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Essential oils composition and bioactivities of two species leaves used as packaging materials in Xishuangbanna, China

Ren Li [a, b, *], Hua-Bin Hu [a, *], Xiu-Fen Li [a, b], Ping Zhang [a], You-Kai Xu [a], Jing-Jing Yang [a, b], Yuan-Fei Wang [c]

[a] Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] Yunnan Traditional Chinese Medical College, Kunming 650500, PR China

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ABSTRACT

This study reports the essential oils chemical composition and antioxidant and antimicrobial potentials of the leaves of *Phrynium pubinerve* Blume and *Thysanolaena latifolia* (Roxb. ex Hornem.) Honda, which are used as natural packaging materials by ethnic groups in Xishuangbanna, southwest China. GC–MS analysis identified 46 and 21 components, representing 88.6% and 93.4% of the essential oils of *P. pubinerve* and *T. latifolia*, respectively. The major constituents for *P. pubinerve* were (Z)-3-hexen-1-ol (17.31%), (E)-2-hexenal (9.01%) and 1-hexanol (8.61%). The major constituents for *T. latifolia* were (Z)-3-hexen-1-ol (28.79%), phytol (12.30) and (E)-β-ionone (9.54%). Both the essential oils and ethanol extracts showed antioxidant activity in DPPH test (IC_{50} values = 192.47–706.07 μg/ml), ABTS assay (IC_{50} values = 35.54–134.97 μg/ml) and FRAP assays. The essential oils showed considerable antimicrobial activity against pathogenic bacteria and spoilage organisms, with MIC and MBC values in the ranges of 64–3072 μg/ml and 64–4096 μg/ml, respectively. The bioactivities of these two plant species validate the traditional use of these two plants, suggesting that both could be new sources of natural antioxidant and antimicrobial agents for the packaging, medical and functional food industries.

1. Introduction

The rapid increase in the production and consumption of plastic packaging has caused serious environmental pollution due to their complex composition and resistance to degradation. Although significant progress has been made in the production of biodegradable materials from renewable natural resources, such as crops (Davis & Song, 2006), it is still a challenge to find biodegradable packages with wide acceptability (Ren, 2003). Essential oils with antioxidant and antimicrobial activities (Alves-Silva et al., 2013; Nedorostova, Kloucek, Kokoska, Stolcova, & Pulkrabek, 2009; Singh, Maurya, de Lampasona, & Catalan, 2006; Ye, Dai, & Hu, 2013) have been used as natural preservative ingredients in packaging applications (Kurek, Moundanga, Favier, Galic, & Debeaufort, 2013; Manso, Cacho-Nerin, Becerril, & Nerin, 2013; Martinez-Abad, Sanchez, Fuster, Lagaron, & Ocio, 2013). Bacteria and oxidation are two major causes of food spoilage, and packaging can be used to reduce these damages. Therefore, the searching for new functional packaging, especially biodegradable packaging with preservative properties, continues.

In Xishuangbanna Dai Autonomous Prefecture, Yunnan province, China, the leaves of *P. pubinerve* Blume (Marantaceae) and *T. latifolia* (Roxb. ex Hornem.) Honda (Poaceae) have been used as natural food packaging materials by ethnic minorities, and the leaves of both species have been used for packaging glutinous rice to make Zongzi (traditional Chinese rice-pudding) in south China (FCEC, 1981, 2002; KIB, CAS, 2003). *P. pubinerve* leaves are also used as a substitute for plastic packages to pack vegetables and meats in the market or the wild, and they are especially applied to pack a traditional dish named Baoshao, which is made by packing fresh minced meats with various spices, and then steamed or roasted before eating (KIB, CAS, 1995). In the high humidity and temperature of southern China, food deteriorates rapidly. So, indigenous

* Corresponding authors. Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, PR China. Tel.: +86 691 8715415; fax: +86 691 8715070.
E-mail addresses: liren@xtbg.ac.cn (R. Li), huhb@xtbg.ac.cn (H.-B. Hu).

