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Chemical composition in different parts of the ethanol extract from zanthoxylum avicennae and antimicrobial activity

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ABSTRACT

Zanthoxylum avicennae is a species of Rutaceae with some bioactivity. The contents of Total flavonols(TF) and Total phenolic acids(TP) and antifungal activities of the six parts of the ethanol extract of Zanthoxylum avicennae were evaluated. Total flavonols (TF) content was estimated by NaNO2-Al(NO)3 colorimetric assay and Total phenolic acids (TP) content was determined using the Folin–Ciocalteu method with a slight modification,while Minimum inhibitory concentration (MIC) was determined using the tube double-dilution technique. The results of the experiments can find the 40% n-BuOH fraction has the strongest antifungal activity, meanwhile having the highest contents of TF and TP,thus providing scientific data for Zanthoxylum avicennae further development and exploitation.

KEYWORDS

Zanthoxylum avicennae; TP; TF; MIC.

INTRODUCTION

Zanthoxylum avicennae (Lam.) DC (Rutaceae) is an 15m high shrub[1] distributed in Fujian, Guangdong, Guangxi, Hainan, Yunnan, India, Indonesia, Malaysia, Philippines, Thailand, Vietnam[2]. Decoction of its stems is used as a stomach tonic and as a counter-poison to snake bite[3]. *Zanthoxylum* L. shown inhibitory action against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,Proteus vulgaris*, *Aspergillus flavus* and *A.parasiticus*[4], At home and abroad on the chemical composition of *Zanthoxylum avicennae* of Hainan of China and Vietnam has done a lot of research[5-10]. Hainan of China has been rich in resources of *Zanthoxylum avicennae*, which has widly variety of biologically active ingredients and extensive pharmacological activity,looking for more of the active ingredient from *Zanthoxylum avicennae*, is able to provide more scientific basis for comprehensive developing and utilization of *Zanthoxylum avicennae*. In this study, the total flavonoids and total phenolic contents of the six parts of the ethanol extract of *Zanthoxylum avicennae* and the determination of minimum inhibitory concentration to determine the active frucation of the six parts of the ethanol extract of *Zanthoxylum avicennae,*which providing a theoretical basis for the development and utilization of *Zanthoxylum avicennae*.

EXPERIMENTAL

Macrofungi materials and Chemicals: *Exserohilum turcicum(E.t), Fusarium graminearum Schwabe (F.s), Fusarium graminearum (F.g), Botrytis cinerea (B.c), Fusarium oxysporum (F.o), Rhizoctonia solani (R.s), Sclerotinia sclerotiorum (S.s)* were provided by the microbiology laboratory in Zhejiang Agriculture and Forestry University. Folin-Ciocalteu reagent; Gallic acid; Rutin; all the chemicals used were of analytical grade.

Sample

Whole plants of *Zanthoxylum avicennae* were collected from Sanya city, Hainan province, China, in September, 2011 by Pro. Huang Shiman of Hainan Univercity. A voucher specimen of this plant has been deposited in our laboratory.

Plant materials and extraction

Aired-dried and powdered whole plants (6 Kg) were extracted with 95% EtoH by percolation. The extract was concentrated by vacuum thin film below 50°C[11]. Then extracts after concentration were dissolved in H_2O and extracted with EtoAc and n-BuOH. Successively. The n-BuOH layer was added to Diaion HP-20 macroporous resin column, then the resin was washed by distilled water to get rid of impurity, then washed by H_2O, 20%, 40% and 60% methanol individually, and obtained H_2O, 20% n-BuOH, 40% n-BuOH and 60% n-BuOH fraction respectively. The water eluate was abandoned, and the other eluates were concentrated for using.

Determination of the contents of TF

Total flavonols (TF) content was estimated by $NaNO_2$-$Al(NO)_3$ colorimetric assay, TF content was calculated using the calibration curve of rutin solution (0.1630 mg/mL), while the absorbance as the abscissa axis and the rutin content as the vertical axis, Y=10.865X-0.0052, R^2=0.9995, as is shown in Figure 1, The sample of the six parts of the ethanol extract of *Zanthoxylum avicennae* were analyzed by this procedure, and expressed as milligrams of rutin equivalent (RE) per gram of the sample.

