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Research Article
Evaluate the Effect of Inhibiting Pathogenic Bacteria and Fungus of Eczematous Dermatitis and Antioxidant Activity of Phenolic from Qinglicao (*Polygonum chinense* L. Var. *chinense*)

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Abstract

Background: QingLiCao has been used for the treatment of skin diseases such as eczematous dermatitis in China-Vietnam border area. However, the mechanism of treatment effect is still unclear. This study aimed to investigate the inhibitory effect of pathogenic bacteria and fungus of eczematous dermatitis and evaluate the antioxidant activity of phenolic contents from QingLiCao, will be helpful to further understand the mechanism of action. **Materials and Methods:** The *in vitro* phenolic antimicrobial activity was determined using disc diffusion and antioxidant activity was evaluated by spectrophotometry. **Results:** The experimental results showed that the phenolics from QingLiCao had excellent antimicrobial activity to pathogenic bacteria and fungus of eczematous dermatitis, similar results were observed for the antioxidant activity. **Conclusion:** The results support the proposition that phenolic from QingLiCao might be a promising anti-eczematous dermatitis natural active ingredients merit further investigations.

Key words: QingLiCao, phenolic, eczematous dermatitis, pathogenic bacteria and fungus, antioxidant activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Eczematous dermatitis is a broad range of common allergic skin diseases, due to its high prevalence, protracted course and often accompanied by serious itching, it can significantly affect the patient's quality of life[1]. The bacterial infection is closely related to the incidence, development and severity of eczematous dermatitis. New research showed that infection of bacteria and fungus played an important role as an aggravating factor in pathogenesis of eczematous dermatitis[2-6].

Traditional natural herbal medicine has unique advantages in the treatment of eczematous dermatitis, it might have fewer side effects as compared with anti-histamine drugs and corticosteroids[7-12]. Although the mechanism of these traditional natural medicines for the treatment of eczematous dermatitis needs further study[13-16], recent studies show that there are certain relationship between the treatment effect on eczematous dermatitis and its antimicrobial and antioxidant activities, which has provided some inspiration[17-20]. In general, the stronger against pathogenic bacteria and fungus and antioxidant activity, the better the treatment effect on eczematous dermatitis. As a traditional natural herbal medicine, QingLiCao has been used for the treatment of skin diseases such as eczematous dermatitis in China-Vietnam border area. In the earlier studies, the extraction of total flavonoids from QingLiCao and *in vitro* antioxidant activities were investigated[21]. In present study, further measured the antimicrobial and antioxidant activities of phenolic from QingLiCao, this will be helpful to provide a scientific evidence for further development QingLiCao on eczema-treatment.

MATERIALS AND METHODS

Materials and reagents: The sample of QingLiCao was purchased from Chinese herbal medicine market in Yulin, China, identified by Professor Li Chen (School of medicine, Guangxi University of science and technology, China). Gallic acid standard (98%) (Bomei Biotechnology Co., Ltd., China). All the others reagents and solvents used were of analytical grade and purchased from Admas-beta Reagent (China) used without further purification.

Preparation of phenolic from QingLiCao: The dried powder of QingLiCao (50 g) was extracted with 60% ethanol-aqueous solution 500 mL at 60°C for 1.5 h as described by Jiang *et al.*[22]. The filtrates were collected and concentrated with a rotary evaporator at 50°C and further to vacuum freeze dried to obtain crude extract as a brown powder. The crude extract was finally purified by using macroporous resin AB-8 (The adsorption process is as follows: 3.0 BV of sample solution at concentration of 2.5 mg mL^{-1} through the column at 1.0 BV h^{-1}, the desorption process is as follows: 2.0 BV 60% ethanol-aqueous solution at 2.0 BV h^{-1} eluted.) to obtain the purification liquid and further to vacuum freeze dried to obtain the purification as a light yellow powder.

Total phenolic content: The total phenolic content was determined by the Folin-Ciocalteu method according to Shi *et al.*[23] with slight modification. The modification was 1.0 mL of diluted sample solution was mixed with 1.0 mL of Folin-Ciocalteu reagent and 2.0 mL of 12% sodium carbonate solution and then diluted to 25 mL with pure water. The absorbance at 765 nm was measured using UV-visible spectrophotometer after 2 h of incubation at 30°C.

Evaluation of antimicrobial activity: The phenolic from QingLiCao minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) were experimentally determined using disc diffusion method as previously described by Khurram *et al.*[24], *Staphylococcus aureus*, *Malassezia furfur* and *Streptococcus hemolyticus* as typical pathogenic bacteria and fungus of eczematous dermatitis were evaluated while normal saline was employed as a negative control.

Evaluation of antioxidant activity: *In vitro* free radical scavenging activity of phenolic from QingLiCao were assessed against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, hydroxyl radical, superoxide radical and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical using the method described by Jiang *et al.*[25] while ascorbic acid was employed as a positive control.

