DOI：10. 13471/j. cnki. acta. snus. 2022E027

Biological activities and detoxification mechanisms of *Clerodendrum chinense* var. *simplex, Marsdenia tenacissima* and *Arundina graminifolia*: The Dai antidotes*

ZHANG Xuefei[1,2], *ZHAO Junke*[2], *HAO Suqi*[2], *DING Qing*[3], *YU Shihui*[2], *MOHAMMAD Imran shair*[2], *WANG Jun*[2], *HU Haiyan*[2]✉

1. *School of Traditional Dai-Thai Medicine, West Yunnan University of Applied Sciences, Jinghong 666100, China*
2. *School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China*
3. *Yunnan Dai Medicine Co., Ltd., Ruili 678699, China*

Abstract: Dai antidotes are the most distinctive medicine and treatment in traditional Dai medicine. *Bin Hao* (*Clerodendrum chinense* var. *simplex*), *Dai Bai Jie* (*Marsdenia tenacissima*) and *Zhu Ye Lan* (*Arundina graminifolia*) are three Dai antidotes widely used for their "detoxifying effects", and their use is rooted in a theoretical system significantly different and much less understood than Western or traditional Chinese medicines. Here, we successively extracted the three Dai antidotes using petroleum ether, ethyl acetate, *n*-butanol, or water, and then prepared their decoctions. The content of total flavonoids in three Dai antidotes ranged from 22.41 to 586.39 mg/g, which is higher than the content of total polyphenols (2.76 to 28.66 mg/g). The various extracts were found to scavenge radicals of DPPH, ·OH and ABTS. They scavenged ABTS radicals much more efficiently than other radicals (IC_{50} > 380 μg/mL). They weakly inhibited the growth of *E. coli, P. aeruginosa* and *S. aureus.* Notably, even at low concentration 60 μg/mL, the extracts can significantly down-regulate the production of NO, TNF-α, IL-1β, and IL-6 by macrophages stimulated with LPS. In conclusion, our results provide the first mechanistic insights into the detoxifying effects of three Dai antidotes, providing a foundation for their optimization and for future research to strengthen Dai medicine through modern scientific practices.

Key words: Dai antidote; *Clerodendrum chinense* var. *simplex*; *Marsdenia tenacissima*; *Arundina graminifolia*; detoxification

CLC number: R29 **Document code:** A **Article ID:** 2097 − 0137(2023)03 − 0089 − 11

As one of the four major ethnomedicines in China (Wang et al. , 2017), Dai medicine dates back more than 2 500 years (Li et al. , 2019) and continues to play an important role in health care in China and many other countries of southeast Asia, especially in the Mekong River valley. "Yajie", is also known as Dai antidotes, refers to the medicine that can regulate the function of four cosmic elements in the body and is widely used among Dai medical practitioners to detoxify body toxins obtained from food, drug or animal

* **Received**: 2022 − 09 − 13 **Accepted**: 2022 − 11 − 04 **Published online**: 2023 − 01 − 30
Supported by Yunnan Provincial Department of Education Science Research Fund Project（2018JS709）; National Natural Science Foundation of China（81973264）; Guangdong Basic and Applied Basic Research Foundation（2019A1515011954, 2020A1515010593）

✉**Corresponding author**: HU Haiyan（*lsshhy@mail.sysu.edu.cn*）

ZHANG Xuefei（*zhangxf27@mail2.sysu.edu.cn*）, ZHAO Junke（*1623928099@qq.com*）, HAO Suqi（*1475338624@qq.com*）, DING Qing（*dq4115222@163.com*）, YU Shihui（*956800457@qq.com*）, MOHAMMAD Imran shair（*shair@mail.sysu.edu.cn*）, WANG Jun(*wjun@mail.sysu.edu.cn*)

bites, etc. It is the most distinctive medicine and treatment method in Dai medicine. (Zhang et al. , 2012). *Bin Hao* (*Clerodendrum chinense* var. *simplex*), *Dai Bai Jie* (*Marsdenia tenacissima*) and *Zhu Ye Lan* (*Arundina graminifolia*) (Fig. 1), are widely used Dai antidotes. Mainly, *Bin Hao* is used to treat cough, sore throat, rheumatic arthralgia and jaundice (Li et al. , 2018), *Dai Bai Jie,* to treat cough, swelling and throat pain(Li et al. , 2014), and *Zhu Ye Lan,* to treat all kinds of poisoning caused by food and medicine, abdominal pain, diarrhea, dizziness and other diseases (Qu et al. , 2011).

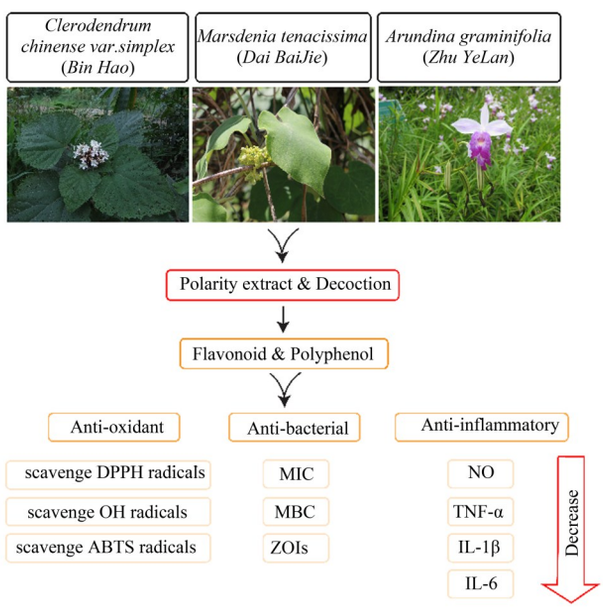


Fig. 1 Schematic representation of research contents of *Bin Hao*, *Dai Bai Jie* and *Zhu Ye Lan*

There are few studies, using modern biomedical techniques to analyze the clinical benefits of Dai antidotes limiting the use of Dai medicine. In fact, the Dai theoretical system remains completely outside from modern medical science and research. Compared with other types of traditional Chinese medicine, which already have a large number of literatures and clinical practice integrated with western medicine, we are unaware the overlap and complementarity between the Dai theoretical system and traditional Chinese medicine or western medicine.

