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Full Length Research Paper

Effect of simultaneous ultrasonic/microwave assisted extraction on the antioxidant and antibacterial activities of burdock leaves

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The effects of different extraction methods on the antioxidant and antibacterial activities of burdock leaves extracts were first investigated. The extracts of burdock leaves were prepared by 10 h maceration extraction (ME) and simultaneous ultrasonic/microwave assisted extraction (UMAE) with a significantly short operation time of 30 s. The UMAE extract exhibited 50.60% inhibition of lipid peroxidation, 51.22% scavenging of DPPH radical at 0.8 mg/ml, 55.21 and 79.80% scavenging of hydroxyl and superoxide anion radicals at 2.5 mg/ml. These activities were much higher than those of ME extract. Moreover, the UMAE extract was more effective against Gram-positive and Gram-negative bacteria than ME extract. The MIC values ranged from 1.5 to 2.5 mg/ml. Among the phenolic compounds identified in the extracts, the occurrence of o-hydrobenzoic acid and ferulic acid in burdock leaves is reported for the first time. The results indicated UMAE to be a new, fast and efficient alternative for preparation of antioxidant and antibacterial agents.

Key words: Antibacterial activity, antioxidant activity, burdock leaves, phenolic compounds, simultaneous ultrasonic/microwave assisted extraction.

INTRODUCTION

A great deal of studies strongly suggests that phenolic compounds have protective effects against many diseases. Thus, phenolic compounds could be used as antibacterial, antioxidant, anti-inflammatory and antiviral agents (Senevirathne et al., 2006). Increasing evidence indicates that consumption of a variety of phenolic compounds may lower the risk of serious health disorders for the antioxidant activities of phenolics (Qiu et al., 2007; Surh, 2002). The antioxidant activity of extracts of many plants including their leaves, bark, roots, fruits and seeds (Kil et al., 2009), has been extensively studied. Burdock, arctium lappa which is a popular vegetable in China and Japan, has been extensively studied for its components (Chen et al., 2004; Hirose et al., 2000; Lou et al., 2009). However, there is less information about burdock leaves of root and seed due to their antioxidant properties, antimicrobial activity and other biochemical activities (Chinese traditional medicine), which are rich in phenolics (Ferracane et al., 2010).

Extraction is one of the most imperative steps for active components preparation and the activity evaluation of various plants (Li et al., 2009; Marghitas et al., 2009; Sun et al., 2009). Most reports focused on investigation of the effect of different solvents on the activity of extract (Kil et al., 2009; Subhasree et al., 2009). However, the influence of different extraction techniques on the activity was rarely studied. Ultrasonic is one of the common methods in enhancing mass transfer. The increasing interest on applying sonochemistry to natural components extraction lies in its advantage on reducing extraction time (Lou et al., 2010), saving in energy, increasing yield, etc. (Wu et al., 2007). Meanwhile, microwave assisted extraction heats the extracts quickly and accelerates the extraction process for adsorption and desorption of the target compounds from matrix. Hence, coupling microwave with

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ultrasonic extraction is a complementary technique and may present many advantages. However, by now, the effect of simultaneous ultrasonic/microwave assisted extraction (UMAE) on the antioxidant, antibacterial activities of target compounds has not been reported.

This study investigates the effect of different extraction techniques on the antioxidant activity, antibacterial activity and active components contents of burdock leaves. Furthermore, major phenolic compounds of burdock leaves were identified.

MATERIALS AND METHODS

Burdock leaves, provided by Xuzhou Wangda Farm and Sideline Products Co., Ltd.. Caffeic acid, chlorogenic acid, quercetin-rhamnoglucoside (rutin), o-hydrobenzoic acid, p-coumaric acid, vanilic acid and ferulic acid were obtained from Sigma (Shanghai, China). All other chemicals were of analytical grade.

Maceration

The dried powder (20 g) of burdock leaves was extracted in a 500 ml flask filled with 70% ethanol solution (400 ml). The extraction was carried out at 30°C, 250 rpm for 10 h. The extracts were filtered and then concentrated in a rotary evaporator at 40°C under vacuum and lyophilised using a freeze-dryer (LGJ-10D, Four-Ring Science Instrument Beijing Co., Ltd., China) to obtain extracts.