Statistical analysis: All of the experimental data were performed in triplicate and the results were expressed as mean±standard deviation. Analysis of variance was performed by one way ANOVA test and a statistically significant ($p<0.05$) was considered.

RESULTS AND DISCUSSION

Total phenolic content of QingLiCao extract: The determination of the total phenolic content was performed using the Folin-Ciocalteu reagent. Gallic acid was used to generate a calibration curve at concentrations of 1-6 μg mL^{-1}, the linear regression equation was $y = 0.1121x+0.0319$ and $R^2 = 0.9990$ shown in Fig. 1.

Table 1: Absorbance value and phenolic purity (%)

Samples	Absorbance value			Phenolic purity (%)		
Crude extract	0.169	0.180	0.171	24.5	26.4	24.8
	0.173±0.006			25.2±1.0		
Purification	0.424	0.450	0.439	70.0	74.6	72.6
	0.438±0.013			72.4±2.3		

Table 2: Antimicrobial activity against three pathogenic bacteria and fungus (mg mL^{-1})

	Crude extract		Purification		Normal saline	
Species	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	6.25	12.5	1.56	3.13	-	-
Malassezia furfur	12.5	25.0	1.56	3.13	-	-
Streptococcus hemolyticus	12.5	25.0	1.56	6.25	-	-

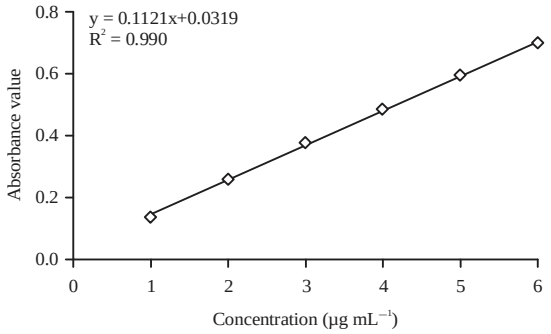

Fig. 1: Gallic acid concentration-absorbance value of the standard curve

According to the measurement result and the linear regression equation, the total phenolic concentration was determined and the phenolic purity was calculated from the relationship:

$$\text{Phenolic purity } (\%) = \frac{nx}{C_0} \quad (1)$$

where, C_0 is the concentration of sample (mg mL^{-1}), n is the dilution times and x is the concentration of phenolic according to the linear regression equation (mg mL^{-1}).

The data in Table 1 showed that the total phenolic purity in the crude extract was 25.2±1.0% and the purification was 72.4±2.3%.

Evaluation of antimicrobial activity: *Staphylococcus aureus*, *Malassezia furfur* and *Streptococcus hemolyticus* are typical pathogenic bacteria and fungus of eczematous dermatitis and the inhibitory capacity is an important indicator for treatment of ezematous dermatitis[3-6]. The phenolic from QingLiCao were investigated and evaluated for the inhibitory capacity to three pathogenic bacteria and fungus and the results shown in Table 2.

As seen from the Table 2, phenolic extract displayed significant antimicrobial activity against three pathogenic bacteria and fungus compared with normal saline. The MIC value of crude extract was 6.25, 12.5 and 12.5 mg mL^{-1}, respectively. However, the MIC only 1.56 mg mL^{-1} was observed in purification. While the MBC value of crude extract were 12.5, 25.0 and 25.0 mg mL^{-1}, respectively compared with 3.13, 3.13 and 6.25 mg mL^{-1} of the purification. According to previous literature, Wu *et al.*[26] and Gao and Zhou[27] revealed the inhibitory effect of Sophora flavescens, honeysuckle, *Phellodendron chinense* Schneid, Cortex dictamni, *Cnidium monnieri* (L.) Cuss., *Angelica dahurica*, *Kochia scoparia* (L.) Schrad and Gentiana scabra Bunge extracts to *Staphylococcus aureus*, however, the MIC more than 100 mg mL^{-1} and the mechanism of inhibitory effect of extracts was not clear. In this experiment, it was obvious that the purification significantly increased the capacity to inhibit pathogenic bacteria and fungus growth due to the higher content of phenolic what it means the phenolic from QingLiCao is one of treat eczematous dermatitis material basis.

