Short communication

Anti-biofilm Activities and Chemical Composition of Essential Oil from Burdock Leaf

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The antibacterial and anti-biofilm activities of essential oil (EO) from burdock leaf have been first investigated. The results indicated EO greatly potential for inhibition the growth of all the bacteria tested. The MIC values for bacteria strains were in the range of 250 – 1000 µg/mL. The EO significantly inhibited the formation of biofilm by *E. coli* and *S. aureus*. The lowest concentrations of EO to completely inhibit biofilm formation were 500 and 250 µg/mL. It could be inferred that the biofilm formation inhibition effect of burdock leaf EO was mainly due to its inhibition effect on the viability of bacteria. Then the composition of EO was analyzed by GC-MS. Sixty three compounds were identified. The EO was dominated by beta-Eudesmol (13.67%). The EO of burdock leaf based on its antibacterial efficiency and its significant inhibition on biofilm formation, could be highly useful in control of foodborne bacterial biofilms.

Keywords: chemical composition, antibacterial, anti-biofilm, essential oil, burdock leaf

Introduction

Biofilm is a stubborn source of pollution in food industry sectors including fresh produce, dairy processing, poultry processing and red meat processing (Chen *et al.*, 2007; Lebert *et al.*, 2007). When the contamination of food occurs, the source of the problem is often the biofilm (Bai and Rai, 2011). A great deal of evidence indicates that biofilm mode of life results in growing resistance to antibacterial agents (Honraet and Nelis, 2006; Quave *et al.*, 2008). Compared to planktonic cells, biofilms are more resistant to antibacterial products. Searching for new strategies for inhibition of biofilm are becoming highly necessary (Kiedrowski and Horswill, 2011; Shunmugaperumal, 2010). In recent years, the efficiency of plant components on inhibition of biofilm has attracted more and more attention (Messier and Grenier, 2011; Sandasi *et al.*, 2010; Xing *et al.*, 2012). Essential oils from plants have been known to possess antibacterial, antifungal and antioxidant activities. There is therefore a great interest for investigating the action of essential oils against food spoilage organisms and foodborne pathogens. The anti-biofilm activity of essential oils was commonly expected. However, the anti-biofilm efficiency of essential oils from

plants has been rarely reported.

Burdock (*Arctium lappa*) is a popular vegetable in China, Japan and many other Asian countries and its leaf has been used as traditional medicine. Many studies suggest burdock leaf exhibit high antibacterial activity (Lou *et al.*, 2011; Lou *et al.*, 2012). However, the effect of essential oil from burdock leaf on formation of biofilm and the chemical composition of essential oil have not been studied.

In the present work, several food-related bacteria have been chosen as objects, and the impacts of burdock leaf essential oil on the formation of biofilms and on the viability of bacteria have been studied. The active compounds contained in the essential oil of burdock leaf were also characterized.

Materials and Methods

Materials Burdock leaves, provided by Xuzhou Wangda Farm and Sideline Products Co., Ltd., were identified by prof. J. Tang.

Preparation of essential oil from burdock leaf Dried burdock leaf (100 g) was ground in a blender. The essential oils were obtained by hydrodistillation at 100°C with a Clevenger-type apparatus for 4 h. The essential oils obtained were separated from water and dried over anhydrous Na₂SO₄, and stored at 4°C.

Antibacterial activity The strains *Staphylococcus au-*

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reus 6538, *Streptococcus pneumoniae* ATCC25922, *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 the essential oils was tested by the pour plate method (Lou et al., 2010; Parvathy et al., 2009). Each experiment was done in triplicates. The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the essential oil capable to inhibit the growth of the bacteria tested.