Simultaneous ultrasonic/microwave assisted extraction

Simultaneous ultrasonic/microwave assisted extraction (UMAE) experiment was carried out with a simultaneous ultrasonic and microwave extracting apparatus. The dried powder (20 g) of burdock leaves was mixed with 70% ethanol (400 ml). Extraction process was performed in the apparatus chamber with simultaneous microwave power of 400 w and ultrasonic power of 50 w for 30 s. The post-treatment of the extracts was the same as that mentioned in maceration.

Determination of total phenols contents

The total phenols contents were determined using the Folin–Ciocalteu method as described by Yoo et al. (2004) and Liu et al. (2008). The total phenols were expressed as gallic acid equivalents.

Antioxidant activity

The inhibition of lipid peroxidation by the extracts was determined using the method of Tsuda et al. (2002). DPPH radical-scavenging activity of UMAE and maceration extraction (ME) extracts was analyzed by the method of Parvathy et al. (2009) and Liang et al. (2009). Hydroxyl radicals scavenging activity was measured according to the method of Tsai et al. (2001). The superoxide anion scavenging activity was determined using chemiluminescence technology as described by Sun et al. (2004).

Antibacterial activity

The strains *Staphylococcus aureus* 6538, *Streptococcus pneumoniae* ATCC49619, *Bacillus subtilis* 9372, *Escherichia coli* ATCC25922, *Shigella dysenteriae* 51302 and *Salmonella typhimurium* 50013 were purchased from China General Microbiological Culture Collection Center (Beijing, China). The antibacterial assay of UMAE and ME extracts was tested by the pour plate method according to the reports of parvathy et al. (2009) and Kil et al. (2009). The inhibitory effect of the extracts was calculated in the following formula:

Inhibition (%) = (1-T/C) × 100%.

Where C is cfu/ml of control and T is cfu/ml of test sample.

The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the extract capable of inhibiting the complete growth of the bacteria.

Identification and quantification of phenolic compounds

Analytical chromatographic analysis was performed using liquid chromatography Agilent Technologies series 1100 (USA), which was equipped with a reversed-phase column, symmetry C18 (250 × 4.6 mm) (Waters, USA). The samples were eluted with a gradient system consisting of solvent A (1% acetic acid, v/v) and solvent B (acetonitrile: methanol, 1:5, v/v), used as the mobile phase, with a flow rate of 1 ml/min. The gradient system started from 100% A at 0 min to 80% A at 10 min, 60% A at 15 min, 50% A at 20 min, and 40% A at 25 min. The peaks of the phenolic compounds were monitored at 280 nm. UV–Vis absorption spectra were recorded on-line from 200 to 700 nm during the HPLC analysis.

Phenolic compounds presented in samples were identified by comparing their retention times and ultraviolet spectra with standard phenolic compounds. Confirmation was performed by co-injection, and comparing the ultraviolet spectra of standards and samples. A calibration curve was plotted using chromatograms peak areas against a known standard's concentration.

RESULTS AND DISCUSSION

Effect of simultaneous ultrasonic/microwave assisted extraction on the antioxidant activities of burdock leaves

To evaluate the antioxidant activity of UMAE and ME extracts against lipid peroxidation, a liposome model system was used. The activities of both the extracts were dose-dependent (Figure 1a). The extract obtained by simultaneous ultrasonic/microwave extraction exhibited higher lipid peroxidation inhibitory activity than the ME extract. At 0.80 mg/ml, the inhibitory activity of lipid peroxidation was 50.60 ± 1.71% for UMAE extract, which was higher than that of ME extract (47.10 ± 1.51%). Meanwhile, at 1.00 mg/ml, the inhibitory activity of UMAE extract (53.50 ± 1.60%) was also higher than ME extract (50.90 ± 1.13%).