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people have accumulated a lot of traditional knowledge on the utilization of local plants to preserve food. The use of *P. pubinerve* leaves as packaging materials and their apparent antiseptic function was reported in the earliest flora of China in 304 AD (KIB, CAS, 1991). The leaves of *P. pubinerve* and *T. latifolia* have also been used as traditional remedies by Dai and Hani people in Xishuangbanna for the relief of coughs, the abatement of fever, detoxification and stomach disorders (A, Wang, & Li, 1999; SATCM, 2005; Pan, Liu, & Xu, 2006).

These two plants have also been widely utilized in some tropical and subtropical Asian countries. For instances, *T. latifolia* inflorescences are used for making brooms and the leaves are valuable fodder in India (Bhardwaj & Gakhar, 2008; Shankar, Lama, & Bawa, 2003). *P. pubinerve* roots are cooked as a vegetable in Meghalaya, Northeast India (Kayang, 2007), and the leaves are also used as packaging materials for vegetables and meat, and as waterproof materials to make rain hats, umbrellas, and to roof huts in India (Bhardwaj & Gakhar, 2008; Shankar et al., 2003; Tag & Das, 2004; Tynsong & Tiwari, 2011). Despite their wide and long history usage, there has been no report on their chemical composition or on the related bioactivities of their essential oils and crude extracts.

Therefore, this study was aimed to justify their traditional knowledge by investigating the chemical composition, and the antioxidant and antimicrobial activities, of the essential oils from the leaves of *P. pubinerve* and *T. latifolia*. If the results support traditional knowledge, this would encourage the development of these plants as biodegradable packaging materials and as natural antioxidant and antimicrobial agents in packaging, medical and functional food industry.

2. Material and methods

2.1. Chemicals and reagents

The following reagents were used in the analyses (supplier companies are given in parenthesis): 2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH, 96%) and 2, 2-Azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS, 98%) (Aladdin Industrial Corporation, Shanghai, China); 2, 4, 6-tripiridyl-s-triazine (TPTZ, 99%) and Dimethylsulphoxide (DMSO) (Sigma–Aldrich, St. Louis, USA); Gallic acid (98%) (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China); 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox, 98%) (Tokyo Chemical Industry Co. Ltd, Tokyo, Japan); Standard Mueller-Hinton agar and broth (MHA and MHB) and Sabouraud agar and broth (SA and SB) (Tianhe Microbial Agents Company, Hangzhou, China). All reagents were analytical standards.

2.2. Plant materials and essential oil extraction

The leaves of *P. pubinerve* and *T. latifolia* were collected from Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences (Menglun Township, Mengla County, Yunnan province, China) in September 2013. Voucher specimens (110216 for *P. pubinerve* and 075482 for *T. latifolia*were) were deposited in the XTBG herbarium (HITBC). The air-dried leaves (300 g) of *P. pubinerve* and *T. latifolia*were were respectively grounded by a laboratory mill and subjected to steam distillation extraction using 2500 ml de-ionized water for 4 h. Diethyl ether (30 ml) was used as the solvent to extract the essential oil simultaneously while the leave samples were steam distilled. The essential oil and solvent were collected together, and then the diethyl ether was removed using a rotary evaporator to give the essential oil (R. Li, Wang, Sun, & Hu, 2014). Ethanol extracts (90%) from powdered leaves (10 g) of *P. pubinerve* and *T. latifolia* were collected separately by following the procedure outlined by Shi, Xu, Hu, Na, and Wang (2011). Both the essential oils and ethanol extracts were stored at −20 °C in the dark for further use.

2.3. Gas chromatography/mass spectrometry (GC–MS) analysis

The analyses were performed using an Agilent Technologies 7890A GC, equipped with an HP-5 MS capillary column (30 m × 0.25 mm; film thickness, 0.25 μm) and a mass spectrometer 5975C (Agilent Technologies, USA) as detector. MS were taken at 70 eV with a mass range of *m/z* 45–500. Helium was used as the carrier gas, at a flow rate of 1 ml/min. Injector and detector (MS transfer line) temperatures were both 250 °C. Column temperatures was gradually increased from 40 °C to 160 °C at 3 °C/min, and increased to 250 °C at 10 °C/min, then held for 10 min finally. A diluted sample of 0.2 μl was injected automatically.