Anti-biofilm activity The inhibitory activity of the essential oil for biofilm formation was assessed mainly according to the method of Figueiredo et al. (Figueiredo et al.; Lebert et al., 2007). *S. aureus* and *E. coli* were incubated overnight in glass bottles at 37°C. The bacterial suspension (1 mL) was inoculated with 9 mL of Luria-Bertani broth (LB broth) containing various concentrations of essential oil in test tubes. After incubation for 24 h at 37°C, the culture medium was discarded and the tubes were washed three times with 300 mL PBS to remove non-adherent bacteria. The sessile cells were sonicated and re-suspended in one milliliter of Saline (0.9%). Then, each suspension (100 µL) was coated on LB agar plates and incubated at 37°C. After 24 h, the colonies were counted and only plates containing between 30 and 300 counts for each dilutions series were counted. For all the assays, a positive control without burdock leaf essential oil and a negative control without inoculation were prepared. IC₅₀ was defined as the lowest agent concentration that showed 50% of inhibition on the formation of biofilm and the IC₅₀ value was calculated according to the experimental results. The procedure was performed in triplicates.

GC/MS analyses The components of the essential oil were identified by GC-MS analyses (Cao et al., 2009; Kumar et al., 2011). Gas chromatography-mass spectrometry (Varian 1200L) was incorporated with a relatively nonpolar capillary column (DB-5, 30 m length, 0.25 mm film thickness, 0.25 mm internal diameter). The injection port and the interface were held at 220 and 260°C, respectively. The temperature was programmed from 50°C to 220°C at 15°C per minute and a hold at 220°C for 25 min with helium as the carrier gas. Mass spectra: electronic impact, ionisation potential 70 eV, ion source temperature 200°C and mass range 35 – 500 Da. Most of the compounds were identified according to Kováts Indexes in reference to n-alkanes (NIST, 2010) and mass spectra (authentic chemicals and Wiley spectral library collection).

Results

Antibacterial activity of essential oil from burdock leaves The essential oil of burdock leaf was tested for the

antimicrobial potential against Gram-positive (*Streptococcus pneumoniae* ATCC25922, *Staphylococcus aureus* 6538) and Gram-negative bacteria (*Shigella dysenteriae* 51302, *Escherichia coli* ATCC25922, *Salmonella typhimurium* 50013). The MIC values were shown in Table 1. Results (Table 1) showed that the essential oil has great potential of antibacterial activity against all of the bacteria tested. The MIC values for bacterial strains were in the range of 250 – 1000 µg/mL.

A comparison of the sensitivities of the bacterial strains to essential oil indicated that the strongest inhibitory effect was against *Staphylococcus aureus*. The essential oil showed MIC value of 250 µg/mL in the case of *Staphylococcus aureus*. The highest MIC value was found for *Salmonella typhimurium*, which was 1000 µg/mL.

Effect of burdock leaf essential oil on the formation of biofilm The inhibitory activities of the essential oil of burdock leaf on the formation of biofilm by *E. coli* are shown in Figure 1. It could be seen that the essential oil significantly inhibited the formation of biofilm by *E. coli*. At a concentration of 125 µg/mL, the inhibition rate of the formation of biofilm was 80.3%. While at a concentration of 250 µg/mL, the inhibitory activity of the essential oil was greatly enhanced (93.6% inhibition). When the concentration of essential oil was 500 µg/mL, 100% inhibition of biofilm formation was achieved. The IC₅₀ value of essential oil that could inhibit 50% of biofilm formation was 54.05 µg/mL.

Figure 2 shows the results of biofilm formation of *S. aureus* inhibited with different concentrations of burdock leaf essential oil. A concentration of 62.5 µg/mL essential oil exhibited an inhibition of 79.5% while a concentration of 125 µg/mL showed an inhibition of 87.0%. This shows a tendency in the significance of reduction in biofilm formation of *S. aureus*. At a concentration of 500 µg/mL, the formation of biofilm by *S. aureus* was completely inhibited (100%). It was calculated that the IC₅₀ of essential oil against the formation and growth of bacterial biofilm was found to be 32.03 µg/mL.

Chemical composition of the essential oil from burdock leaf The results above suggested that the essential oil of burdock leaf exhibited high antibacterial activity. So the compositions of essential oil from the leaf were analyzed by GC-MS. Sixty two compounds of the leaf oil were identified.