The lipid peroxidation inhibitory activities of the burdock leaves extracts in this study are probably due to chain termination of free radicals. The lipid peroxidation inhibition activity of rutin and chlorogenic acid was higher (Figure 2). At 0.06 mg/ml, the inhibitory activity of chlorogenic acid was 59.58 ± 1.76%, while the inhibitory

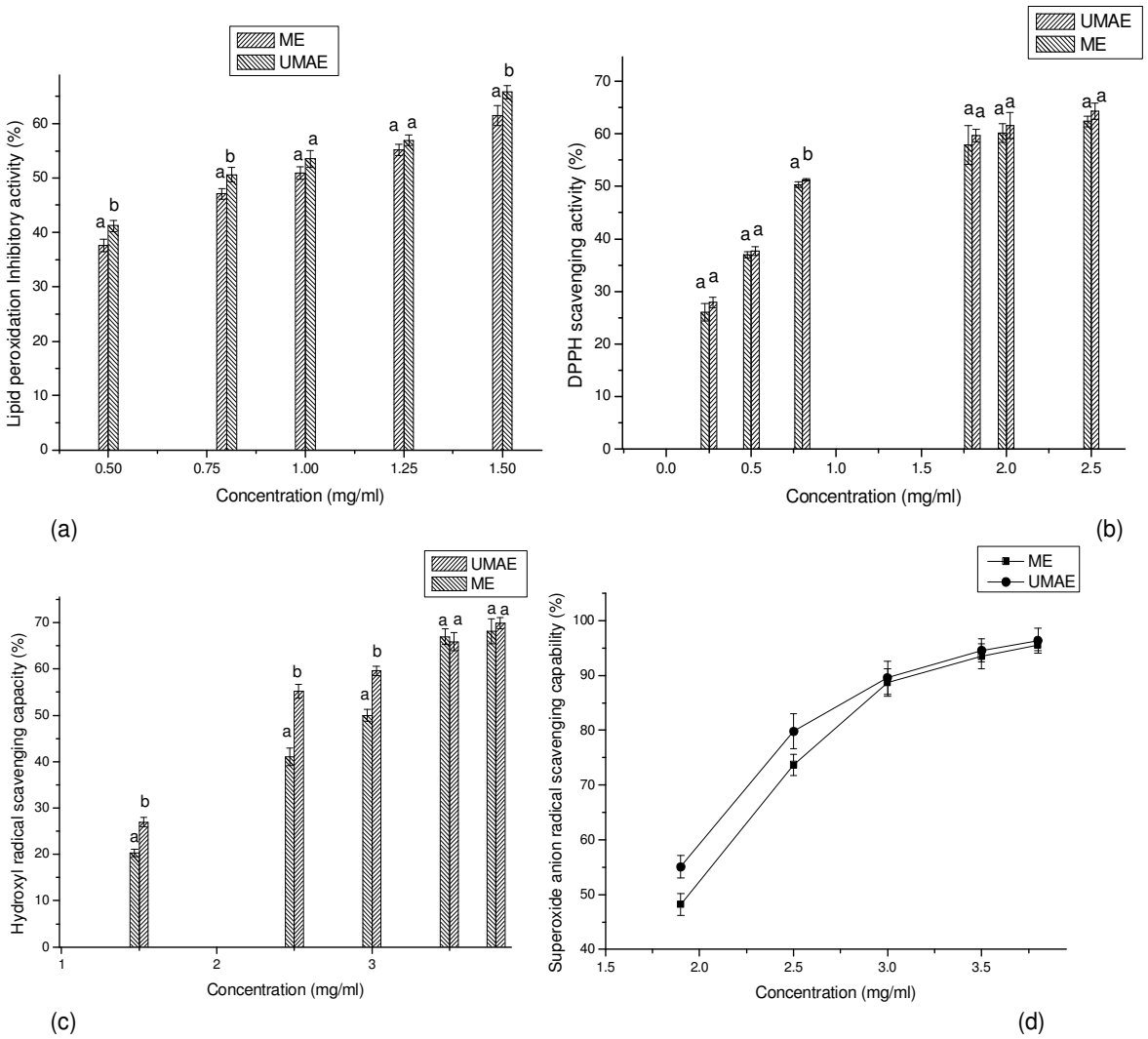

Figure 1. Antioxidant activities of UMAE and ME extracts from burdock leaves. (a) Lipid peroxidation inhibitory activity of the extracts, (b) DPPH radical-scavenging ability, (c) Hydroxyl radical scavenging capability and (d) Superoxide anion radical scavenging capability.

activity of rutin was 50.23 ± 1.57%. Antioxidant activity determined using DPPH radical scavenging assay is often expressed in terms of IC_{50}, the concentration of extract needed to achieve 50% scavenging of DPPH radical under experimental condition. In this study, it was found that the IC_{50} of UMAE extract, ME extract, chlorogenic acid and rutin were 0.80, 1.10 0.04 and 0.06 mg/ml respectively. Thus the scavenging ability of UMAE extract was much higher than that of ME extracts.