Dai medical theory holds that disease is related to imbalance among the four cosmic elements in the body, and that such imbalance can arise due to the presence of toxins (Zhang et al. , 2012). This imbalance may be attributed to imbalance between free radicals and anti-oxidant defenses (Oszmianski et al. , 2020) or imbalance between pro- and anti-inflammatory factors, such as in excessive inflammation or infection with pathogens. All these imbalances can lead to a disease initiation and progression. Therefore, the present study used standard and well-established laboratory methods to assess the *in vitro* antioxidant, anti-bacterial and anti-inflammatory effects of the three Dai antidotes. In addition, the material basis of three Dai antidotes were explored by determining their contents of total polyphenols and flavonoids, as described in Fig. 1. The results may help explain the clinical efficacy of these traditional medicines, providing a modern scientific foundation for understanding and developing Dai medicine for the treatment of various diseases as well as expanding their use in other regions of the world.

1 Methods

1. 1 Plant material, reagents and cells

The *Bin Hao, Dai Bai Jie* and *Zhu Ye Lan* were purchased from the Institute of Ethnic Medicine (Xishuangbanna, Yunnan Province) and identified by Mrs. Lin Yanfang, chief expert of Dai Medicine. They were washed and dried for two weeks in the shade. Before extraction, the plants were cut into small pieces and crushed using a floor-standing continuous feed grinder (DF-35, Wenling Linda Machinery, Zhejiang, China).

DPPH, ABTS, hydrogen peroxide (H_2O_2), rutin, gallic acid and potassium persulfate were purchased from Aladdin (Shanghai, China); Vitamin C was obtained from Solarbio Science & Technology Co. , Ltd. (Beijing, China). Ethylparaben was purchased from Sigma-Aldrich (USA). Luria-Bertani (LB) broth was purchased from Hopebio (Qingdao, China); *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *S. aureus* (ATCC 25923), were obtained from Huankai Guangzhou Microbial. RAW 264. 7 macrophage cells were purchased from the Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China).

Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco-Thermo Fisher Scientific (Grand Island, NY,

USA). LPS was purchased from Sigma Chemical (St. Louis, USA). NO Griess reagent was acquired from Beyotime Institute of Biotechnology (Shanghai, China). IL-1β, IL-6 and TNF-α ELISA kits were purchased from Boster Biological Technology (Wuhan, China). All other reagents were purchased as analytical reagent grade and used without further purification.

1. 2 Extraction of Dai antidotes

Crushed *Bin Hao*, *Zhu Ye Lan* and *Dai Bai Jie* were extracted three times with 95% ethanol at a mass-to-volume ratio of 1:10 for 2 h at 98 ℃. The extracts were filtered, combined, and evaporated under reduced pressure to obtain crude ethanol extract. The polar extracts were obtained from the corresponding crude extract by successive extraction with the same volume of solvents of increasing polarity: petroleum ether, ethyl acetate, *n*-butanol and distilled water. Then, we obtained the petroleum ether extract (PE), ethyl acetate extract (EE), *n*-butanol extract (BE) and water extract (WE), respectively.

Aqueous decoctions (DE) of the three Dai antidotes were prepared by mixing 100 g dried *Bin Hao*, *Zhu Ye Lan* or *Dai Bai Jie* with 1 000 mL of distilled water and boiling for 0. 5 h and repeat three times. The decoctions were filtered with gauze, combined and concentrated to 50 mL, giving crude Dai antidotes 2 g/mL.

1. 3 Determination of total flavonoids content

The total flavonoids content of the samples was determined as described by Hossain et al. (2011). Briefly, 200 μL of samples, 200 μL of ethanol and 40 μL of *w*=10% $NaNO_2$ was mixed and allowed to stand for 7 min. Then, 40 μL of *w*=5% $Al(NO_3)_3$ solution was added. After 7 min, 400 μL of 1 mol/L NaOH and 120 μL ethanol was added to the solution. The absorbance of obtained mixture was measured at 510 nm. In the same way, the standard solution was prepared with rutin in a series of concentration gradients, and the standard curve was drawn to calculate the flavonoids content. The total flavonoids content of different extracts was expressed as mg of rutin equivalents per g of dry weight of plant material (mg/g).

The linear equation was

$$y = 0.001\ 69x - 0.011\ 21,$$

and the correlation coefficient $R^2 = 0.996$.

1. 4 Determination of total polyphenols content

The total polyphenols content of Dai antidotes was determined by using Folin-Ciocalteu reagent as described by Hossain et al. (2014). Briefly, 0. 2 mL of samples, 6 mL of ethanol and 0. 5 mL of Folin reagent were mixed to the 10 mL volumetric flask. After 5 min, 1. 5 mL of 20% (*w*) Na_2CO_3 was added, dilute with water to volume and incubated at room temperature for 60 min, then absorbance was measured at 765 nm. In the same way, the standard solution was prepared with gallic acid in a series of concentration gradients, and the standard curve was drawn to calculate the polyphenols content, the total polyphenols content of different extracts was expressed as mg of gallic acid equivalents per g of dry weight of plant material (mg/g).

The linear equation was

$$y = 0.\ 037\ 4x - 0.\ 026\ 02,$$

and the correlation coefficient $R^2 = 0.\ 999\ 5$.

1. 5 *In vitro* anti-oxidant activity

The *in vitro* anti-oxidant activities of different polar extracts and decoctions of the three Dai antidotes were evaluated based on ability to scavenge DPPH free radicals, ·OH radicals and ABTS radicals, and vitamin C was used as positive control. Absorbance was determined on an ultraviolet spectrophotometer (UV-2600, Techcomp, Shanghai, China).

The scavenging effects on DPPH free radical was determined by the method as described by Dong et al (2017) with modifications, 3. 0 mL of DPPH(0. 1 mmol/L)was intermingled with 1 mL of each sample and allowed to stand at 37 ℃ for 30 min. The absorbance was then measured at 517 nm.

The scavenging effects on ·OH was determined based on Fenton's reaction as described by Aquino-Martins et al(de Queiroz et al. , 2019) with modifications. 1 mL of samples with different concentrations was mixed with 1 mL 9 mmol/L $FeSO_4$ solution, 9 mmol/L salicylic acid ethanol solution, and 8. 8 mmol/L H_2O_2 solution, respectively, and incubated at 37 ℃ for 30 min, then absorbance was measured at 510 nm.

The protocol of scavenging effects on ABTS free radical was adapted from Re et al. (1999), ABTS reagent (7. 0 mmol/L) was mixed with 2. 45 mmol/L potassium persulfate in a volume ratio of 1:1, and al-

lowing the mixture to stand in the dark at room temperature overnight to obtain an ABTS stock solution. Then the ABTS stock solution was diluted with deionized water to obtain ABTS working solution with an absorbance value of 0. 70 ± 0. 05 at 734 nm. 4. 0 mL of ABTS working solution was intermingled with 1 mL of each sample, and incubated at 37 ℃ in dark for 30 min. The absorbance was then measured at 734 nm.