2.4. Identification of the components

The components were identified by comparing calculated experimental GC retention indices, which were determined with reference to homologous series of n-alkanes C_7–C_{30} under identical experimental conditions, with the GC retention indices reported in the National Institute of Standards and Technology Standard Reference Database (NIST, 2011), by matching their mass spectra with those recorded in the NIST 08 database (NIST, Gaithersburg, MD, USA) and mass spectra with published data. The percentage composition of individual components was computed by the normalization method from the GC peak areas, assuming an identical mass response factor for all compounds.

2.5. Antioxidant activity

2.5.1. Free radical scavenging ability by DPPH assay

The DPPH radical scavenging activity was determined using the method as previously reported (Brandwilliams, Cuvelier, & Berset, 1995). Each sample (1 ml) with various concentrations (62.5–500 μg/ml) was added to 2 ml of freshly prepared DPPH solution (0.06 mM). The mixture was shaken vigorously and allowed to incubate at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 517 nm. The results were expressed as mean percentage inhibition from three replicates. Gallic acid and Trolox were served as positive controls. IC_{50} value was calculated as the concentrations of the samples that could inhibit 50% of the DPPH radicals in parallel experiments.

2.5.2. Free radical scavenging ability by ABTS assay

The free radical scavenging activity was determined by the ABTS assay (Re et al., 1999). Seven mM $ABTS^{\bullet+}$ solutions were prepared and reacted with 2.45 mM aqueous ammonium persulfate solution. The mixture was then kept in shade at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of about 0.7 (±0.02) at 732 nm. A reagent blank reading was taken. After addition of 2.0 ml of diluted $ABTS^{\bullet+}$ solution to 0.5 ml of the dissolved samples (10–80 μg/ml), the absorbance reading was taken exactly 6 min after initially mixing in the dark. The results were expressed as mean percentage inhibition from three replicates. Gallic acid and Trolox were served as positive controls. The concentrations of the samples that could inhibit 50% of the ABTS radicals (IC_{50}) were also calculated in parallel experiments.

2.5.3. Ferric reducing antioxidant power (FRAP) assay

The reducing power potential was determined using the FRAP assay (Benzie & Strain, 1996). In brief, 2 ml of the FRAP reagent,

prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (v/v/v), was added to 25 μl of the dissolved sample solutions (1–10 mg/ml) and reacted thoroughly at 37 °C for 10 min. The absorbance was taken at 593 nm. The results were calculated from the standard curve of Gallic acid and Trolox, expressed as Gallic acid equivalent antioxidant capacity (GAEAC) in μg Gallic acid/ml and Trolox equivalent antioxidant capacity (TEAC) in mM Trolox/l, respectively.

2.6. Antimicrobial activity

2.6.1. Microbial strains and culture media

The samples were individually tested against seven microbial strains. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC Y0109) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, China); *Aspergillus fumigatus*, *Acinetobacter baumannii*, and *Klebsiella pneumonia* were provided by Kunming General Hospital. Standard Mueller-Hinton agar and broth (MHA and MHB), and Sabouraud agar and broth (SA and SB) were used as the bacterial and fungal culture media.

2.6.2. Antimicrobial screening

The samples were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20 mg/ml, and sterilized by filtration through 0.45 μm Millipore filters. The agar well diffusion method (Dickson, Houghton, Hylands, & Gibbons, 2006) was used to screen the inhibitory zone of the samples. Under aseptic conditions, 20 μl of suspensions containing 1.5×10^9 colony forming units (CFU)/ml of bacteria and 1.0×10^6 CFU/ml of fungi were seeded on MHA or SA, respectively. Wells (6 mm diameter) were cut from the agar and 50 μl of sample solution was delivered into them. After incubation for 24 h at 37 °C, the diameters of the inhibition zones, measured in millimeters, were examined. Negative controls, whose values were deducted accordingly, were prepared with the same solvents used to dissolve the plant extracts.

