased from 70 % to 90 %, the ABTS, DPPH radical scavenging activities, MCA and ORAC values increased by 13.37 %, 54.04 %, 37.65 % and 27.04 %, respectively. Similar findings were also reported for *Bixa orellana L.* extracts (Quiroz et al., 2019). Therefore, the conventional extraction of *A. remota* Benth leaves powder using 90 % ethanol was relatively more efficient than 70 % ethanol.

Impact of ultrasonic power on the yield, TPC, TFC and antioxidant activities

Table 3 shows the effect of ultrasounic power on the TPC, TFC, and antioxidant activities (Table 4) of *A. remota* leaves extracts. It was found that ultrasonic power had significant ($p<0.05$) effect on the TPC and TFC. In addition, the antioxidant activities of the extract were significantly ($p<0.05$) increased as the ultrasonic power was increased from 50% to 70% at constant sonication time, with the exception of metal chelating activity. When ultrasonic extraction was carried out at of 70 % power for 10 min of sonication time, there was a notable increment in yield, TPC and TFC by 19.37 %, 41.23 % and 39 %, respectively when it was compared to other ultrasonic power and time treatments and conventional extraction (control). Similarly, the ABTS, DPPH, FRAP, and ORAC values increased by 20.4 %, 82.18 %, 42.2 %, and 53.57 %, respectively, compared to the results obtained with 50 % power with 10 min extraction. These results are in line with the polyphenol extraction from crude pollen (Oroian et al., 2020), *Nepheliuml appaceum* (Maran et al., 2017), and grape seeds (Da Porto et al., 2013), *Litopenaeus vannamei* (Gulzar & Benjakul, 2018).

Table 1. Effect of conventional extraction solvent and solute-to-solvent ratio on extract yield, TPC and TFC from *A. remota* leaves.

Ethanol Conc. (%)	Solute-to-solvent ratio	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
70	1:20	21.01 ± 0.92^{a}	30.66 ± 0.110^{c}	77.21 ± 1.38^{c}
70	1:40	18.90 ± 0.65^{b}	33.0 ± 0.60^{b}	84.53 ± 3.18^{b}
90	1:20	18.85 ± 0.53^{b}	37.69 ± 0.65^{a}	90.59 ± 0.90^{a}
90	1:40	18.26 ± 0.19^{b}	37.15 ± 1.60^{a}	88.91 ± 2.16^{a}

Note: Different superscript letters in a column indicate significant difference ($p<0.05$). GAE: gallic acid equivalent, QE: quercetin equivalent.

Table 2. Effects of conventional extraction solvent and solute-to-solvent ratio on the antioxidant activities of *A. remota* extract

Ethanol conc. (%)	Solute-to-solvent ratio	DPPH (mg TE/g)	ABTS (mg TE/g)	FRAP (mg TE/g)	MCA (µmol EDTA/g)	ORAC (mg TE/g)
70	1:20	45.89 ± 5.06^{b}	222.16 ± 2.77^{b}	142.43 ± 3.84^{a}	12.17 ± 2.65^{c}	26.28 ± 1.22^{c}
70	1:40	91.82 ± 7.50^{a}	$252.80\pm6{,}94^{a}$	145.84 ± 8.97^{a}	16.96 ± 0.09^{b}	32.74 ± 1.05^{b}
90	1:20	99.84 ± 5.31^{a}	256.45 ± 3.22^{a}	139.71 ± 4.41^{a}	19.52 ± 1.44^{a}	36.02 ± 1.45^{a}
90	1:40	87.79 ± 8.52^{a}	231.70 ± 1.10^{b}	118.11 ± 9.93^{b}	7.16 ± 1.23^{d}	27.03 ± 1.12^{c}

Note: as in Table 1

Table 3. Impact of ultrasonic power and time on extract yield, TPC and TFC of *A. remota* leaves.

UAE power (%)	Extraction time (min)	Extract yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Control		18.85 ± 0.53^{b}	37.69 ± 0.63^{c}	90.59 ± 0.93^{cd}
50	10	16.73 ± 0.01^{c}	25.94 ± 0.32^{e}	76.38 ± 1.25^{d}
50	20	17.17 ± 0.64^{c}	33.69 ± 0.8^{d}	113.63 ± 8.81^{b}
60	10	16.64 ± 0.58^{c}	34.86 ± 0.29^{d}	83.12 ± 11.11^{c}
60	20	17.30 ± 2.30^{c}	40.69 ± 0.65^{b}	123.9 ± 1.62^{a}
70	10	20.75 ± 0.07^{a}	44.14 ± 1.59^{a}	126.67 ± 6.29^{a}
70	20	19.87 ± 1.54^{ab}	38.39 ± 1.16^{c}	117.90 ± 4.14^{ab}

Note: as in Table 1

It is reported that the efficacy of the vibration wave depends on the intensity of the process, increasing ultrasonic power led to the increase in extraction efficiency of phenolic compounds (Oroian et al., 2020). In addition, it is might be due to a cavitation effects (Dadi et al., 2019) which occur when an ultrasonic wave cycle involves in compression and rarefaction. The compression and rarefaction cycles are affected by the amplitude of the ultrasonic waves. As a result, the higher the ultrasonic amplitude, the more cavities were attained, and thus the extraction yield improved (Gulzar & Benjakul, 2018; Yang et al., 2017).