Table 1. Minimal Inhibitory Concentration (MIC) values of burdock leaf essential oil against foodborne pathogens.

Organisms	MIC (µg/mL)
<i>Streptococcus pneumoniae</i>	500
<i>Staphylococcus aureus</i>	250
<i>Shigella dysenteriae</i>	500
<i>Escherichia coli</i>	500
<i>Salmonella typhimurium</i>	1000

Fig. 1. Inhibition of *E. coli* biofilm formation by essential oil from burdock leaf. Error bars mean standard deviation of the mean.

Fig. 2. Inhibition of the formation of *S. aureus* biofilm by burdock leaf essential oil. Error bars mean standard deviation of the mean.

Table 2. The main components and their relative contents (%) of burdock leaf essential oil.

Peak	RT (min)	Area (%)	Compounds
1	10.00	1.77	5-Methyl-2-hexanone
2	10.14	0.27	3-Methyl-5-hexanone
3	10.74	1.02	3-Methyl-3,4-divinylcyclohexene
4	11.18	0.35	6-Methyl-2-heptanone
5	11.86	0.62	p-Ethyltoluene
6	12.10	1.06	2-octanone
7	12.63	0.35	2,2,6-trimethylcyclohexan-1-one
8	12.91	1.59	6-Octen-2-one, (6Z)-
9	13.04	2.02	6-Methyl-5-hepten-2-one
10	13.90	1.23	cis-3-Hexen-1-ol
11	14.10	0.43	1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-(1-methylethylidene)-
12	14.21	0.70	trans-2-Hexen-1-ol
13	14.81	1.63	1-octen-3-ol
14	15.00	0.52	5-hepten-2-ol, 6-methyl
15	15.31	0.35	3,5,5-Trimethyl-2-hexene
16	15.58	0.28	2-undecyne
17	15.88	0.58	Bornylene
18	16.12	1.61	Linalool
19	16.48	0.31	p-pentadecanol
20	16.75	0.40	3,5-Heptadien-2-one,6-methyl-, (3E)-
21	17.02	0.93	trans-2-Octen-1-ol
22	17.14	0.28	β-Cyclocitral
23	17.34	0.29	Tricyclol3.3.0.0 (2.8)octan-3-one.8-methyl-
24	17.45	0.44	1,3-Cyclohexadiene-1-carboxaldehyde,2,6,6-trimethyl-
25	17.50	1.29	acetophenone
26	17.55	0.87	Acetic acid, 2-chloro-,nonyl ester
27	17.85	0.37	2.7-octadien-1-ol
28	18.14	2.37	borneol
29	18.38	1.06	Propiophenone
30	18.52	0.45	3-Buten-1-ol, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E)-
31	18.75	0.73	4,8-Dimethyl-1-nonanol
32	19.42	0.31	3,4-Dimethylbenzaldehyde
33	19.49	1.01	β-Damascenone
34	19.61	1.01	7,8-Dihydro-b-ionone
35	19.93	0.48	Aromadendrene,dehydro-
36	20.09	0.59	Benzyl alcohol
37	20.49	1.18	Phenylethyl alcohol
38	20.78	0.95	β-Ionone
39	21.01	0.71	4-(2,6,6-trimethyl-cyclohex-a-enyl)-butan-2-ol
40	21.36	0.51	3(2H)-Furanone, 2-hexyl-5-methyl-

