Antibacterial and antioxidant properties of various solvents extracts of *Abutilon theophrasti* Medic. leaves

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Abstract: This paper described the extraction procedure of six extracts from *Abutilon theophrasti* Medic. leaves and evaluated antioxidant and antibacterial activity of different extracts by hydroxyl radical, DPPH radical scavenging, broth micro-dilution and agar-well diffusion methods. The six extracts were prepared by the two extraction procedures: (I) water was the extraction solvent; (II) 90% alcohol extract was extracted by petroleum ether, chloroform, ethyl acetate and *n*-butanol in turn. Extract yields were 7.34%, 7.31%, 0.45%, 0.12%, 2.70% and 5.68% for extract I to VI. It was revealed that the various extracts had effective antibacterial activity against four test strains from *Staphylococcus aureus* (ATCC 25923), *Streptococcus* (ATCC 49619), *Escherichia coli* (ATCC 25922) and *Salmonella* (ATCC 01303); meanwhile, the six extracts demonstrated potent antioxidant activity, achieved by hydroxyl radical and DPPH radical scavenging assay. Minimum inhibitory concentrations (MICs) for the bacterial species ranged from 2.21 to 539.46 mg/ml, diameter of inhibition zone ranged from 2.08 to 15.05mm. The scavenging ·OH and DPPH· rates were 62.37% to 81.86% with the concentration 0.06 to 1.89mg/ml and 37.80% to 81.23% with the concentration 1.07 to 35.52mg/ml. According to the results, these extracts have antioxidant and antibacterial activity. In view of all the facts collectively, the six extracts will become natural and nontoxic antioxidant and antibacterial agent, and be applied in food and pharmaceutical industries for the prevention or treatment caused by microorganisms and free radicals.

Keywords: *Abutilon theophrasti* Medic., antibacterial property, antioxidant property, MIC/DPPH.

INTRODUCTION

With the continuous improvement of living standards, the quest for natural food and drug additives has become an increasing concern. Markets and Consumers' demand for healthier foods and drugs has been the initiative for many researchers seeking for natural alternatives such as oxidation inhibitor, bacteriostatic agent, coloring agent, flavoring agents.

Abutilon theophrasti Medic., a medicinally important plant belongs to the Family Malvaceae (Fu & Hong, 1993), was early recorded by Compendium of Materia Medica and Map of Materia (Gu GY & Jiang Y, 2009; Liu, H *et al*., 2010). Now, there are many flora, local flora and records of Chinese materia medica embodying it. It is widely grown in tropics and sub tropics areas of the world, particularly in China, India, Japan, Vietnam, Europe and North America but nowadays cultivated throughout many regions of the world. Owing to many pharmacological effects such as detoxication, dispelling wind, analgesia and anti-inflammatory, roots, seeds, leaves and other parts of *A. theophrasti*, have been widely applied in folk (Gu & Jiang, 2009; Liu *et al*., 2010; Su, Yang, Zhang, & Zhang, 2010). The main medicinal constituents are flavonoids, phenolic, tannin and fatty acid extracted from the whole plant of *A. theophrasti* leaves is a rich of phenolic and flavonoids part (Sikorska & Matlawska, 2008; Tian, Wang, Sheng, & Zhao, 2012). At present, natural phenolic and flavonoids are attracting more and more food, pharmaceutical and nutrition researchers' attention.

The classical extraction process of natural products is usually water extraction and alcohol precipitation, or after water/ alcohol extraction, petroleum ether, chloroform/ dichloromethane, ethyl acetate and *n*-butanol extraction in turn. Meanwhile, in view of the difference of the effective chemical composition in medicinal materials, other extraction methods with various solvents such as hexane, methanol and mixed solvent (khoudja, Boulekbache-Makhlouf & Madani, 2014; Sarikurkcu *et al*., 2009; Wang, Wang, &Li, 2013) also have been reported. Dichloromethane was adopted to extract the effective constituent in fruits, flowers and leaves of *Lawsonia inermis* L. from Jaffna (Jeyaseelan, Jenothiny, Pathmanathan & Jeyadevan, 2012); *A. squamosa* L. plant extracts were obtained by methanol: distilled water (8:2), acetone: distilled water (1:1), ethanol: distilled water (1:1) and boiling distilled water (El-Chaghaby Ahmad & Ramis, 2014).