DPPH radical scavenging activity: The DPPH radical scavenging activity of different concentration samples and positive control shown in Fig. 2. The DPPH radical scavenging capacity of crude extract, purification and ascorbic acid showing the dose-effect relationship with significantly increase at the concentration range (50-150 μg mL^{-1}) while displayed a moderate increase at a higher concentration (greater than or equal to 200 μg mL^{-1}). The purification displayed a superior antioxidant effect to eliminate DPPH radicals compared with crude extract and ascorbic acid at the concentration range (50-150 μg mL^{-1}) $p < 0.05$. However, there is no significant difference when the concentration was greater than or equal to 200 μg mL^{-1} as the concentration continues to increase, it means that the DPPH scavenging

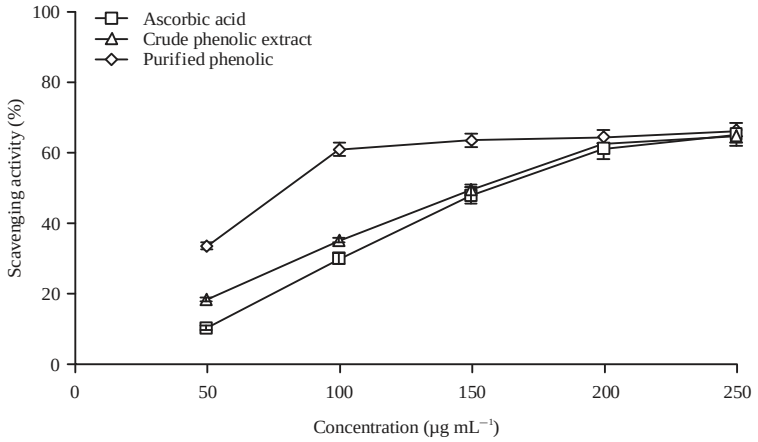

Fig. 2: DPPH radical scavenging activity

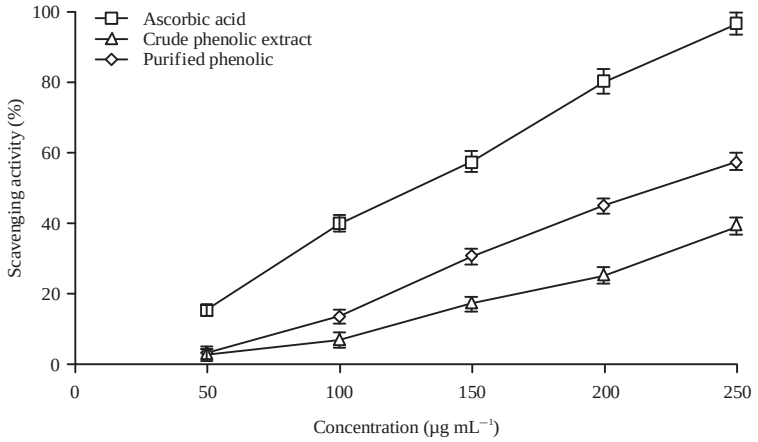

Fig. 3: Hydroxyl radical scavenging activity

capacity was saturated under current experimental conditions. The results showed that phenolic from QingLiCao have strong antioxidant capacity; many phenolic extracts have been reported to similar results[17-19].

The mechanism of scavenging DPPH radical of phenolic was caused by the fact that the phenol or its phenolic anion can transfer an electron or a hydrogen atom to DPPH and the DPPH radical was reduced to a non-radical form[21]. This can explain why the purification exhibited higher DPPH radical scavenging ability at the test concentration range than crude extract.

Hydroxyl radical scavenging activity: It could be seen from Fig. 3 that the capacity of hydroxyl radical scavenging of phenolic from QingLiCao and ascorbic acid gradually increased with increasing concentration of the sample, similar results were previously observed by Mao *et al.*[28] and Zhang *et al.*[29]. The scavenging ability of ascorbic acid on hydroxyl radical was significantly greater than the others, the scavenging activity was 97.1% when the concentration at 250 μg mL^{-1}. Moreover, there is a significant difference between purification and crude extract ($p<0.05$), the former has higher scavenging activity at the same concentration: The scavenging activity of purification was 57.8% compared with crude extract 39.5% when the concentration at 250 μg mL^{-1}.

Hydroxyl radical scavenging activity is important parameter for evaluating antioxidant activity. There were close connection between the hydroxyl radical scavenging activity and phenolic content due to the phenolic hydroxyl groups were easily oxidized by hydroxyl radical[28,29]. As a result, phenolic from QingLiCao exhibited excellent hydroxyl radical scavenging capacity and the capacity increased as the phenolic content increased.