The scavenging DPPH, ·OH and ABTS free-radical effect according to the following equations

$$\text{Scavenging effect} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% ,$$

where A_0 is the absorbance of the control, A_1 is the absorbance of the samples, A_2 is the absorbance of the sample background.

1. 6 *In vitro* anti-bacterial activity

The anti-bacterial activity of three Dai antidotes were evaluated by determining the MICs, MBCs and ZOI against *E. coli*, *P. aeruginosa* and *S. aureus*, and ethylparaben was used as positive control.

The MICs was determined by microtiter broth dilution method. In brief, 100 μL of bacteria suspension with the dilution of 1 ∶ 10 was inoculated in the 96-well plates, the extracts were diluted serially, then 100 μL of the diluted extracts solutions were added subsequently. The inoculated microplates were incubated under microaerobic conditions at 37 ℃ for 24 h with shaking (100 r/min). The lowest concentration resulting in no visible growth of tested organisms was recognized as MICs.

For determination of MBCs, 10 μL of the bacterial suspension and sample (which shown no visible growth) inoculated onto the appropriated agar and incubated at 37 ℃ for 24 h. The lowest concentration that completely prevented microbial growth in LB broth agar was recognized as MBCs.

To assess ZOI in an agar diffusion model, 50 μL of bacterial lawns were prepared on a nutrient agar plate using the spread plate method. After soaking the sterile double-layer circular filter paper (diameter 6 mm) in each sample solution for 2 h, the filter paper was removed, dried, and gently put it on the corresponding position of the plate. Then, these petri dishes were incubated at 37 ℃ for 24 h. Negative controls were DMSO and H_2O. Then, the ZOI diameter was measured by digital calipers, and was recorded in cm.

1. 7 *In vitro* anti-inflammatory activity

1. 7. 1 Cell culture The RAW 264. 7 cells were cultured in DMEM supplemented with φ=10% FBS and antibiotics (streptomycin 100 U/mL and penicillin 100 U/mL) in a humidified atmosphere of φ=5% CO_2 at 37 °C.

1. 7. 2 Determination of NO, IL-1β, IL-6, TNF-α production According to the literature (Yan et al. , 2021), RAW 264. 7 cells, were seeded in 96 well plates at a density of 1×10^4 cells/mL and incubated for 24 h. Then, the cells were incubated with respective extracts of three Dai antidotes and exposed to LPS (1 μg/mL) for 24 h. The blank control cells were treated with DMEM only. LPS-induced NO production was determined by using Griess reagent, and the absorbance at 540 nm was measured using a microplate reader (Molecular Devices, Flex Station 3). The generation of IL-1β, IL-6, TNF-α was determined by using ELISA Kit, and the absorbance at 450 nm was measured.

1. 8 Statistical analysis

Statistical analysis were performed using SPSS Statistics for Windows, software version 25. 0 (SPSS Inc. , Chicago, IL, USA). A one-way analysis of variance (ANOVA) and the least significant difference test were employed to analyze the data.

2 Results

2. 1 The contents of total flavonoids and polyphenols

As shown in Fig. 2, the contents of total flavonoids in three Dai antidotes (22. 41 to 586. 40 mg/g) were much higher than that of total polyphenols (2. 76 to 28. 66 mg/g), and *Zhu Ye Lan* was higher than *Bin Hao* and *Dai Bai Jie*. In general, with the increase polarity of solvents, the total polyphenols and flavonoids content decreased and the contents in decoctions was higher than that of water extracts.

2. 2 Antioxidant activity

2. 2. 1 Ability to scavenge DPPH radicals All extracts showed dose-dependent DPPH scavenging activity (Fig. 3). In the case of *Bin Hao*, as the concentration increases, extracts with lower polarity showed greater scavenging activity than that higher

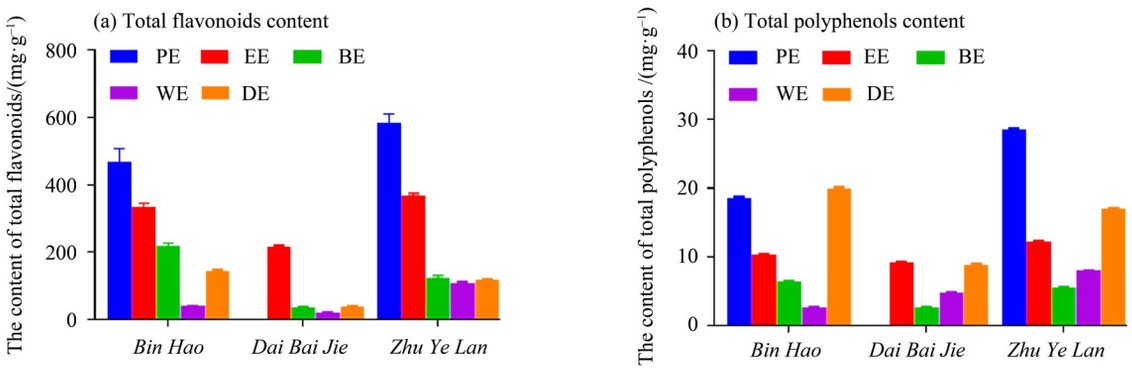


Fig. 2 Contents of total flavonoids and polyphenols in three Dai antidotes

polarity. While, the DE showed the weakest scavenging at every tested concentration (Fig. 3a).

In the case of *Dai Bai Jie*, more polar extracts showed less scavenging activity (Fig. 3b). The DE showed greater scavenging ability than WE, but less than that of EE or BE.

In case of *Zhu Ye Lan*, more polar extracts also showed less scavenging activity (Fig. 3c). The DE showed greater scavenging activity than any of the polar extracts.