Peak	RT (min)	Area (%)	Compounds
41	22.00	0.46	Caryophyllenyl alcohol
42	22.17	0.27	Cyclohexanemethanol,4-ethenyl-a,a,4-trimethyl-3-(1-methylethenyl)-, (1R,3S,4S)-
43	22.30	0.72	7-Methoxy-2,2,4,8-tetramethyltricyclo[5.3.1.0(4,11)]undecane
44	22.54	0.32	2-Pentadecanone,6,10,14-trimethyl-
45	22.67	0.77	cedrol
46	22.92	1.85	1,8(2H,5H)-Naphthalenedione, hexahydro-8a-methyl-, cis-
47	23.08	1.09	2-Naphthalenemethanol,1,2,3,4,4a,5,6,7-octahydro-a,a,4a,8-tetramethyl-, (2R,4aR)-
48	23.18	3.21	agarospirol
49	23.24	1.12	Cyclohexanecarboxylicacid, ethenyl ester
50	23.27	1.06	hinesol
51	23.41	0.42	megastigmatrienone
52	23.59	0.53	α -eudesmol
53	23.70	13.67	β -Eudesmol
54	23.82	0.49	isoaromadendrene epoxide
55	24.05	1.28	2,6-Heptadien-1-ol,2-methyl-6-[(1S)-4-methyl-3-cyclohexen-1-yl]-, (2E)-
56	24.26	0.81	2,4-Di-tert-butylphenol
57	24.40	0.50	Cycloisolongifolene,8,9-dehydro
58	25.63	0.73	Longipinocarveol, trans-
59	26.08	0.33	2,6-Heptadien-1-ol,2-methyl-6-[(1S)-4-methyl-3-cyclohexen-1-yl]-, (2E)-
60	26.56	1.07	Diisobutylphthalate
61	27.20	0.49	g-Gurjunenepoxide-(2)
62	27.46	0.28	Ethanone,1-(2-hydroxy-4,6-dimethoxyphenyl)-

Quantitative and qualitative analytical results are shown in Table 2.

It is found that 62 components have been identified from the burdock leaf essential oil, while the remaining part of the oil has not been identified. The major compounds of the essential oil were β -eudesmol (13.67%), agarospirol (3.21%), borneol (2.37%), 6-Methyl-5-hepten-2-one (2.02%), 1,8(2H,5H)-Naphthalenedione, hexahydro-8a-methyl-, cis- (1.85%) and 5-Methyl-2-hexanone (1.77%).

Discussion

Plant essential oils have been used for thousands of years all over the world (Ozkan *et al.*, 2010; Valeriano *et al.*, 2012). A great many of reports have suggested that some of the essential oils are useful in food preservation (Bendimerad *et al.*, 2005; Mimica-Dukic *et al.*, 2004) and also exhibit pharmacological properties such as antibacterial (Lo Cantore *et al.*, 2009), spasmolytic (Olivier *et al.*, 2009), antioxidant (Güllüce *et al.*, 2003) and anticancer activities (Ashour, 2008). The essential oils can be useful in food industry if they could successfully substitute the synthetic antibacterial agents which are responsible for potential toxicity.

In this study the antibacterial properties of burdock essential oil were evaluated against food related strains. A comparison of the sensitivities of the bacterial strains to essential oil indicated that the strongest inhibitory effect was against *Staphylococcus aureus*. The essential oil showed MIC value of 250 μ g/mL in the case of *Staphylococcus aureus*. The

highest MIC value was found for *Salmonella typhimurium*, which was 1000 μ g/mL. In the previous reports, it has been noticed that, essential oil were found more active against Gram positive bacteria when compared to Gram negative (Kalembe and Kunicka, 2003), which partially support our findings.

Essential oils have already been known to exhibit antimicrobial properties (Bajpai *et al.*, 2009; Filippi *et al.*, 2006). However, the effect of essential oils on the formation of biofilms has been less investigated. To the best of our knowledge, the effect of burdock leaf essential oils on the formation of bacterial biofilm has not been reported before. As shown in the study, the essential oil from burdock leaf significantly inhibited the formation of biofilm both by *E. coli* and *S. aureus*. Previous studies have indicated that bacteria in the biofilm mode of growth are much more resistant to antibacterial agents than their planktonic counterparts (Harmsen *et al.*, 2010; Lewis, 2001). In this work, the inhibition effect of burdock leaf essential oils against the growth of bacteria was also been evaluated. The lowest concentrations of burdock leaf essential oil, which did not show any growth of *E. coli* and *S. aureus*, are found to be 500 and 250 μ g/mL. The results showed that the lowest biofilm inhibition concentrations of burdock leaf essential oil against *E. coli* and *S. aureus* were also 500 and 250 μ g/mL. Therefore, the lowest concentrations of burdock leaf essential oil to completely inhibit biofilm formation were same as the minimum concentration to inhibit the growth of bacteria. So it could be