2.6.3. Minimal inhibitory and bactericidal concentration (MIC and MBC) assay

MIC and MBC were determined against tested microbial strains (CLSI, 2006; NCCLS, 1999). The MICs and MBCs were both determined with starting inoculums of 1.0×10^6 CFU/ml for bacteria and 1.0×10^4 CFU/ml for fungi, incubated at 37 °C for 24 h, and examined for growth in daylight. The serial dilution method was used for the determination of the MICs of the samples. For the MIC assay, samples were dissolved in dimethylsulfoxide (DMSO) and sterilized by filtration through 0.45 μm Millipore filters. 100 μl of an appropriate culture medium, sample solutions and inoculums were dispensed onto a 96-well plate. MIC values were the lowest concentration of a given sample that completely inhibited the visible microbial growth. For the MBC assay, 10 μl samples taken from the clear wells of the microbroth susceptibility studies were placed onto the surfaces of MHA or SA plates. The MBC was defined as the concentration of drug that resulted in >99.9% mortality of the bacterium relative to the concentration of bacterium that was present in test wells at 0 h (NCCLS, 1999). The MICs and MBCs of Amikacin, Fluconazole, Vancomycin, Tigecycline, Ciprofloxacin and Cefotaxime were also determined in parallel experiments as positive controls. The culture medium and solvent (DMSO) was employed as negative controls and their values were deducted accordingly.

2.7. Statistical analysis

All experiments were performed in triplicate and expressed as mean values ± standard deviations (SD). The 50% inhibition concentration (IC_{50}) was calculated by Probit regression analysis of SPSS 17.0 for Windows (SPSS Inc. Chicago, USA). The one-way

Table 1
Chemical composition of the essential oils of *Phrynium pubinerve* and *Thysanolaena latifolia* (EOPP and EOTL).

No	RI_{cal}	RI_{lit}	Compound	EOPP (%)	EOTL (%)
1	798	800	Hexanal	2.69	1.70
2	831	830	Furfural	1.55	–
3	850	850	(E)-2-Hexenal	9.01	–
4	855	855	(Z)-3-Hexen-1-ol	17.31	28.79
5	867	862	(E)-2-Hexen-1-ol	–	1.29
6	869	865	1-Hexanol	8.61	2.60
7	893	889	2-Heptanone	0.54	–
8	904	901	2-Heptanol	1.14	–
9	937	942	3-Hepten-2-one	0.10	–
10	954	952	4-Ethyloctane	0.42	–
11	959	966	Benzaldehyde	–	1.38
12	991	993	2-Pentylfuran	0.63	–
13	1023	1021	o-Cymene	0.40	–
14	1033	1035	2,6,6-Trimethylcyclohexanone	0.25	–
15	1043	1049	Benzeneacetaldehyde	–	2.77
16	1057	1060	γ-Terpinene	0.59	–
17	1089	1091	p-Cymenene	0.43	–
18	1091	1089	o-Methoxyphenol	0.24	–
19	1096	1001	Pinane	1.90	–
20	1100	1103	Linalool	1.99	–
21	1105	1105	Nonanal	–	2.56
22	1106	1110	2,6-Dimethylcyclohexanol	0.95	2.67
23	1119	1119	Dehydro sabinene ketone	2.39	–
24	1132	1130	5,6-Dimethyldecane	0.30	–
25	1140	1141	L-(E)-Pinocarveol	1.11	–
26	1149	1152	1,4-Dimethyl-4-acetylcyclohexene	1.80	–
27	1176	1179	Terpinen-4-ol	4.56	–
28	1185	1183	α-4-Trimethyl-benzenemethanol	2.08	–
29	1190	1187	ι-α-Terpineol	1.09	–
30	1195	1193	Myrtenol	0.52	–
31	1198	1201	Safranal	0.70	1.57
32	1219	1217	β-Cyclocitral	0.81	4.19
33	1236	1237	2,3-Dihydrobenzofuran	0.40	–
34	1255	1251	β-Cyclo-homocitral	0.43	–
35	1315	1320	2-Methoxy-4-vinylphenol	3.87	4.60
36	1350	1355	Dehydro-ar-ionene	0.37	–
37	1358	1359	Eugenol	–	4.34
38	1382	1386	β-Damascenone	1.63	0.72
39	1398	1400	Tetradecane	0.27	–
40	1412	1414	E-β-Damascone	–	1.03
41	1426	1428	α-Ionone	4.01	–
42	1450	1451	Dihydropseudoionone	0.68	6.47
43	1485	1489	(E)-β-Ionone	4.67	9.54
44	1509	1509	β-Bisabolene	–	1.11
45	1529	1525	Dihydroactinidiolide	0.83	–
46	1568	1569	E-Nerolidol	0.17	–
47	1681	1680	8-Heptadecene	0.47	–
48	1787	1780	Phenanthrene	0.32	–
49	1848	1847	Hexahydrofarnesyl acetone	0.97	1.69
50	1873	1873	Diisobutyl phthalate	1.31	1.35
51	1924	1927	Farnesyl acetone	0.89	0.77
52	2090	2095	Methyl linolenate	0.20	–
53	2123	2125	Phytol	2.97	12.30
Total				88.57	93.44
Aliphatic alcohols				60.50	60.94
Aromatics				6.89	14.44
Monoterpene hydrocarbons				4.92	–
Oxygenated monoterpenes				13.10	5.77
Sesquiterpene hydrocarbons				0.17	–
Diterpenes				2.97	12.30