Plant-base antimicrobial agents are sensitive against many common microorganisms such as *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli* and *Salmonella*. In research of the antimicrobial properties, there were comparative more reports about natural plant extracts

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(Cushnie, & Lamb, 2011; Khee, & Foong-Yee, 2011; Rodríguez-Rojas, Rodríguez-Beltrán, Couce, &Blázquez, 2013). *Leea indica* leaf extract showed significant zone of inhibitions compared to positive controls Ampicillin and Tetracycline against Gram-positive *Bacillus megate rium*, *Bacillus subtilis*, *Staphylococcus aureus* and *Bacillus cereus*, and Gram-negative *Salmonella paratyphi*, *Salmonella typhi*, *Pseudomonas aeroginosa*, *Shi-gella dysenteriae*, *Vibrio cholerae* and *Escherichia coli* (Rahman, Imran, & Islam, 2013).

On account of the character of delaying or preventing oxidation of an oxidizable substrate, antioxidants widely used in food, medicine, feed, chemical industry and other fields, its production technology has been brought to the attention (Lahouar *et al*., 2014). In recent years, natural plant extracts have played an important role in many industry because of their ability to scavenge free radicals (Changwei *et al*., 2011; Kim, *et al*., 2010; Okoth, Chenia, & Koorbanally, 2013; Sivasothy *et al*., 2013). The scavenging DPPH radical, superoxide radical and hydroxyl radical methods were adopted for evaluation the antioxidant activities of alkali and polysaccharide extracts from sclerotium of P. *tuber-regium* (Wu *et al*., 2014). ABTS, DPPH and FRAP assays were used for evaluation the antioxidant activities of the seeds and sprouts of mung beans, radish, broccoli and sunflower (Pajak *et al*., 2014).

However, the antibacterial and antioxidant properties of the different extracts of *A. theophrasti* leaves have not been investigated thoroughly. Therefore, the present research was carried out to investigate the antibacterial and antioxidant activities of sequentially extracted by water and organic solvent extracts of *A. theophrasti*. leaves.

MATERIALS AND METHODS

Apparatus and reagents

Positive control of Gentamicin and vitamin C (HPLC purity＞98.0%) were from China Institute of veterinary Drug and Control (Beijing, China). Mueller-Hinton agar and Nutrient broth were purchased from Beijing Aoboxing biological technology co., LTD (Beijing, China). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Chemie (Steinheim, Germany) and other chemicals and solvents were of analytical grade from China. The strains included *Staphylococcus aureus* (ATCC 25923), *Streptococcus* (ATCC 49619), *Escherichia coli* (ATCC 25922) and *Salmonella* (ATCC 01303). The standard strains were obtained from Culture collection center of China supervision of veterinary drug.

Collection and preparation of plant material

A. theophrasti leaves were gathered from Jilin province in China (No.131028). The leaves were washed cleanly by flowing water and then dried naturally in a shady and dry place during 20 days and ground to finely powder for further utilization.

Preparation of plant extracts

A. theophrasti leaves extracts I, II, III, IV, V and VI were obtained by different procedures as the following diagram (fig. 1 and 2). After extraction, the extracts were finally evaporated to dryness. The solid residue, were stored in a refrigerator (4^{o}C). The extraction yields were then calculated as a percent of the used powder.