The DPPH radical-scavenging activities of both the extracts from burdock leaves increased with the increase of concentration (Figure 1b). At 0.80 mg/ml, scavenging ability of UMAE extracts was higher (51.22 ± 0.25%) than that of ME extracts (50.31 ± 0.51%). At 0.5 mg/ml, radical scavenging ability of UMAE and ME extracts were 37.66 ± 0.80% and 37.00 ± 0.60%, respectively. Increased concentrations of all tested samples exhibited concentration dependent hydroxyl radical scavenging activities (Figure 1c). The UMAE extract exhibited higher hydroxyl radicals scavenging capability of 26.94 ± 1.02% than ME extract (20.31 ± 0.81%) at the same concentration of 1.50 mg/ml. At 2.50 mg/ml, the hydroxyl radicals scavenging ability of UMAE extract was 55.21 ± 1.50%, which was much higher than that of the ME extract (41.01 ± 1.89%) ($p < 0.05$). As for chlorogenic acid and rutin, the scavenging ability of hydroxyl radicals reached 50% at about 0.1 mg/ml (Figure 2). UMAE extract could significantly scavenge the superoxide anion radical, inhibited the chemical luminescence reaction and decreased the chemiluminescence intensity.

Figure 1d shows the percentage inhibition of superoxide anion radicals by UMAE and ME extracts at

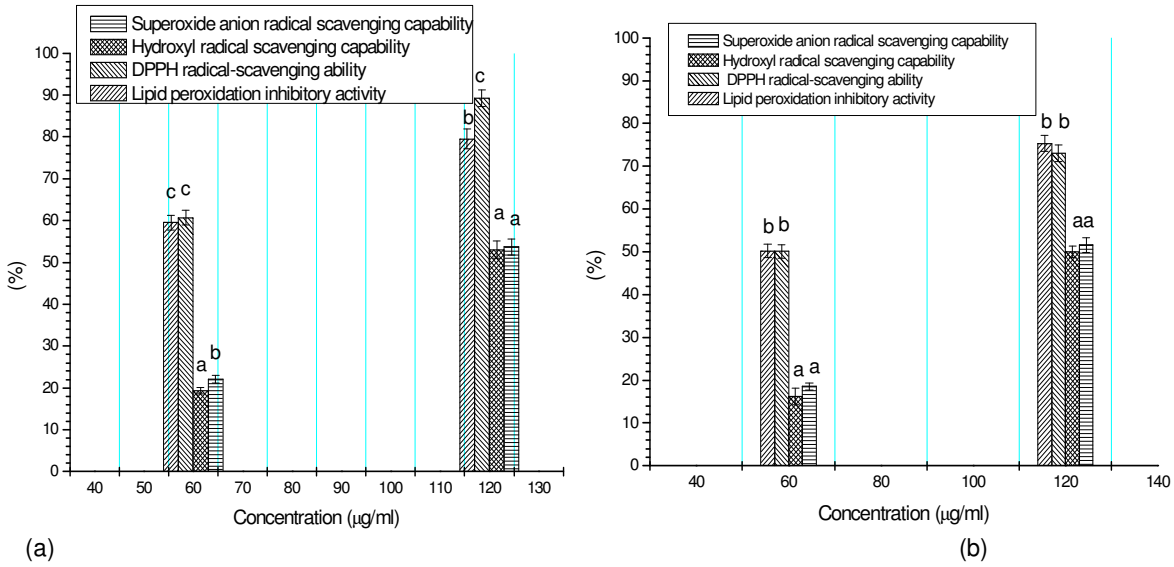

Figure 2. Antioxidant activity of chlorogenic acid and rutin. (a) chlorogenic acid, and (b) rutin.