A dash (–) indicate the absence of component in the oil.
RI_{cal} refers to the retention index experimentally calculated using C_7–C_{30} alkanes.
RI_{lit} refers to the retention index taken from NIST database.

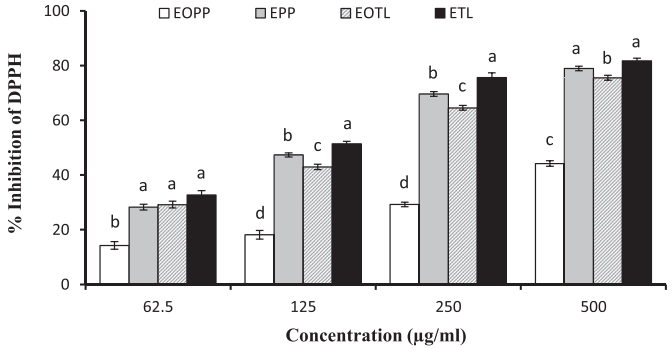

Fig. 1. DPPH• radical scavenging activity of the essential oils and ethanol extracts of *Phrynium pubinerve* and *Thysanolaena latifolia* (EOPP, EPP, EOTL and ETL) at concentration from 62.5 to 500 μg/ml. Values are means of triplicates ± SD. The bars with different letters are significantly different ($P < 0.05$).

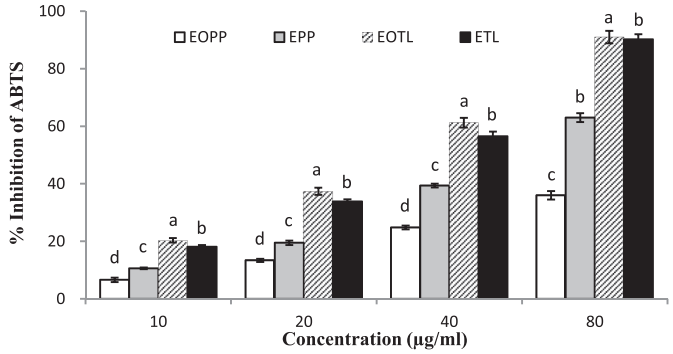

Fig. 2. ABTS•+ radical scavenging activity of the essential oils and ethanol extracts of *Phrynium pubinerve* and *Thysanolaena latifolia* (EOPP, EPP, EOTL and ETL) at concentration from 10 to 80 μg/ml. Values are means of triplicates ± SD. The bars with different letters are significantly different ($P < 0.05$).

ANOVA analysis with a Dunnett's multiple comparison test was performed using SPSS 17.0. Differences were accepted as significant at $P < 0.05$.