Antibacterial activity assay

Broth micro-dilution assay

MIC values of all the strains were measured using a modified Eloff (1998) micro-well dilution method. *S. aureus* (ATCC 25923), *Streptococcus* (ATCC 49619), *E. coli* (ATCC 25922) and *Salmonella* (ATCC 01303), cultured overnight by Mueller-Hinton agar (MHA), were adjusted to the concentration of 1×10^{8} CFU/ml, and diluted 1:100 by sterile nutrient broth. The various extracts were dissolved in 1% DMSO, and diluted with the method of ten serial twofold dilution. Briefly, every well of 96-well plate was added with 100µl of the different extract solution and 100µl bacteria inoculums. 100µl of 0.1% DMSO and gentamicin were used as negative and positive controls, respectively. After covered, the plates were incubated at 37^{o} for 24h. The MIC values were the lowest concentration of samples suppressing bacteria growth in a certain environment, and used for the quantitative determination of antibacterial activity *in vitro*. The experiment was repeated in triplicate.

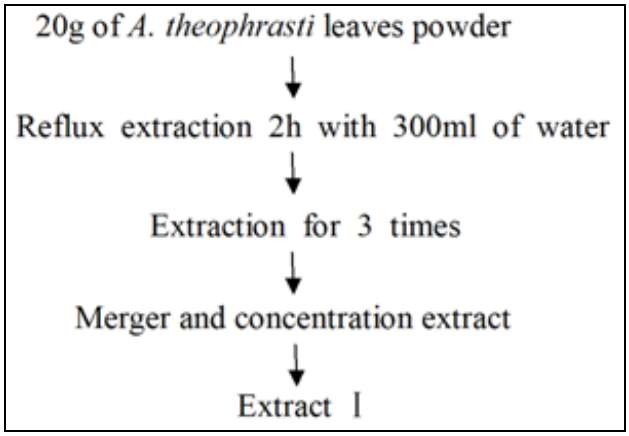

Fig. 1: Extraction procedure diagram of extract I

Agar-well diffusion method

Bacteria Suspensions were cultivated by MHA plates. The 6 mm diameter of wells were padded with 50µl of different extracts with the concentration 69.94, 472.78, 297.87, 34.26, 1079.48 and 1136.70mg/ml for extract I to VI and gentamicin (0.002mg/ml). The plates, which were inoculated by *S. aureus*, *Streptococcus*, *E. coli* and *Salmonella*, were incubated for 24h in constant

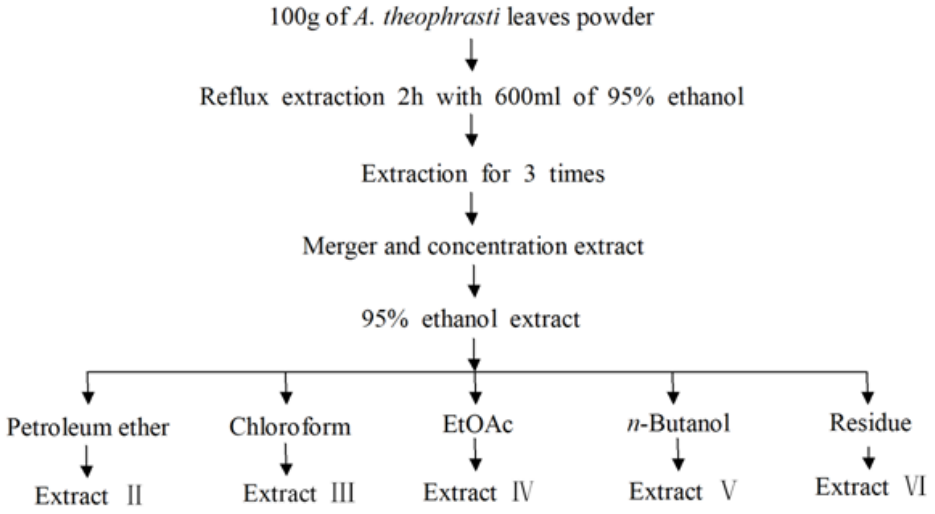

Fig. 2: Extraction procedure diagram of extract II, III, IV, V and VI

temperature incubator at 37□. The diameter of the growth inhibition zone around the well was an index of antimicrobial property. The experiment was repeated in triplicate.