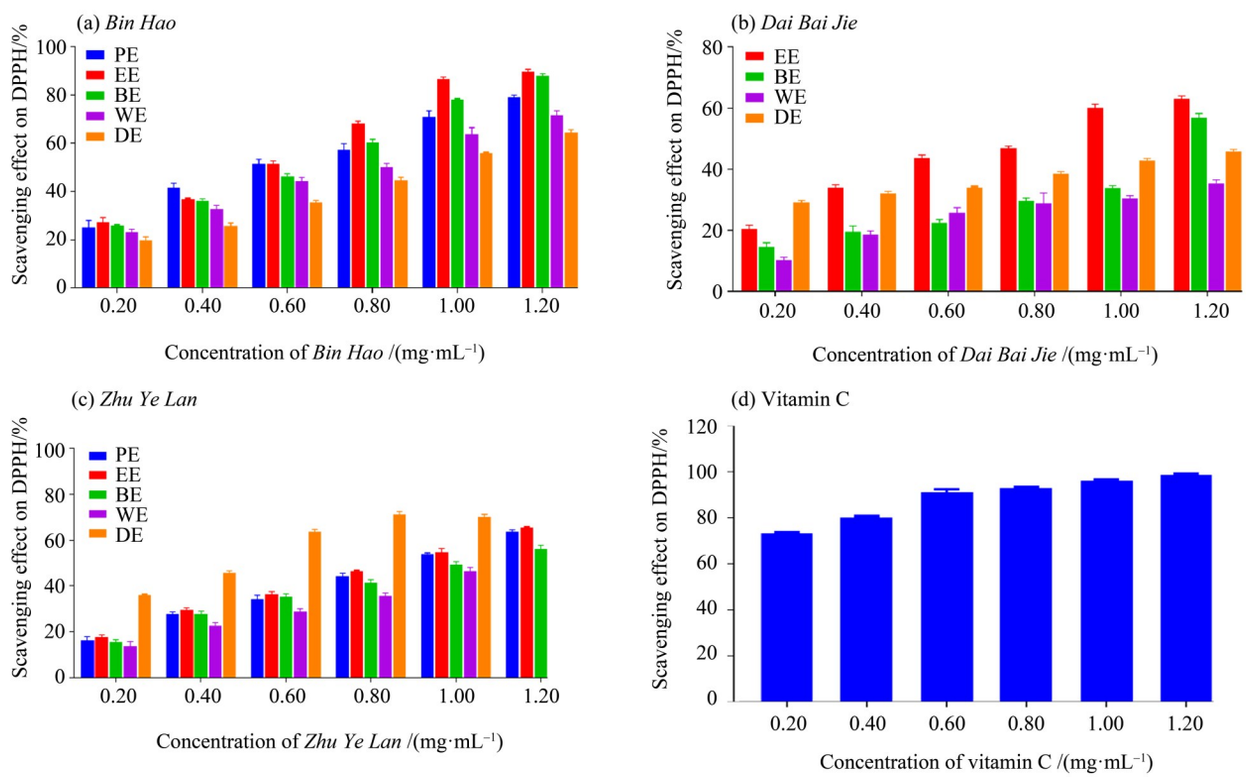


Fig. 3 Ability of various extracts of the Dai antidotes to scavenge DPPH radicals

Furthermore, the activity was quantified in terms of the half-maximal inhibitory concentration (IC_{50}), the results showed that the scavenging DPPH radicals' ability of *Bin Hao* alcohol extracts was better than that of the other two Dai antidotes (Table 1). However, the scavenging DPPH radicals' ability of *Zhu Ye Lan* decoction was better than that of *Bin Hao* or *Dai Bai Jie*. The scavenging DPPH radicals' activity of three Dai antidotes were weaker than vitamin C (IC_{50} 0. 09 mg/mL).

2. 2. 2 Ability to scavenge ·OH radicals Extracts from the three Dai antidotes showed a weak, dose-dependent scavenging activity of ·OH radicals (Fig. 4). In case of *Bin Hao*, polar extracts and decoction

Table 1 Quantitation of DPPH radical scavenging activity by three Dai antidotes mg/mL

Dai antidote	IC_{50}				
	PE	EE	BE	WE	DE
Bin Hao	0. 53	0. 48	0. 53	0. 67	0. 86
Zhu Ye Lan	0. 88	0. 83	1. 01	1. 17	0. 38
Dai Bai Jie	ND[1)]	0. 75	1. 34	2. 55	2. 18

1）Not done.

showed similar scavenging activity (Fig. 4a). In the case of *Dai Bai Jie*, as the polarity increased, the scavenging activity of extracts was decreased (Fig. 4b). The scavenging activities of DE and EE were similar. Similarly, in the case of *Zhu Ye Lan*, greater polarity was associated with weaker scavenging (Fig. 4c), and the scavenging activities of DE were also like EE.

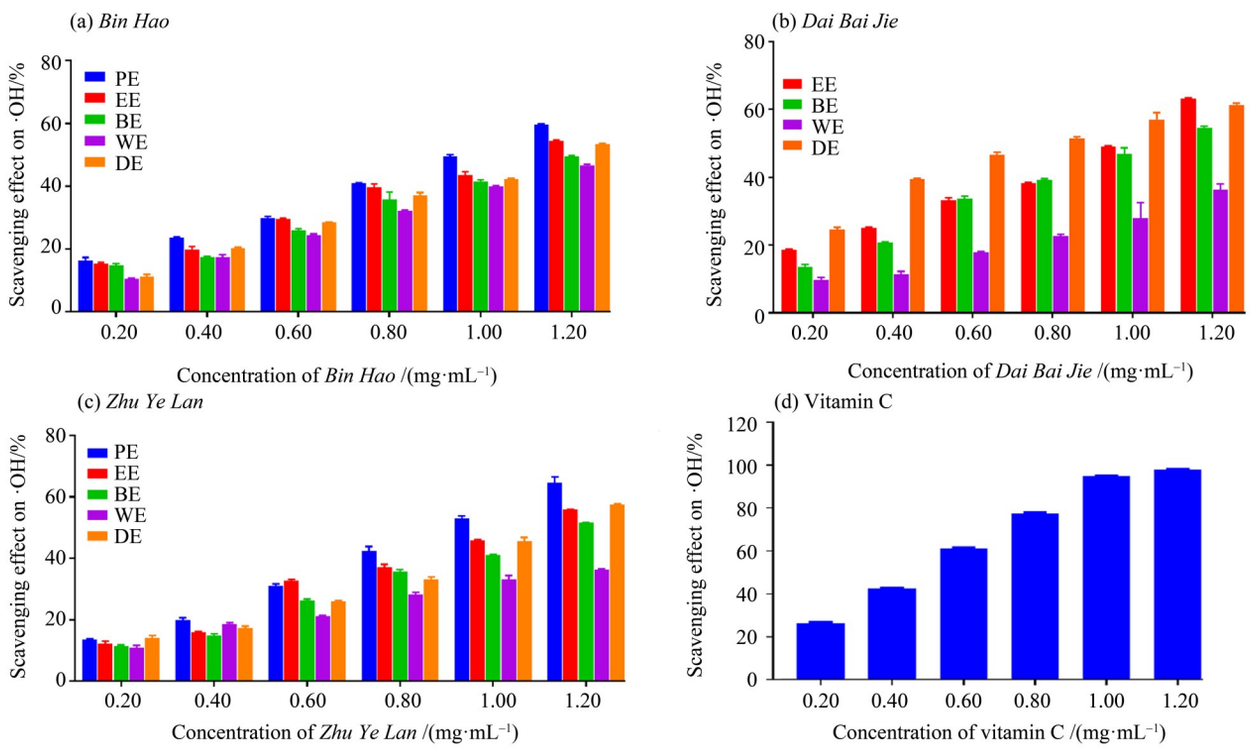


Fig. 4 Ability of Dai antidotes to scavenge ·OH

Comparison of IC_{50} values showed that polar extracts of the three Dai antidotes scavenged ·OH with similar efficacy (Table 2), which were weaker than vitamin C (IC_{50} 0. 42 mg/mL). While for WEs and DEs IC_{50} values ranged from 0. 71 mg/mL for *Dai Bai Jie* DE to >2 mg/mL for WEs of *Zhu Ye Lan* and *Dai Bai Jie*.