different concentrations. For both extracts, the increase of superoxide anion scavenging activity was rapider at low concentrations, while slow at high concentrations. The superoxide anion scavenging of UMAE extract reached 55.08 ± 2.01% at 1.90 mg/ml, which was similar to that of the ME extract at 2.10 mg/ml. While the scavenging capabilities of chlorogenic acid and rutin were 53.70 ± 1.90% and 51.60± 1.70% at the concentration of 0.12 mg/ml (Figure 2). ME extract exhibited 73.68 ± 1.92% scavenging against superoxide anion, which was much lower than that of UMAE extract (79.80 ± 3.19%) at the same concentration of 2.50 mg/ml ($p < 0.05$). It was also reported that the IC_{50} value of TBSF melanin was 1879 ± 90.9 μg/ml (Tu et al., 2009).

The observation that UMAE extract effectively lowered the cascade of oxidation reactions induced by superoxide anion radical indicated effective chain breaking anti-oxidant activity of UMAE extract from burdock leaves.

Effect of UMAE on antibacterial activities

Furthermore, UMAE extract, ME extract, rutin and chlorogenic acid were tested for their antimicrobial potential against three Gram-positive (*S. pneumoniae* ATCC49619*, B. subtilis* 9372*, S. aureus* 6538) and three Gram-negative bacteria (*S. dysenteriae* 51302*, E. coli* ATCC25922, *S. typhimurium* 50013). (Figure 3a to f) shows the inhibition of bacteria at different concentrations of UMAE and ME extracts. The results indicated the differences in the antimicrobial properties of the extracts. In general, the UMAE extract was more effective than the ME extract. A comparison of the sensitivities of the bacterial strains to the extracts indicated that the stronger inhibitory effect was against *S. pneumoniae*. UMAE and ME extracts showed 60.70 and 52.60% inhibition, respectively, in the case of *S. pneumoniae* at the concentration of 1.00 mg/ml. At 1.50 mg/ml, UMAE extract showed 100% inhibition while ME extract showed only 83.60% inhibition, which was statistically lower ($p<0.05$) than the inhibition shown by UMAE extract. The minimum inhibitory concentrations (MIC) of UMAE and ME extracts against *S. pneumoniae* were found to be 1.50 and 1.80 mg/ml, respectively.

In the case of *B. subtilis*, UMAE extract showed 67.70% inhibition at 1.50 mg/ml whilst ME extract of the same concentration showed 45.50% inhibition. UMAE and ME extracts exhibited 100% inhibition at 2.00 and 2.50 mg/ml, respectively, indicating that the MIC values of UMAE and ME extracts were 2.00 and 2.50 mg/ml, respectively. But the MIC values of chlorogenic acid and rutin were 0.04 and 0.04 mg/ml (Table 1), respectively. In the case of *S. aureus,* UMAE and ME extracts showed 68.57 and 55.98% inhibition, respectively, at the concentration of 1.00 mg/ml. UMAE extract showed 100% inhibition at 1.50 mg/ml, but for ME extract, 100% inhibition was obtained at 2.00 mg/ml. The MIC values of UMAE and ME extracts were 1.50 and 2.00 mg/ml, respectively.

The antimicrobial potential of the extracts, rutin and chlorogenic acid against Gram-negative bacteria was also investigated (Figure 3d to f and Table 1). The inhibition by these extracts against *Shigella* showed that, at the concentration of 1.00 mg/ml, ME extract inhibited 50.31% of growth, which was much lower than UMAE extract (71.56%). At 1.50 mg/ml, UMAE extract showed 100% inhibition while ME extract showed only 70.30% inhibition, which was statistically lower ($p<0.05$) than the