Antioxidant activity assay

Hydroxyl radical scavenging activity assay

The hydroxyl radical scavenging activity was determined by the Deng method (Deng *et al*., 2012) with some modification. 1.0ml of different samples were added with 3.0ml of 2mmol/L $FeSO_4$, 3.0ml of 1mmol/L H_2O_2 and 3.0ml of 6mmol/L salicylic acid-ethanol and then the mixture was incubated for 15min at 37°C. The hydroxyl radical was assayed at 510nm. The DMSO and vitamin C were chose as the blank control and positive control, respectively. The assay was done in triplicate. The hydroxyl radical scavenging activity was calculated as:

$$\text{Scavenging rate}(\%) = \frac{A_{\text{blank control}} - A_{\text{sample}}}{A_{\text{blank control}}} \times 100\%$$

Where $A_{\text{blank control}}$ was the absorbance of DMSO and A_{sample} was the absorbance of the different extract or vitamin C sample added with reaction solution.

Measurement of DPPH· radical scavenging activity

The DPPH· radical scavenging activity was evaluated by the method proposed by Moure *et al*. (2001) with a few modifications. Briefly, 2ml of 0.1mM ethanolic solution of DPPH· radicals were added to 1.0ml of the various extracts with 1ml of DMSO. The absorbance of the mixture was assayed at 517 nm after 30 min of incubation at room temperature in the dark. Vitamin C was used as the positive control and DMSO as the blank control. Each sample was measured in triplicate. The scavenging effect was calculated according to the following equation of hydroxyl radical scavenging activity assay.

STATISTICAL ANALYSIS

The results were showed by mean ± standard error. Statistical analysis was performed with ANOVA. Differences among groups were considered significance level of P values <0.05 (SPSS19.0 for WINDOWS; IBM Co., USA).

RESULTS

The obtained extraction yields for the different tested solvents extracts are listed in table 1. The antibacterial activities of six extracts of *A. theophrasti* leaves against the employed bacteria were evaluated by the MIC and inhibition zone values. The results of the antibacterial properties of various extracts of *A. theophrasti* leaves, assayed by broth micro-dilution and agar-well diffusion methods are given in table 2 and table 3, respectively. Table 4 shows the hydroxyl radical scavenging activity and DPPH radical scavenging activity of the six extracts of *A. theophrasti* leaves with vitamin C used as reference antioxidants.

DISCUSSION

Extraction methods

The extraction yield was influenced usually by the extraction factors, such as solvents, time and temperature of extraction as well as physical and chemical properties of the sample. On the basis of literature and traditional extraction and separation experience of natural product, water and 90% ethanol solution were chose in the present study. In turn, the extract, extracted by 90% ethanol solution, was extracted again by petroleum ether, chloroform, ethyl acetate and n-butanol.

Antibacterial activity

Minimum inhibitory concentration (MIC)

As shown in table 2, the MIC determined by broth micro-

dilution method shows that the different extracts from *A. theophrasti* leaves have antibacterial activity towards tested *Staphylococcus aureus* (ATCC 25923), *Streptococcus* (ATCC 49619), *Escherichia coli* (ATCC 25922) and *Salmonella* (ATCC 01303). According to the MIC, the extract V is the best antibacterial agent with MIC 2.21mg/ml. Meanwhile, the extract have higher antibacterial activity to *Escherichia coli* and other three strains with MIC 4.25mg/ml and 17mg/ml, respectively. Overall, the antibacterial results for different extracts (table 2) indicates that the extract I, II, III are found more effective than compared to IV, V, VI.

According to the references, the reports are few on antibacterial activity of the six extracts from *A. theophrasti* leaves to the four tested strains. The results in table 2 demonstrated that the extract I, II, III have better antibacterial activity. This is an indication that the three extract are likely to be the potential of antibiotic alternatives.