2. 2. 3 Ability to scavenge ABTS free radicals All extracts and decoctions of the three Dai antidotes strongly scavenged ABTS radicals (Fig. 5). Scavenging activity differed substantially between extracts of different polarity from the same Dai antidote, while activity was similar between extracts of similar polarity from different Dai antidotes.

Table 2 Quantitation of ·OH scavenging activity by three Dai antidotes mg/mL

Dai antidote	IC_{50}				
	PE	EE	BE	WE	DE
Bin Hao	1. 01	1. 15	1. 32	1. 40	1. 18
Zhu Ye Lan	0. 92	1. 09	1. 22	2. 27	1. 19
Dai Bai Jie	ND[1)]	0. 98	1. 09	2. 15	0. 71

1）Not done.

Comparison of IC_{50} values showed that in general, scavenging strength was greater for *Zhu Ye Lan* than for the other two Dai antidotes. All the BEs showed the strongest scavenging ability (IC_{50}<35 μg/mL, Table 3), among which *Bin Hao* and *Zhu Ye Lan* showed greater scavenging activity than vitamin C (IC_{50} 18. 18 μg/mL). DEs scavenged more weakly than polar extracts (IC_{50}>400 μg/mL).

2. 3 Anti-bacterial activity

2. 3. 1 MICs and MBCs As shown in Table 4, ethylparaben showed good bactericidal effects against

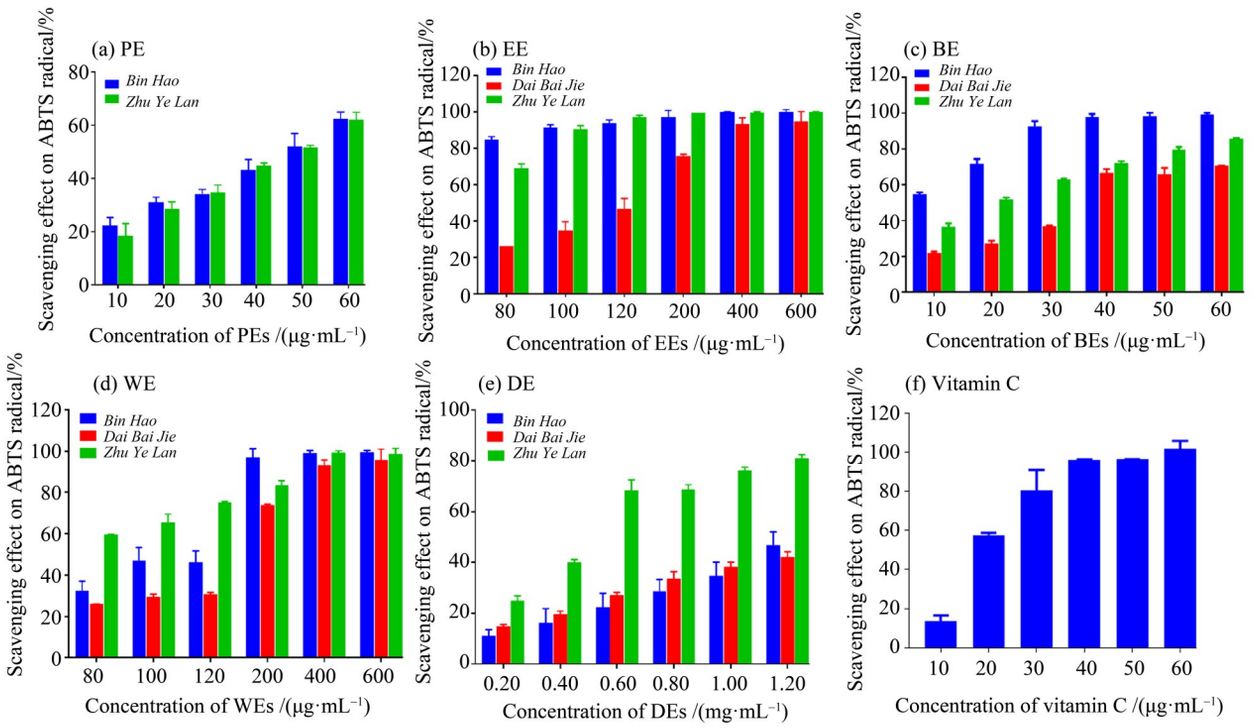


Fig. 5 Ability of Dai antidotes to scavenge ABTS radicals

Table 3 Quantitation of ABTS radical scavenging activity by three Dai antidotes μg/mL

Dai antidote	IC_{50}				
	PE	EE	BE	WE	DE
Bin Hao	45. 95	38. 28	9. 539	108. 4	1523
Zhu Ye Lan	45. 57	70. 60	17. 45	65. 46	445. 6
Dai Bai Jie	ND[1)]	126. 9	33. 15	143. 3	174 8

1) Not done.

three bacteria, among which the anti-bacterial activity against *E. coli* was stronger than *P. aeruginosa* and *S. aureus*. All polar extracts of *Bin Hao* showed bactericidal effects against the three strains, with activity weakening with greater polarity. They were most effective against *E. coli*, DE showed bactericidal activity only against *E. coli*, while WE showed anti-bacterial activity, but no bactericidal effects.

Table 4 MICs and MBCs of ethylparaben and *Bin Hao* extracts against three bacteria [1)] mg/mL

Bacterium	ethylparaben		PE		EE		BE		WE		DE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	0. 195	1. 563	0. 391	3. 125	0. 391	3. 125	0. 195	3. 125	3. 125	–	0. 781	6. 25
P. aeruginosa	0. 391	3. 125	3. 125	25. 0	1. 563	12. 5	1. 563	12. 5	12. 5	–	3. 125	–
S. aureus	1. 0	–	1. 563	6. 25	3. 125	6. 25	12. 5	25. 0	6. 25	–	1. 563	–

1) "–": Not detected.

All polar extracts of *Dai Bai Jie* except BE showed bactericidal effect against only *E. coli* (Table 5). BE had the strongest anti-bacterial effects against all three bacteria, while DE showed no bactericidal effect against any of them.