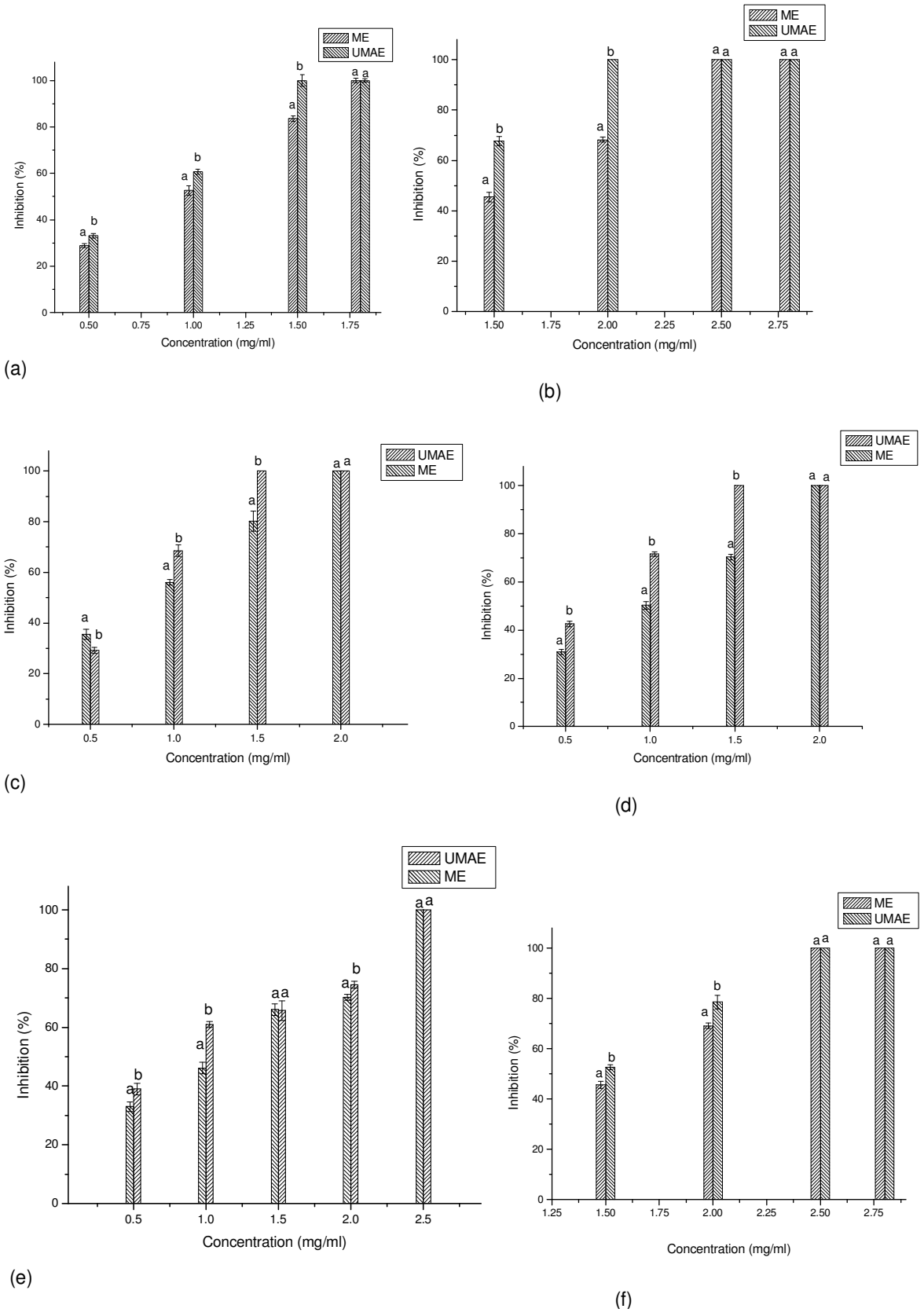

Figure 3. Anti-bacterial activities of UMAE and ME extracts against bacteria. (a) *Streptococcus pneumoniae* ATCC25922, (b) *Bacillus subtilis* 9372, (c) *Staphylococcus aureus* 6538, (d) *Shigella dysenteriae* 51302, and (e) *Escherichia coli* ATCC25922, (f) *salmonella typhimurium* 50013.

Table 1. The MIC values (mg/ml) of chlorogenic acid and rutin against bacteria.