3. Results and discussion

3.1. Chemical composition of the essential oils

The essential oils were collected by simultaneous distillation extraction with a yield of 0.025% (w/w) and 0.030% (w/w) on a dry weight basis for *Phrynium pubinerve* and *Thysanolaena latifolia*, respectively. In total, 46 and 21 components, representing 88.57–93.44% of the essential oils of the two species were identified by GC–MS (Table 1). The essential oil samples are found rich in aliphatic alcohols, aromatics, oxygenated monoterpenes and diterpenes. The major constituents for *P. pubinerve* were (Z)-3-hexen-1-ol (17.31%), (E)-2-hexenal (9.01%) and 1-hexanol (8.61%), and for *T. latifolia*were (Z)-3-hexen-1-ol (28.79%), phytol (12.30) and (E)-β-ionone (9.54%) (Table 1). As far as we know, little study has reported the essential oil compositions of genus *Phrynium* and *Thysanolaena* plants, this study provided reference information for the chemical taxonomy and potential application of these two genus species.

3.2. Antioxidant activity

Oxidation reaction is a well-known cause of food spoilage, and in vitro antioxidant test are designed to estimate the antioxidant potentials of various chemical and biological samples. In this study, all essential oil and ethanol extract samples showed antioxidant activity in the DPPH, ABTS and FRAP assays. The presence of Phytol, 2-Methoxy-4-vinylphenol and Eugenol, which have been reported with high antioxidant capacity (Fukai et al., 2009; Gulcin, 2011; Pramod, Ansari, & Ali, 2010; Wang et al., 2013), could explain the antioxidant activity of the essential oils. Moreover, the essential oil from *T. latifolia*, with a higher content of both these compounds, exhibited stronger antioxidant potentials than that from *P. pubinerve*.

Table 2
The IC_{50} values of the essential oils and ethanol extracts of *Phrynium pubinerve* and *Thysanolaena latifolia* (EOPP, EPP, EOTL and ETL) by DPPH and ABTS assays.[a]

Samples (μg/ml)	DPPH assay	ABTS assay
EOPP	706.07 ± 4.29	134.97 ± 3.09
EPP	220.65 ± 4.71	60.57 ± 1.25
EOTL	243.17 ± 3.67	35.54 ± 0.86
ETL	192.47 ± 5.50	38.02 ± 1.24
Gallic acid	4.04 ± 0.05	1.89 ± 0.01
Trolox	4.62 ± 0.06	15.19 ± 0.98

[a] The IC_{50} values were calculated by the SPSS 14.0 software using probit regression analysis. All values were performed in triplicate and expressed as means ± SD.

3.2.1. DPPH radical scavenging activity

The scavenging ability of the essential oil and ethanol extract samples on DPPH free radical was showed in Fig. 1. The tested samples showed a dose dependent scavenging power. Especially, the inhibition percentage of the ethanol extract of *Thysanolaena latifolia* increased from 32.70 % to 81.75 % as its concentration rising from 62.5 to 500 μg/ml, indicating that it has generally better scavenging ability. Lower IC_{50} value means better antioxidant activity. As can be seen in Table 2, the ethanol extracts showed relatively stronger DPPH radical scavenging activity than the essential oils with lower IC_{50} values. The IC_{50} values of all samples were higher than Gallic acid and Trolox, indicating that they have lower DPPH radical scavenging activity than these compounds.

3.2.2. ABTS radical scavenging activity

As shown in Fig. 2, the essential oil and ethanol extract samples showed clear ABTS free radical scavenging ability. The ABTS radical inhibition percentages increased from 6.59 to 90.20 % as sample

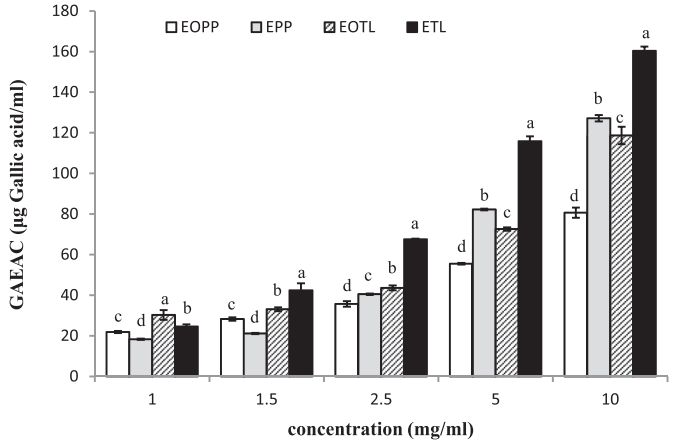

Fig. 3. Gallic acid equivalent of antioxidant capacity (GAEAC) of the essential oils and ethanol extracts of *Phrynium pubinerve* and *Thysanolaena latifolia* (EOPP, EPP, EOTL and ETL) at concentration from 1 to 10 mg/ml by FRAP assay. Values are means of triplicates ± SD. The bars with different letters are significantly different ($P < 0.05$).