Zhu Ye Lan extracts also showed stronger effects against *E. coli* than the other two bacteria (Table 6). All polar extracts showed bactericidal effect against *E.*

Table 5 MICs and MBCs of *Dai Bai Jie* extracts against three bacteria [1)] mg/mL

Bacterium	EE		BE		WE		DE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	0. 391	6. 25	0. 195	1. 563	1. 563	12. 5	1. 563	–
P. aeruginosa	3. 125	–	0. 781	6. 25	6. 25	–	3. 125	–
S. aureus	25. 0	–	12. 5	25. 0	6. 25	–	3. 125	–

1）"–"：Not detected.

coli, while PE also showed bactericidal effect against *P. aeruginosa*, and EE and BE also showed bactericidal effect against *S. aureus*. DE did not show bactericidal effect against any of the bacteria.

Table 6 MICs and MBCs of *Zhu Ye Lan* extracts against three bacteria [1)] mg/mL

Bacterium	PE		EE		BE		WE		DE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	0. 391	3. 125	1. 563	12. 5	0. 391	6. 25	3. 125	25. 0	0. 391	–
P. aeruginosa	3. 125	25. 0	6. 25	–	3. 125	–	12. 5	–	1. 563	–
S. aureus	1. 563	–	3. 125	6. 25	12. 5	25. 0	6. 25	–	0. 781	–

1）"–"：Not detected.

The inhibitory effect of all three Dai antidotes to *E. coli* was better than that of the other two strains. *Bin Hao* and *Zhu Ye Lan* had similarly effective, while *Dai Bai Jie* was less effective. WEs and DEs showed the weakest anti-bacterial activity. All the three Dai antidotes showed weaker anti-bacterial activity than that of the positive control.

2. 3. 2 Zones of inhibitions The ZOIs for different Dai antidotes and extracts generally mirrored the trends observed with MICs and MBCs (Fig. 6). Anti-bacterial effect was stronger against *E. coli* than the other two bacteria, and DEs showed negligible anti-bacterial effect.

2. 4 Anti-inflammatory activity

All the three Dai antidotes inhibited NO, TNF-α, IL-1β or IL-6 production in at low extract concentration of 60 μg/mL(Fig. 7) . The inhibition effect of *Bin Hao* on NO production was stronger than *Dai Bai Jie* and *Zhu Ye Lan.* The greater polarity of *Dai Bai Jie* and *Zhu Ye Lan* was associated with weaker inhibition of NO production, EE of *Dai Bai Jie* and PE/EE of *Zhu Ye Lan* significantly inhibited the production of NO((Fig. 7a).

As shown in Fig. 7b, greater polarity of *Bin Hao* extracts was associated with stronger inhibition: WE and DE suppressed production by nearly 70%, whereas BE caused negligible inhibition. As for *Dai Bai Jie*, all extracts significantly inhibited the production of TNF-α. While DE of *Zhu Ye Lan* negligibly inhibited TNF-α production.

As displayed in Fig 7c, EE, BE and DE from *Bin Hao* inhibited IL-1β production by nearly 80%, while PE and WE inhibited it by approximately 70%. Extracts of *Dai Bai Jie* showed weaker inhibition: DE, BE, EE and WE inhibited production by 60% −80%. In contrast, extracts of *Zhu Ye Lan* reduced IL-1β production to nearly undetectable levels.

All extracts and decoctions of all three Dai antidotes strongly inhibited IL-6 production (Fig. 7d). In the case of *Bin Hao,* inhibition increased with polarity, with inhibition ranging from 60% to 100%. Similarly, inhibition by extracts from *Dai Bai Jie* or *Zhu Ye Lan* was greater with greater polarity, with inhibition ranging from 80% to 100%.

3 Discussion

Oxidative stress has been associated with various diseases(de Queiroz et al. , 2019), and many drugs exert therapeutic effects by scavenging free radicals. DPPH, ·OH and ABTS free radical scavenging assay are commonly used to evaluate antioxidant activity. Among them, DPPH and ABTS assay are simple, but the chemical properties of DPPH and ABTS free radical are quite different from the biological environ-