Bacteria	Chlorogenic acid	Rutin
Bacillus subtilis	0.04	0.04
Escherichia coli	0.08	0.08
Shigella dysenteriae	0.04	0.04

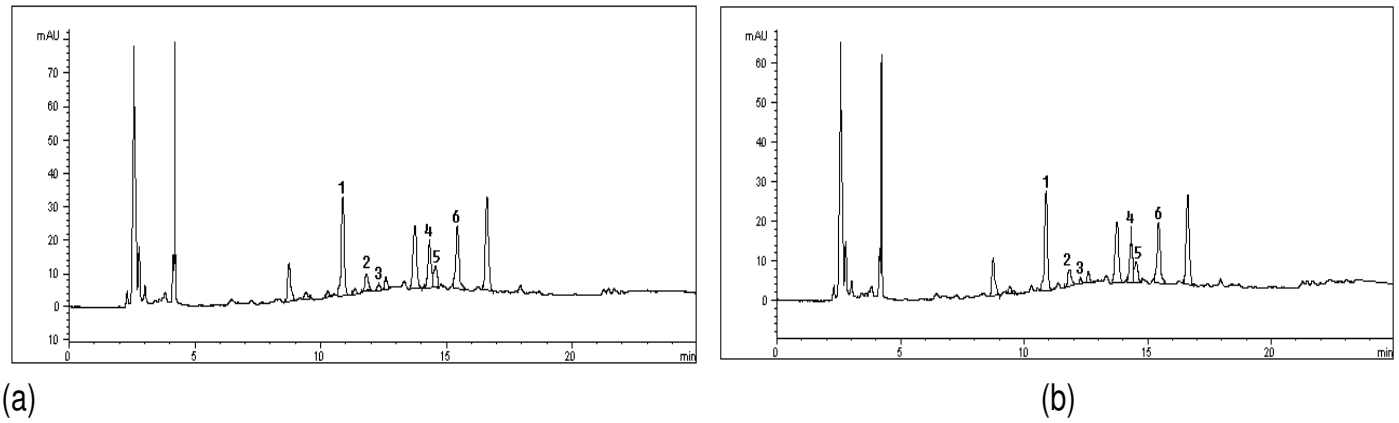

Figure 4. HPLC chromatogram of phenoilc compounds in (a) UMAE extract and (b) ME extract at 280 nm.

inhibition by UMAE extract. The MIC value of UMAE extract against *Shigella* (1.50 mg/ml) was much lower than that of ME extract (2.00 mg/ml). In the case of *E. coli*, UMAE extract showed 61.02% inhibition at 1.00 mg/ml, while the inhibition effect of ME extract of the same concentration was much lower (46.11%). UMAE and ME extracts both showed 100% inhibition at 2.50 mg/ml, suggesting the MIC values of the extracts to be 2.50 mg/ml. However, rutin and chlorogenic acid both showed 100% inhibition at the concentration of 0.08 mg/ml (Table 1). For *S. typhimurium,* UMAE and ME extract showed 45.70 and 52.70% inhibition at 1.50 mg/ml. At 2.50 mg/ml, UMAE and ME extract both showed 100% inhibition. The MIC values of UMAE and ME extracts against *S.* typhimurium were both 2.50 mg/ml.

In this work, higher antimicrobial activity of UMAE extract against *S. typhimurium, Shigella, E. coli, S. aureus, S. pneumoniae* and *B. subtilis* was observed. The lower MIC of UMAE extract than ME extract may be due to the fast separation of the active compounds by UMAE, which significantly decreased the damages to the extract. Moreover, the phenolic compounds in UMAE and ME extracts may contribute to the antimicrobial activity.

Effect of UMAE on phenolic content

The contents of total phenolics in UMAE and ME extracts ± 0.13 mg/g GAE, respectively. UMAE is more effective than ME in the extraction of phenolics. There was a high correlation between the antioxidant capacity and phenolic contents. UMAE extract exhibited the higher antioxidant capacities, and possessed the higher total phenolic content at the same time. The correlation between antioxidant capacities and phenolics was so evident that the capacities observed were probably due to the high phenolic content of the extracts.

Analysis and identification of phenolic compounds

The HPLC chromatograms recorded at 280 nm for the UMAE and ME extracts are shown in (Figure 4a and b), respectively. HPLC-DAD profiles for both extracts roughly contained the same type of phenolic compounds, even though differences in concentration of each individual compound were observed (Table 2). The compounds have been identified according to their retention time and the spectral characteristics of their peaks compared to those of standards, as well as by spiking the sample with standards. It is found that UMAE and ME extracts contain chlorogenic acid (Peak 1), o-hydrobenzoic acid (Peak 2), caffeic acid (Peak 3), rutin (Peak 6), p-coumaric acid (Peak 5), ferulic acid (Peak 4), with high area in rutin and chlorogenic acid in both two extracts.