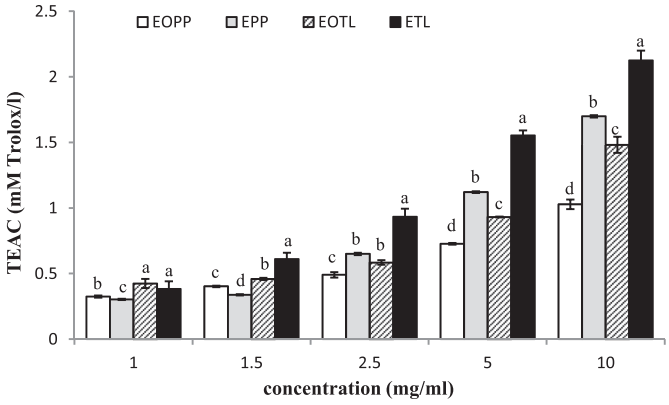

Fig. 4. Trolox equivalent of antioxidant capacity (TEAC) of the essential oils and ethanol extracts of *Phrynium pubinerve* and *Thysanolaena latifolia* (EOPP, EPP, EOTL and ETL) at concentration from 1 to 10 mg/ml by FRAP assay. Values are means of triplicates ± SD. The bars with different letters are significantly different ($P < 0.05$).

Table 3
Inhibition zone diameter (mm) of the essential oils and ethanol extracts of *Phrynium pubinerve* and *Thysanolaena latifolia* (EOPP, EPP, EOTL and ETL).[a]

Strains	EOPP	EPP	EOTL	ETL
S. aureus	12.67 ± 0.60	–	12.00 ± 0.00	–
A. baumannii	14.33 ± 0.57	10.00 ± 0.00	11.67 ± 0.57	9.67 ± 1.15
E. coli	15.33 ± 0.57	10.33 ± 0.63	11.00 ± 0.00	11.00 ± 0.00
K. pneumoniae	12.00 ± 0.00	–	13.33 ± 0.67	10.67 ± 1.15
P. aeruginosa	11.67 ± 0.57	–	12.00 ± 0.00	12.00 ± 0.33
A. fumigatus	11.33 ± 1.53	–	9.00 ± 0.00	–
C. albicans	14.67 ± 1.16	–	10.33 ± 0.57	–

A dash (–) indicate no antimicrobial activity.
[a] All values were performed in triplicate and expressed as means ± SD.

concentrations increased from 10 to 80 μg/ml, indicating that their scavenging power increased with concentration. The IC_{50} values of the essential oils and ethanol extracts were higher than Gallic acid and Trolox, while that of *Thysanolaena latifolia* (35.54–38.02 μg/ml) were only about 2–3 times of Trolox (15.29 μg/ml), indicating that it has strong ABTS free radical scavenging activity (Table 2).

3.2.3. Ferric reducing power

The reducing power of the samples measured by the FRAP assay and expressed as GAEAC (μg Gallic acid/ml) and TEAC (mM Trolox/l) increased with the increase in sample concentrations (Figs. 3 and 4). Both essential oils showed a potential reducing antioxidant power, with GAEAC in the range of 18.32–160.38 (μg Gallic acid/ml) and TEAC in the range of 0.30–2.12 (mM Trolox/l) at sample concentrations of 1–10 mg/ml, respectively.

3.3. Antimicrobial activity

3.3.1. Antimicrobial screening

The two essential oils were active against all tested pathogenic bacteria and spoilage organisms, with the inhibition zones in the range of 9.00–15.33 mm at concentrations of 20 mg/ml, while the ethanol extracts were only active against *A. baumannii*, *E. coli*, *Klebsiella pneumonia* and *P. aeruginosa* (Table 3). Thus the essential oils have much broader antimicrobial activities than the ethanol extracts.