Inhibition zone (IZ)

The antibacterial activities were categorized by Chan *et al* (2007), as follows: (I) strong: for inhibition ≥70%, (II) moderate: for inhibition 50-70%, (III) weak: for inhibition < 50%. The antibacterial activity of the tested extracts shows different selectivity for each strain. According to the criterion reported by Chan *et al* (2007), the extracts and δ are found to have the strong inhibition against *S.aureus* with inhibition 95% and 93%, respectively. Meanwhile, extracts I and VI exhibits to moderate inhibition (54% and 50%). Extracts β and ε show moderate inhibition against *Streptococcus* with inhibition 68% and 57%, respectively. However, the antibacterial activities of six extracts toward the other tested organisms are found to be weak. It is further proved that the six extract had certain antibacterial activity, which will provide research basis and reference for solutions of the problem of antibiotic resistance in clinical practice.

Antioxidant activity

Antioxidant activity by the hydroxyl radical method

At the similar scavenging OH· rate, the concentrations of extracts I, V and VI were 0.12, 1.80 and 1.89, respectively. The hydroxyl radical scavenging rate of extract III is slightly lower than that of extract IV, but its concentration is extract IV more than eight times. The scavenging rate of extract is lower with relatively lower concentration. The difference of chemical components in various extracts is probably the main reason, which caused the experiment results above. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant activity (Parimala, M., & Shoba FG 2013). The study shows that the six extracts of *A. theophrasti* leaves have certain ability for scavenging hydroxyl radical.

Antioxidant activity by the DPPH method

As showing in the table 4, the ordering of DPPH radical scavenging ability was: vitamin C > Extracts II, IV > I > III > V, VI. At the concentration of 1.62 and 1.07 mg/ml, the DPPH radical scavenging activity of extract II and IV were 81.23% and 76.72%, respectively. It indicates that the extract II and IV may be excellent DPPH radical scavengers comparing to other four extracts. Although the scavenging activity of the six extracts is lower than that of vitamin C standard, this needs to be fully clarified by further assay methods and using additional concentration of extracts (Parimala, M. & Shoba FG, 2013).

The research indicates that the six extracts might have a better antioxidant activity. Thus, in comparison with other synthetic antioxidants, the different extracts of *A. theophrasti* leaves are rich sources of natural antioxidant compounds that could potentially be used widely in the food and pharmaceutical industries.

CONCLUSION

In the present study, the extraction procedure, antibacterial and antioxidant activities of the various extracts from the *A. theophrasti* leaves were evaluated systematically. The six extracts were prepared by heating reflux extraction and liquid-liquid extraction methods. The optimum extraction procedures were based on two traditional extraction methods: (i) water was the extraction solvent; (ii) 90% alcohol extract was extracted by petroleum ether, chloroform, ethyl acetate and *n*-butanol in turn. The antibacterial property of the various extracts was determined successfully by broth micro-dilution and agar-well diffusion methods. The antioxidant activity of the six extracts were demonstrated by hydroxyl radical and DPPH radical scavenging. Based on the all results, it is revealed that the traditional heating reflux extraction and liquid-liquid extraction methods, which are effective methods for extraction of chemical compositions in Traditional Chinese medicine, were simple, low-cost and easy to operate. Meanwhile, the six extracts from *A. theophrasti* leaves exhibit antibacterial and antioxidant activity, this research revealed clearly that these extracts could be a natural and nontoxic antibacterial and antioxidant agent. Further work is to assess whether *A. theophrasti* leaves could be used as a source of natural antibacterial and antioxidant agent for pharmaceutical and food applications.

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Table 1: Extracts characteristics and yields obtained of the different extraction solvents.