Chlorogenic acid and rutin were detected as the major phenolic components in the UMAE extract, contributing about 16.22 and 15.35% to the total amount of phenolics,

Table 2. Chromatographic and spectral characteristics of the phenolic compounds detected in the UMAE and ME extracts.

Peak number	Compounds	Λmax (nm)	Amount [a b] (mg/g)	
			UMAE extract	ME extract
1	Chlorogenic acid	332	7.16±0.18^{B}	6.25±0.16^{A}
2	O-hydrobenzoic acid	254	4.72±0.16^{A}	4.81±0.17^{A}
3	Caffeic acid	324	1.47±0.15^{B}	1.08±0.13^{A}
4	Ferulic acid	328	1.82±0.13^{B}	1.11±0.14^{A}
5	P-coumaric acid	310	1.26±0.18^{A}	1.03±0.19 ^{A}
6	Rutin	359	6.78±0.29^{A}	5.82±0.28^{A}

[a] The amount was expressed as mg of compound per gram of each extract on dry weight basis, [b] Values are the means of three replicates ± standard deviation. Means in the same row with different letters are significantly different ($p < 0.05$).

from burdock leaves were 44.16 ± 0.35 and 39.85 and showing the levels of 7.16 and 6.78 mg/g dry weight (DW) in UMAE extract, respectively (Table 2). Similarly, the two compounds were the major phenolic components in the ME extract, and the content of them was a bit lower. O-hydrobenzoic acid was also predominant, but slightly higher in ME extract (4.81 mg/g DW) than in UMAE extract (4.72 mg/g DW). In addition, ferulic acid, p-coumaric acid and caffeic acid in UMAE extract (1.82, 1.26 and 1.47 mg/g DW) were higher than those in the extract of ME (1.11, 1.03 and 1.08 mg/g DW), respectively (Table 2). UMAE and ME extracts from burdock leaves possess similar composition. Chlorogenic acid, rutin, caffeic acid,were previously reported from burdock leaves (Liu et al., 2005). This study shows, for the first time, that ferulic acid and o-hydrobenzoic acid are in burdock leaves.

The results earlier stated showed that the antioxidant activity of chlorogenic acid and rutin was very high (Figure 2). The previous studies also reported that phenolic compounds such as ferulic acid, p-coumaric acid and caffeic acid exhibited significant antioxidant activities (Arimboor et al., 2008; Cheng et al., 2007; Medina et al., 2007; Ou et al., 2009). High antioxidant activities exerted by the extracts of burdock leaves were probably due to the phenolics, especially chlorogenic acid and rutin. As shown in (Table 1), chlorogenic acid and rutin exhibited strong antibacterial activity (MIC ranging from 0.04 to 0.08 mg/ml). The high antimicrobial activity of the extracts against *S. typhimurium, Shigella, E. coli, S. aureus, S. pneumoniae* and *B. subtilis* is probably due to the combined action of chlorogenic acid and rutin presented in the extracts (Antonio et al., 2010; Ayaz et al., 2008).

Chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, o-hydrobenzoic acid and rutin contained in the extract may have a synergistic interaction to inhibit the growth of bacteria.

Conclusion

In conclusion, the UMAE extract of burdock leaves exhibited much higher antioxidant activities and antibacterial capacity than ME extract. The activities of the extracts are probably due to the combined action of chlorogenic acid rutin and other phenolic compounds present in the extracts. The extract prepared by simultaneous ultrasonic/microwave assisted extraction (UMAE) requiring an extraction time of 30 s exhibited higher antioxidant, antibacterial activities and had higher total phenolic content than the ME extract, indicating significant improvement in extraction efficiency and remarkable shortening of processing time by UMAE, as well as the prospect of UMAE as a new and efficient technique for preparation of antioxidant and antimicrobial extracts. Moreover, UMAE provides a new sample preparation method for the evaluation of antioxidant and antibacterial activity of plants.

In addition, among the phenolic compounds identified, the occurrence of o-hydrobenzoic acid and ferulic acid is reported for the first time.

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