3.3.2. Minimal inhibitory and bactericidal concentration (MIC and MBC)

The essential oils were active against all tested microbial strains, with MIC ranging from 64 to 3072 μg/ml and MBC ranging from 64 to 4096 μg/ml (Table 4). They were particularly active against spoilage organisms, including *A. fumigates* and *C. albicans*, with MIC ranging from 64 to 1024 μg/ml and MBC ranging from 64 to 2048 μg/ml, respectively. Particularly, the essential oil of *Phrynium pubinerve* showed 8 to 16 times lower MIC and MBC values (64 and 64 μg/ml) than the positive control Tigecycline (512–1024 μg/ml) against *A. fumigates*. The essential oils also displayed obviously antimicrobial activity against the food-borne pathogens *S. aureus*, *E. coli* and *P. aeruginosa*, with MIC and MBC values in the range of 64–4096 μg/ml. Furthermore, the essential oils showed strong antimicrobial activity against the conditioned pathogens, *Klebsiella pneumoniae* and *A. baumannii*, which have shown multiple drug resistance, and could cause serious infections, such as pulmonary infection. Specifically, the *P. pubinerve* essential oil had 2 times lower MIC and MBC values (128–256 μg/ml) than the positive control Amikacin (256–512 μg/ml) against *K. pneumonia*. Based on the MIC and MBC values, the *P. pubinerve* essential oil has stronger antimicrobial activity than the one from *Thysanolaena latifolia*. This higher antimicrobial might due to the high content of (E)-2-Hexenal and Terpinen-4-ol, which have not been found in *T. latifolia* and have been credited with broad antimicrobial activity (De Lucca, Carter-Wientjes, Boue, Lovisa, & Bhatnagar, 2013; Lanciotti et al., 2003; Loughlin, Gilmore, McCarron, & Tunney, 2008; Morcia, Malnati, & Terzi, 2012).

4. Conclusions

In this study, the chemical composition, and antioxidant and antimicrobial activities of essential oils from *P. pubinerve* and *T. latifolia* have been reported. Our results showed that leaves of both plants had antioxidant and antimicrobial activities, which is consistent with the traditional use of both species as naturally biodegradable package materials with antiseptic functions. Furthermore, their high antimicrobial activities against pulmonary

Table 4
MIC and MBC (μg/ml) of the essential oils and ethanol extracts of *Phrynium pubinerve* and *Thysanolaena latifolia* (EOPP, EPP, EOTL and ETL).[a]

Strains	EOPP		EPP		EOTL		ETL		Control[b]	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	512	1024	–	–	512	1024	1024	2048	0.25	0.5
A. baumannii	256	512	–	–	512	1024	–	–	0.5	1
E. coli	128	256	2048	2048	3072	4096	3072	4096	0.05	0.25
K. pneumoniae	128	256	2048	3072	2048	2048	3072	5120	256	512
P. aeruginosa	64	128	–	–	1024	2048	2048	6144	0.25	1
A. fumigatus	64	64	–	–	1024	2048	512	512	512	1024
C. albicans	256	256	–	–	512	1024	–	–	0.5	1

A dash (–) indicate no antimicrobial activity.
[a] All values were performed in triplicate.
[b] Positive control: Vancomycin for *S. aureus*; Fluconazole for *A. baumannii* and *C. albicans*; Cefotaxime for *E. coli*; Amikacin for *K. pneumonia*; Ciprofloxacin for *P. aeruginosa*; Tigecycline for *A. fumigatus*.

infection-causing bacteria and food-borne pathogens agree with the traditional use of both taxa as indigenous remedies for the relief of coughs, detoxification and stomach disorders. In conclusion, we support the continued use of leaves of both plants as biodegradable package materials and indigenous remedies in the ethnic villages of Xishuangbanna, Yunnan Province, China and other countries where the two plants exist. In particular, the leaves of *P. pubinerve* should be further promoted and applied as biodegradable package material alternative to plastic packaging. The leaf essential oils from both taxa could be further developed as easy available natural antioxidant and antimicrobial agents in the packaging, medical and functional food industries.

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