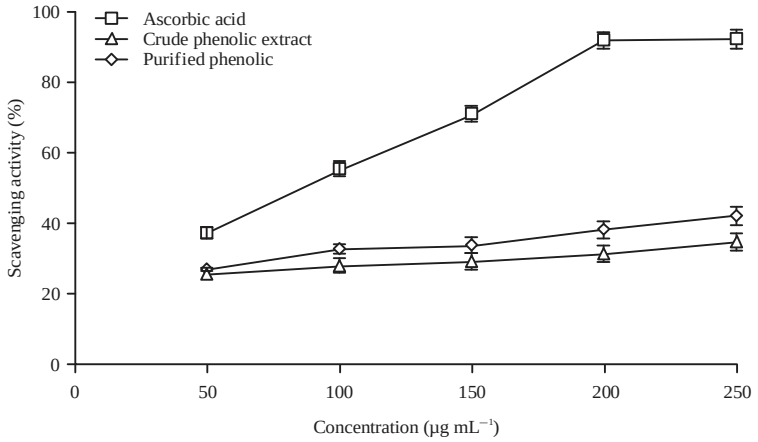

Fig. 4: Superoxide radical scavenging activity

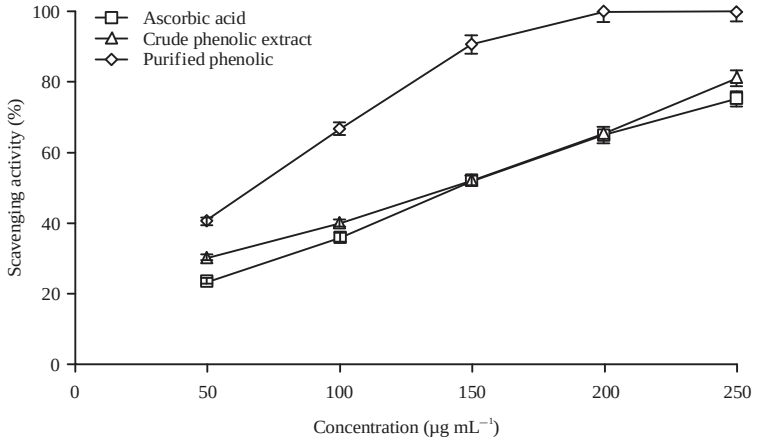

Fig. 5: ABTS radical scavenging activity

Superoxide radical scavenging activity: The determined results of eliminating superoxide radical scavenging activity showed in Fig. 4. As could be seen from the figure, there were significant concentration-dependent increase in scavenging activity. Ascorbic acid showed the highest inhibitory capacity compared with that of others and the scavenging activity nearly 90% when the concentration at greater than or equal to 200 μg mL^{-1}. The superoxide radical scavenging activity of crude extract was much lower than ascorbic acid and the purification showed the moderate scavenging activity compared with the crude extract with a $p<0.05$. The scavenging activity of ascorbic acid for superoxide radical was 92.1% compared with crude extract's 34.4% and purification's 42.1% at concentration of 250 μg mL^{-1}. Similar results were in agreement with the reported by Shi *et al.*[23], Mao *et al.*[28] and Zhang *et al.*[29].

Studies had shown that superoxide anion was a kind of free radical with strong oxidative properties. It had cytotoxicity and could combine with other free radicals to further produce a strong poisoning effect on biological tissues[22]. Therefore, phenolic on superoxide radicals scavenging activity played an important role in preventing biological damage[23,29]. Although the elimination rate of superoxide radicals of phenolic was much lower than that of ascorbic acid, the superoxide radicals scavenging activity increased with increasing phenolic concentration, this means phenolic from QingLiCao showing that the capacity of scavenge superoxide radicals and preventing biological damage.

ABTS radical scavenging activity: The ABTS radical scavenging activity of the phenolic from QingLiCao and ascorbic acid were measured as the percent to eliminate ABTS radicals and the results was shown in Fig. 5, there was also a significant dose-effect relationship for the capacity of ABTS radical scavenging activity. The purification showed the highest scavenging capacity as compared with the others, while the measurements values of ascorbic acid and crude extract were very closely. The scavenging activity of

purification was 100% compared with crude extract's 81.1% and ascorbic acid's 75.3% when the concentration at 250 μg mL^{-1}, $p<0.05$. These findings were in agreement with the results reported by Mao *et al.*[28] and Zhang *et al.*[29], Shon *et al.*[30] and Yu *et al.*[31].

The ABTS radical scavenging activity was one of the popular indirect methods of determining the antioxidant activity of compounds[30]. The ABTS reacts with oxidants to get ABTS+·free radicals (blue-green) and phenolic reduce ABTS+· to colorless ABTS[31]. There were close connection between the ABTS radical scavenging activity and phenolic content, the purification contained more phenolic and exhibited more significantly capacity to scavenge ABTS radical. The experimental results indicated that the phenolic from QingLiCao have a good scavenging effect on ABTS radicals.

CONCLUSION

It was concluded that the phenolic from QingLiCao were closely related to the inhibitory effect of pathogenic bacteria and fungus of eczematous dermatitis and antioxidant capacity. The study results provide a further understanding of the phenolic from QingLiCao was one of treatment for eczematous dermatitis material basis and worthy of further study.

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SIGNIFICANCE STATEMENT

This study discovered that the phenolic from QingLiCao have excellent inhibitory capacity against the pathogenic bacteria and fungus of eczematous dermatitis, at the same time, also exhibited strong antioxidant capacity. The results provide a further understanding of the phenolic from QingLiCao.

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