No.	Solvent	Extract characteristics[a]	Extract yields (%)[b]
Extract I	Water	Light brownish solid	7.34
Extract II	Petroleum ether	Dark green semisolid	7.31
Extract III	Chloroform	Dark brownish semisolid	0.45
Extract IV	Ethyl acetate	Light brownish semisolid	0.12
Extract V	*n*-Butanol	Dark brownish solid	2.70
Extract VI	Residue	Dark brownish solid	5.68

[a]The color and status of the extract. [b]Extract yields(%)=Weight of extract/Weight of dry medicinal materials×100%.

Table 2: Antibacterial activity of the different extract from *A. theophrasti* leaves using broth micro-dilution method.

No.	MIC (mg/ml)[a]			
	S. aureus (ATCC 25923)	*Streptococcus* (ATCC 49619)	*E. coli* (ATCC 25922)	*Salmonella* (ATCC 01303)
Gentamicin	0.002	0.004	0.002	0.004
Extract I	17.03±0.45[b]	8.76±0.10	34.85±0.16	8.80±0.15
Extract II	45.97±0.11	11.68±0.15	46.59±0.45	11.62±0.21
Extract III	37.35±0.55	37.48±0.23	148.22±0.73	74.38±0.65
Extract IV	17.24±0.53	17.41±0.27	4.25±0.12	17.52±0.37
Extract V	136.21±1.14	539.46±2.19	269.53±2.07	2.21±0.12
Extract VI	143.08±1.32	284.51±0.54	570.31±6.39	143.15±1.08

[a]MIC, Minimum inhibitory concentration. [b]The values in this table represent the mean ± SD (*n*=3).

Table 3: Antibacterial activity of the different extract from *A. theophrasti* leaves using agar-well diffusion method.

No.	IZ[a]			
	S.aureus (ATCC 25923)	*Streptococcus* (ATCC 49619)	*E. coli* (ATCC 25922)	*Salmonella* (ATCC 01303)
Gentamicin	15.87±0.32[b]	10.43±0.81	14.14±0.47	16.61±0.62
I	8.59±0.43(54f)++c	3.05±0.52(29) +	2.88±0.75(20) +	3.57±1.11(21) +
II	15.05±0.45(95)+++e	2.71±0.37(26) +	4.20±1.08(30) +	3.04±0.29(18) +
III	6.79±0.38(43) +d	7.06±0.48(68)++	6.03±0.60(43) +	5.94±0.20(36) +
IV	3.83±0.55(24)+	2.08±0.66(20) +	3.22±0.57(23) +	3.82±0.52(23) +
V	14.72±0.47(93)+++	3.17±0.73(30) +	5.70±0.59(40) +	6.85±0.40(41) +
VI	7.86±0.41(50) ++	5.98±0.45(57)++	1.87±0.35(13) +	2.16±0.47(13) +

[a]IZ, diameter of inhibition zone (mm) excluding diameter of well (6 mm). [b]All values in this table represent the mean±SD (*n*=3). [c+]Weak inhibition. [d++]Moderate inhibition. [e+++]Strong inhibition. [f]Values in parentheses are the inhibition percentages compared to standard antibacterial agent.

Table 4: The scavenging effect on OH and DPPH· of different extracts and Vc.

Sample	Antioxidant activity			
	The concentration of extracts (mg/ml)[a]	The scavenging OH· rate (%)[b]	The concentration of extracts (mg/ml)	The scavenging DPPH· rate (%)
Extract I	0.12	81.86±0.47	2.19	65.37±0.74
Extract II	0.17	63.37±0.45	1.62	81.23±0.65
Extract III	0.50	72.78±0.30	9.31	62.11±0.44
Extract IV	0.06	77.18±0.70	1.07	76.72±0.52
Extract V	1.80	79.52±0.95	33.73	37.80±0.36
Extract VI	1.89	79.28±0.35	35.52	51.97±0.87
Vitamin C	0.09	97.12±0.54	0.11	96.63±0.47

[a] The concentration of different extracts. [b]The values of the scavenging rate represent the mean ±SD (*n*=3).

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