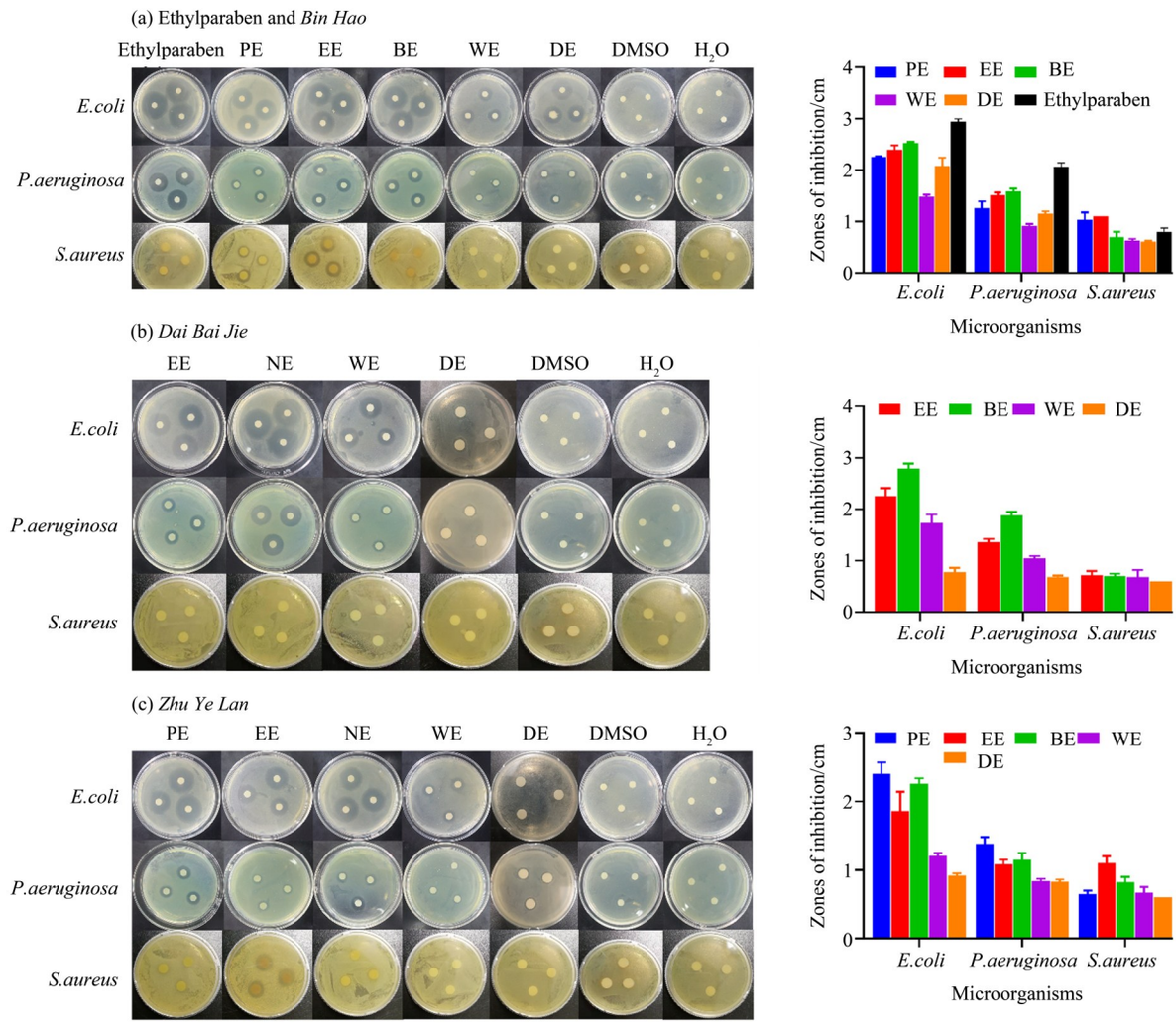
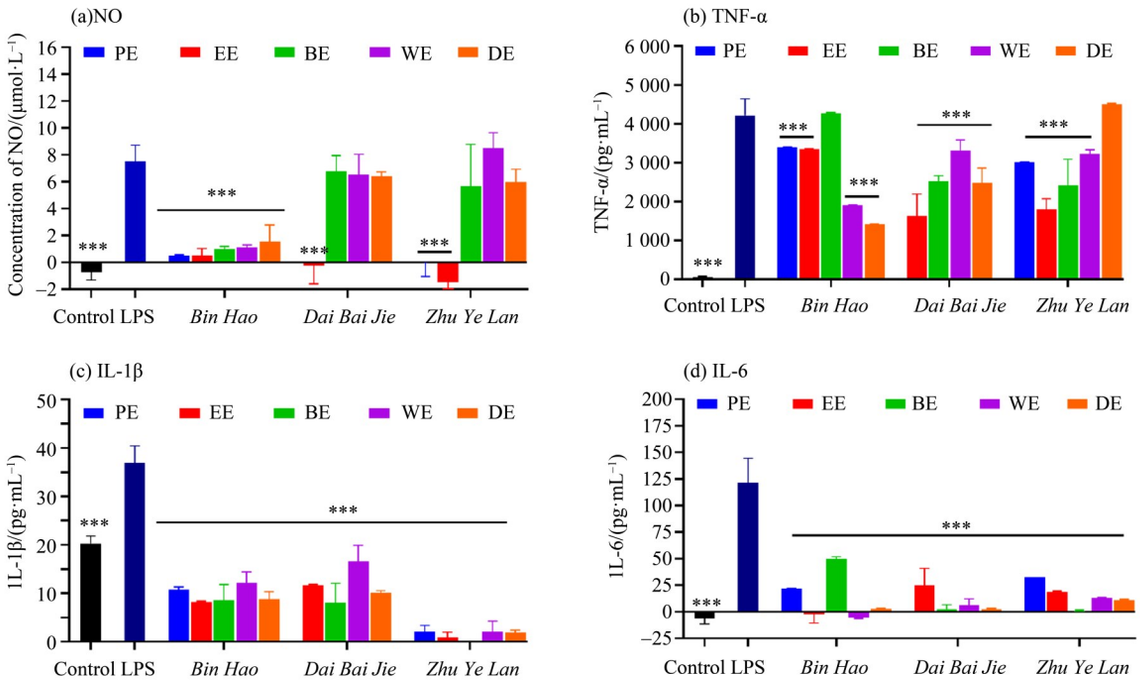


Fig. 6 Inhibition zone assay to assess anti-bacterial activity of various Dai antidotes

ment. ·OH as a common free radical in organism metabolism, which is more close to physiological characteristics (Dong et al. , 2017). Therefore, we comprehensively evaluated the antioxidant activity of three Dai antidotes by investigating their scavenging ability on DPPH, ·OH and ABTS free radicals. The results suggested that the three Dai antidotes showed dose-dependent ability to scavenge DPPH, ·OH and ABTS radicals. They scavenged ABTS radicals most effectively. These results suggested that anti-oxidation may help explain the clinical benefits of Dai antidotes.

Infections, such as those involving the bacteria *E. coli*, *P. aeruginosa* and *S. aureus*, can cause a range of health problems (Wang et al. , 2021). Many medicines can inhibit bacterial growth. Here we showed that the three Dai antidotes, at least at higher extract concentrations, showed some anti-bacterial activity against the three pathogenic strains. This activity was stronger when extracts were less polar. Most WEs and DEs did not show bactericidal effects. The relative inefficacy of the Dai antidotes may reflect low intrinsic bactericidal activity, as well as the presence of sugars in the extracts, which may aid bacterial growth. We conclude that the observed detoxifying effects of the Dai antidotes are not due primarily to anti-bacterial effects.

The signaling molecule NO mediates and regulates inflammatory responses (Yin et al. , 2019), while the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 can lead to tissue damage when their levels become excessive or remain chronically high. Using bacterial LPS to stimulate the production of NO and these cytokines (Gao et al. , 2020), we found that all three Dai antidotes inhibited their production. NO production was inhibited most strongly by *Bin Hao*, while IL-1β production was inhibited most strongly by *Zhu Ye Lan*. The inhibition of three Dai antidotes against IL-6 production were similar. These results may imply



***P<0. 001 *vs*. cultures treated only with LPS.

Fig. 7 Ability of Dai antidotes to inhibit the production of NO, TNF-α, IL-1β and IL-6

that the detoxifying effects of Dai antidotes is related to their anti-inflammatory activity.

As polyphenols and flavonoids have significant antioxidant and anti-inflammatory activities. The results showed that with the increase of polarity the contents decreased, which was correlated with the antioxidant and anti-inflammatory activities of three Dai antidotes. These results suggested that Dai antidotes may have antioxidant and anti-inflammatory activities by containing more polyphenols and flavonoids.