Impact of sonication time on yield, TPC, TFC and antioxidant activities

One of the factors influencing the yield of polyphenols is sonication time. The extract yield was not significantly ($p>0.05$) influenced by increasing sonication time from 10 to 20 min at constant ultrasonic power (Table 3). Moreover, the TPC and TFC of the extract were significantly influenced by sonication time (Table 3). Similarly, the antioxidant activities (DPPH radical scavenging activity, ORAC, FRAP, and MCA) were also significantly ($p<0.05$) influenced by sonication time (Table 4). It was found that 10 min of sonication time at 70 % ultrasonic power was sufficient to attain the extraction's maximum efficiency.

This shows that 10 min of sonication time is sufficient for the sonication wave to allow the solvent to penetrate the cell membrane and increase the mass transfer rate. This result was in line with the findings of polyphenol extraction using ultrasonication of rice protein (Yang et al., 2017), crude pollen (Oroian et al., 2020) and oil from *Litopenaeus vannamei* (Gulzar & Benjakul, 2018). This shown that the optimal ultrasonic extraction condition for extracting bioactive compounds from *A. remota* leaves is 70 % power for 10 min of sonication time.

LC/MS analysis of bioactive compounds profile in *A. remota* leaves extract

Table 5 shows the bioactive compounds profile in *A. remota* leaves extract extracted by UAE at 70 % ultrasonic power and 10 min of sonication time. The LC/PAD/MSD analysis was carried out in negative mode since it is often more sensitive than the positive mode. This can be attributed to their proclivity to rapidly produce negative ions for profiling the bioactive, more particularly the polyphenolic components (Olatunde et al., 2018). The molecular formula, m/z, and quantity of bioactive compounds were used to identify the compounds in the extract.

The most prominent compound in *A. remota* extract was isoacteoside, followed by Okanin 4'-O-(4",6"-di-O-acetylglucoside), Hesperetin 7-(2,6-dirhamnosylglucoside) and Isoacteoside (Table 5). These compounds are well-known possessing high antioxidant activities. Some phenolics were also found to be glycosides, which were linked with sugar. Free phenolic acids are rare (Bartnik et al., 2017). This result showed that *A. remota* leaves is a promising source of natural antioxidants.

Table 4. Impact of ultrasonic power and time on antioxidant activities of *A. remota* extract

UAE power (W)	Extraction time (min)	DPPH (mg TE/g)	ABTS (mg TE/g)	FRAP (mg TE/g)	MCA (µmol EDTA/g)	ORAC (µmol TE/g)
Control		99.84 ± 5.31^{a}	256.45 ± 3.22^{b}	139.71 ± 4.41^{e}	19.51 ± 1.44^{a}	36.02 ± 1.45^{f}
50	10	17.84 ± 3.95^{d}	210.15 ± 4.63^{c}	165.45 ± 9.03^{d}	14.76 ± 1.99^{b}	41.96 ± 1.64^{ef}
50	20	74.37 ± 3.83^{b}	201.51 ± 8.97^{c}	117.43 ± 4.88^{f}	20.65 ± 3.81^{a}	43.66 ± 1.24^{de}
60	10	52.66 ± 0.96^{c}	250.17 ± 8.57^{b}	199.49 ± 7.19^{b}	12.24 ± 3.62^{b}	49.49 ± 0.98^{d}
60	20	67.64 ± 7.47^{b}	241.51 ± 0.38^{b}	186.95 ± 5.35^{c}	21.01 ± 2.09^{a}	83.97 ± 1.07^{c}
70	10	100.11 ± 8.48^{a}	263.97 ± 3.55^{a}	286.19 ± 4.71^{a}	22.97 ± 0.06^{a}	91.51 ± 1.28^{b}
70	20	93.11 ± 4.18^{a}	210.28 ± 3.24^{c}	116.09 ± 2.30^{f}	22.04 ± 0.54^{a}	101.07 ± 1.3^{a}

Different superscript letters in a column indicate significant difference ($p<0.05$). Control: Conventional extraction (90% of ethanol and solute-to-solvent ratio 1/20). TE: Trolox equivalent, EDTA: Ethylenediaminetetraacetic acid equivalent.