inferred that the biofilm formation inhibition effect of burdock leaf essential oil were mainly due to its inhibition effect on the viability of bacteria. In the anti-biofilm activity tests, no viable cell was observed in the discarded cultured media, and this was mainly due to the inhibition activity of essential oil against the growth of bacteria. When the concentration of essential oil was 500 and 250 µg/mL, the growth of *E. coli* and *S. aureus* was completely inhibited, thus no viable cell was observed and the formation of biofilm was completely inhibited.

Essential oils, well known to possess both *in vitro* and *in vivo* antibacterial activities, are fairly complex mixtures. In general, they exhibit selective toxicity to a variety of pathogenic microorganisms and they are relatively safe to animals and humans. In complex mixtures, synergism of individual components in essential oil is also expected, therefore microbial pathogens could hardly develop resistance towards them.

Previous studies reported that compounds such as β -eudesmol (Costa *et al.*, 2008; Kumar *et al.*, 2011), borneol (Ngan *et al.*, 2012; Al-Farhan *et al.*, 2010; Elaissi *et al.*, 2011), 1-octen-3-ol (Kim *et al.*, 2004; Yu *et al.*, 2004), linalool (Ngan *et al.*, 2012; Alviano *et al.*, 2005; Singh *et al.*, 2008), benzyl alcohol (Lucchini *et al.*, 1990), phenylethyl alcohol (Corre *et al.*, 1990), α -eudesmol (Costa *et al.*, 2008; Wang *et al.*, 2005) exhibited significant antibacterial activities. The high antimicrobial activity of the essential oil of burdock leaf against streptococcus pneumoniae, *S. aureus*, *S. dysenteriae*, *E. coli* and *S. typhimurium* was probably due to the combined action of β -eudesmo, borneol, 1-octen-3-ol, linalool, benzyl alcohol, phenylethyl alcohol and α -eudesmol present in burdock leaf essential oil.

The essential oil of burdock leaf was found to have the wide spectrum of antimicrobial activity, being effective against both Gram-positive and Gram-negative bacteria. Moreover, the essential oils could significantly inhibit the formation of biofilm by *S. aureus* and *E. coli*. The high antimicrobial and anti-biofilm efficacy of essential oil is the consequence of interaction between minor and major components and conditioned by the activity of their components. The effective dose *in vitro*, however, needs to be enhanced when applied to food systems to achieve the same effect (Burt, 2004; Ozturk and Ercisli, 2007).

Conclusion

This study demonstrated that burdock leaf essential oil possess remarkable antibacterial activities against all the foodborne bacteria tested with MIC values ranging from 250 – 1000 µg/mL. Moreover, the essential oil of burdock leaf significantly inhibited the formation of biofilm, and the IC₅₀ was found to be 54.05 µg/mL and 32.03 µg/mL for *S. aureus*

and *E. coli*, respectively. At a concentration of 250 and 500 µg/mL, the formation of biofilms by *S. aureus* and *E. coli* was completely inhibited. The biofilm formation inhibition effect of burdock leaf essential oil were chiefly due to its inhibition effect on bacterial viability. The high antimicrobial and anti-biofilm efficacy of essential oil is probably due to the combined action of β -eudesmol, borneol, 1-octen-3-ol, linalool, benzyl alcohol, phenylethyl alcohol and α -eudesmol present in the essential oil. Combining such results we could propose the future use of burdock leaf essential oil, to reduce food related bacteria growth and control the biofilm of the foodborne pathogens in food. Further research is needed in order to determine if the essential oil from burdock leaf could efficiently substitute the synthetic antibacterial agents or, perhaps be used in combination with other agents.

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