4 Conclusions

Our analysis of the commonly used Dai antidotes showed that all three were effective at scavenging radicals of DPPH, ·OH and ABTS, as well as inhibiting the production of NO, TNF-α, IL-1β and IL-6 by macrophages in response to LPS trigger. However, the Dai antidotes showed weak bactericidal activity against Gram-positive or -negative bacteria. We found that the anti-oxidant, anti-bacterial and anti-inflammatory activities of the Dai antidotes depended on the polarity of the solvent used to extract them. In addition, the contents of total flavonoids and total polyphenols in three Dai antidotes were correlated with the antioxidant and anti-inflammatory activities. These results may also pave the way for the study of components in Dai antidotes and related in-depth research. Our results begin to provide a modern scientific perspective on the clinical efficacy of Dai medicines, and they provide a guide for future studies to optimize the extraction of active compounds from Dai antidotes and other medicines. Ultimately, these studies should examine the safety, efficacy and mechanisms of action of Dai medicines in preclinical models.

Acknowledgements

We thanks to Mrs. Yanfang Lin, Chief Expert of Dai Medicine and Mrs. Xiaohua Li from School of Traditional Dai-Thai Medicine, West Yunnan University of Applied Sciences for identification of plant material.

References:

de QUEIROZ AQUINO-MARTINS V G, MOURA de MELO L F, PEREIRA SILVA L M, et al, 2019. In vitro antioxidant, anti-biofilm, and solar protection activities of *Melocactus zehntneri* (Britton & Rose) Pulp extract [J]. Antioxidants, 8(10):439.

DONG Y R, CHENG S J, QI G H, et al, 2017. Antimicro-

bial and antioxidant activities of *Flammulina velutipes* polysacchrides and polysacchride-iron (III) complex [J]. Carbohydr Polym, 161: 26-32.

GAO X H, ZHANG S D, WANG L T, et al, 2020. Anti-inflammatory effects of neochlorogenic acid extract from mulberry leaf (*Morus alba* L.) against LPS-stimulated inflammatory response through mediating the AMPK/Nrf2 signaling pathway in A549 cells [J]. Molecules, 25(6): 1385.

HOSSAIN M A, KALBANI M S A A, FARSI S A J A, et al, 2014. Comparative study of total phenolics, flavonoids contents and evaluation of antioxidant and antimicrobial activities of different polarities fruits crude extracts of *Datura metel* L [J]. Asian Pacific J Trop Dis, 4 (5): 378-383.

HOSSAIN M A, MIZANUR RAHMAN S M, 2011. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple [J]. Food Res Int, 44(3): 672-676.

LI B, HUANG S M, PANG X H, et al, 2018. Optimization of extraction process of *Clerodendrum philippinum* Schauer var. *simplex* mlodenke total flavonoids (CPTF) by central composite design-response surface methodology [J]. Med Plant, 9(3): 17-20.

LI H T, KANG L P, GUO B L, et al, 2014. Original plant identification of Dai nationality herb "Daibaijie" [J]. China Journal of Chinese Materia Medica, 39(8): 1525-1529.

LI Y, MA G, LV Y, et al , 2019. Efficacy of Obcordata A from *Aspidopterys obcordata* on kidney stones by inhibiting NOX_4 expression [J]. Molecules, 24(10): 1957.

OSZMIANSKI J, WOJDYLO A, JUSZCZYK P, et al, 2020. Roots and leaf extracts of *Dipsacus fullonum* L. and their biological activities [J]. Plants, 9(1): 78.

QU R, LUO W, ZHANG L W, et al, 2011. Determination of the contents of trace elements in *Arundina graminifolia* (D.Don) Hochr [J]. Med Plant, 2(6): 57-58+61.

RE R, PELLEGRINI N, PROTEGGENTE A, et al, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay [J]. Free Radic Biol Med, 26 (9/10): 1231-1237.

WANG L, SUO S, LI J, et al, 2017.An investigation into traditional Chinese Medicine Hospitals in China: Development trend and medical service innovation [J]. Int J Health Policy Manag, 6(1): 19-25.

WANG Q E, GE L, WANG L, et al, 2021. Formulation optimization and in vitro antibacterial ability investigation of azithromycin loaded FDKP microspheres dry powder inhalation [J]. Chin Chem Lett, 32(03): 1071-1076.

YAN Z B, WANG D, AN C M, et al, 2021. The antimicrobial peptide YD attenuates inflammation via miR-155 targeting CASP12 during liver fibrosis [J]. Acta Pharm Sin B, 11(1): 100-111.

YIN L, HAN H, ZHENG X, et al, 2019. Flavonoids analysis and antioxidant, antimicrobial, and anti-inflammatory activities of crude and purified extracts from *Veronicastrum latifolium* [J]. Ind Crops Prod, 137: 652-661.

ZHANG C, CHONGSUVIVATWONG V, KEAWPRADUB N, et al, 2012. Analysis of prescription database extracted from standard textbooks of traditional Dai medicine [J]. J Ethnobiol Ethnomed, 8: 34.

傣药“雅解”：宾蒿、傣百解及竹叶兰的生物活性及其解毒机制

张雪飞[1,2]，赵俊可[2]，郝素旗[2]，丁青[3]，于世慧[2]，伊木朗[2]，王军[2]，胡海燕[2]

1. 滇西应用技术大学傣医药学院，云南 景洪 666100
2. 中山大学药学院，广东 广州 510006
3. 云南傣药有限公司，云南 瑞丽 678699

摘要： 傣药“雅解”是傣族传统医学中最具特色的药物和治疗方法。其中，宾蒿（*Clerodendrum chinense* var. *simplex*）、傣百解（*Marsdenia tenacissima*）和竹叶兰（*Arundina graminifolia*）作为3种常见的“雅解”被广泛使用。本研究使用石油醚、乙酸乙酯、正丁醇或水依次萃取3种“雅解”的乙醇提取物获得各极性部位，并制备3种“雅解”的水煎剂。经测定3种“雅解”的总黄酮质量分数为22.41 ~ 586.39 mg/g，高于总多酚质量分数（2.76 ~ 28.66 mg/g）。3种“雅解”的提取物均可清除 DPPH、·OH 和 ABTS 自由基，其中对 ABTS 自由基的清除率高于其他自由基（IC_{50} > 380 μg/mL）。它们对大肠杆菌、铜绿假单胞菌和金黄色葡萄球菌的抑制作用较弱。此外，3种“雅解”具有一定的抗炎作用，在 60 μg/mL 的质量浓度下即可显著下调脂多糖刺激巨噬细胞产生的NO、肿瘤坏死因子-α、白细胞介素-1β 和白细胞介素-6。综上所述，本研究通过现代科学手段初步揭示了3种“雅解”的解毒作用机理，为“雅解”的进一步优化与研究奠定了基础。

关键词： 傣药“雅解”；宾蒿（*Clerodendrum chinense* var. *simplex*）；傣百解（*Marsdenia tenacissima*）；竹叶兰（*Arundina graminifolia*）；解毒作用

（责任编辑　张　冰）