Table 5. Bioactive compounds profile in *A. remota* Benth extract

Name	Formula	m/z	Mass	RT	Abundance (10^6)
Verbasoside	$C_{20}H_{30}O_{12}$	461.17	462.17	8.75	0.55
Rhamnetin 3-rhamnoside	$C_{22}H_{22}O_{11}$	461.11	462.12	21.4	0.56
Aesculin	$C_{15}H_{16}O_9$	339.07	340.08	9.29	0.59
Physagulin E	$C_{36}H_{50}O_{14}$	705.31	706.32	26.6	0.70
Citrusin E	$C_{17}H_{24}O_9$	371.13	372.14	22.1	0.71
Rhodojaponin I	$C_{24}H_{36}O_8$	451.23	452.24	31.0	0.75

7-Hydroxy-4-methylphthalide O-[arabinosyl-(1->6)-glucoside]	$C_{20}H_{26}O_{12}$	457.13	458.14	11.9	0.83
Nigakilactone E	$C_{24}H_{34}O_8$	449.22	450.23	31.8	0.88
3-(3,4-Dihydroxyphenyl)-1-propanol 3'-glucoside	$C_{15}H_{22}O_8$	329.12	330.13	14.8	0.90
APIIN	$C_{26}H_{28}O_{14}$	563.14	564.15	14.1	0.91
Stachyoside A	$C_{21}H_{34}O_{15}$	525.18	526.19	7.80	0.96
Glucocaffeic acid	$C_{15}H_{18}O_9$	341.09	342.10	8.88	1.17
Leonuridine	$C_{15}H_{24}O_9$	347.13	348.14	8.59	1.19
Leonoside A	$C_{35}H_{46}O_{19}$	769.26	770.26	20.1	1.62
Saponarin	$C_{27}H_{30}O_{15}$	593.15	594.16	13.6	1.68
cis-3-Hexenyl b-primeveroside	$C_{17}H_{30}O_{10}$	393.18	394.18	5.96	1.70
Morroniside	$C_{17}H_{26}O_{11}$	405.14	406.15	8.27	1.80
Antirrhinoside	$C_{15}H_{22}O_{10}$	361.11	362.12	5.74	1.81
Diosmetin	$C_{16}H_{12}O_6$	299.06	300.06	30.8	1.88
Camellianin A	$C_{29}H_{32}O_{15}$	619.17	620.17	19.6	1.98
Barbatoflavan	$C_{24}H_{28}O_{13}$	523.15	524.15	15.3	2.02
3',5,6-Trihydroxy-3,4',7,8-tetramethoxyflavone 3-glucoside	$C_{25}H_{28}O_{14}$	551.14	552.15	14.2	2.17
10-Hydroxymorroniside	$C_{17}H_{26}O_{12}$	421.14	422.14	5.72	2.24
1-Octen-3-yl primeveroside	$C_{19}H_{34}O_{10}$	421.21	422.22	22.9	2.29
Norrubrofusarin 6-beta-gentiobioside	$C_{26}H_{30}O_{15}$	581.15	582.16	12.4	2.35
(1x,2x)-Guaiacylglycerol 3-glucoside	$C_{16}H_{24}O_{10}$	375.13	376.14	16.3	2.51
[6]-Gingerdiol 3,5-diacetate	$C_{21}H_{32}O_6$	379.21	380.22	30.4	2.55
(-)-Matairesinol 4'-[apiosyl-(1->2)-glucoside]	$C_{31}H_{40}O_{15}$	651.23	652.24	24.0	3.52
3,4,5-Trimethoxyphenyl glucoside	$C_{15}H_{22}O_9$	345.12	346.13	12.7	4.92
Luteolin 4'-glucoside 7-galacturonide	$C_{27}H_{28}O_{17}$	623.13	624.13	16.3	5.59
Hesperetin 7-(2,6-dirhamnosylglucoside)	$C_{34}H_{44}O_{19}$	755.24	756.25	19.0	5.74
6"-(4-Carboxy-3-hydroxy-3-methylbutanoyl) hyperin	$C_{27}H_{28}O_{16}$	607.13	608.14	18.0	7.35
Nigakilactone C	$C_{24}H_{34}O_7$	433.22	434.23	36.7	7.64
Okanin 4'-O-(4",6"-di-O-acetylglucoside)	$C_{25}H_{26}O_{13}$	533.13	534.14	17.6	13.28
Hesperetin 7-(2,6-dirhamnosylglucoside)	$C_{34}H_{44}O_{19}$	755.24	756.25	17.7	15.63
Isoacteoside	$C_{29}H_{36}O_{15}$	623.20	624.21	18.3	18.49

CONCLUSION

The extraction of *A. remota* Benth leaves powder was effective when 90% ethanol with a solute-to-solvent ratio of 1:20 (w/v) was used. In addition, the study showed that ultrasonic power and sonication time during ultrasonic extraction of *A. remota* leaves have significant impact on the extract yield, bioactive compounds and antioxidant activities. The highest total phenolic content (44.14 mg GAE/ g) and flavonoids content (126 mg QE/g), and antioxidant activities 100.11 mg TE/g, 263.97, mg TE/g and 286.19 mg TE/g for DPPH, FRAP and ABTS, respectively, were found ultrasonic extraction was carried out at 70 % ultrasonic power for 10 min. In addition, it was shown that ultrasonic extraction at 70 % power for 10 min had better extraction efficiency than the conventional extraction. Therefore, ultrasonic assisted extraction for *A. remota* Benth leaves improves the extract yield, bioactive compounds and antioxidant activities that can be used in the food industry.

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