Determination of the contents of TP

Total phenolic acids (TP) content was determined using the Folin-Ciocalteu method[12] with a slight modification, while the absorbance as the abscissa axis and the gallic acid (GA) content as the vertical axis, Y=64.746X+0.0827, R^2=0.9992, as is shown in Figure 2, The sample of the six parts of the

ethanol extract of *Zanthoxylum avicennae* were analyzed by this procedure, and expressed as milligrams of gallic acid equivalent (GAE) per gram of the sample.

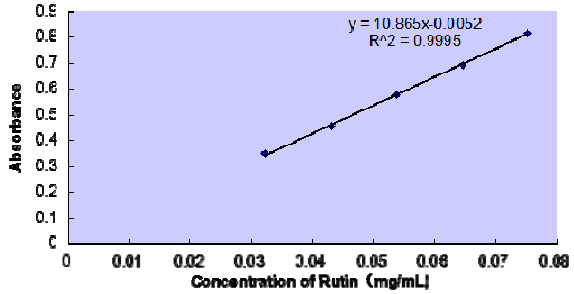

Figure 1 : The standard curve of Rutin

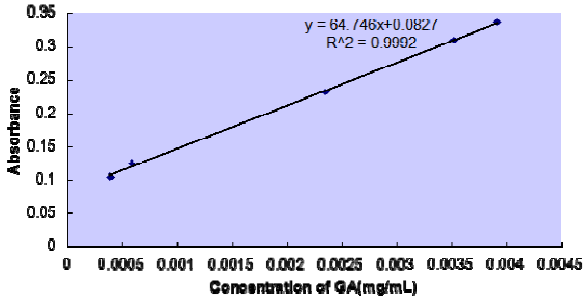

Figure 2 : The standard curve of GAE

Determination of the MIC: Minimum inhibitory concentration (MIC) was determined using the tube double-dilution technique[13], The antifungal effects of the six parts of the ethanol extract of *Zanthoxylum avicennae* were tested against *Exserohilum turcicum (E.t), Fusarium graminearum Schwabe (F.s), Fusarium graminearum(F.g), Botrytis cinerea(B.c), Fusarium oxysporum(F.o), Rhizoctonia solani(R.s), Sclerotinia sclerotiorum(S.s)*. Sets of slant tubes were prepared with potato dextrose agar (PDA) to which appropriate volumes of a solution containing 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 $\mu g \cdot mL^{-1}$ of the sample powder in DMSO had been added.The MIC was assessed as the lowest concentration of the six sample able to inhibit the visible growth of the microorganisms. The experiments were conducted in triplicate.

The Experimental Results and Its Analysis: Flavonoid has play a great role in anti-inflammatory[14], anti-tumor[15-16], antioxidant[17], inhibit fungal[18] as well as lower blood and liver cholesterol[19]. etermination the total flavonoid content of the six parts of the ethanol extract of *Zanthoxylum avicennae* was estimated by $NaNO_2$-Al $(NO)_3$ colorimetric assay, compared to other parts, the 40% n-BuOH fraction contains the highest content of total flavonoids, as is shown in Figure 3(A). Phenolic compounds are widely found in plant secondary metabolites of fruits, vegetables and cereals, a variety of biological activity, phenolic acids on plant pathogenic fungi inhibitory activity, on Microcystis aeruginosa have allelopathic Especially to play a very important role in the prevention of cardiovascular, cancer and aging. Determination the total Phenolic content of the six parts of the ethanol extract of *Zanthoxylum avicennae* was estimated by Folin-Ciocalteu assay with a slight modification, compared to other parts, the 40% n-BuOH fraction contains the highest content of total phenolic acids, as is shown in Figure 3 (B).

The determinant of MIC: Minimum inhibitory concentration (MIC) was determined using the tube double-dilution technique. The MIC data of the six parts of the ethanol extract of *Zanthoxylum avicennae* is showed in TABLE-1. From TABLE1, it can be concluded that the antifungal effect of 40% n-BuOH fraction is the strongest against *Fusarium graminearum Schwabe*, *Fusarium graminearum*, *Fusarium oxysporum*, as the MIC data is 3.91; the antifungal effect of ethyl acetate fraction is just

weaker than 40% n-BuOH fraction,but the effect against *Fusarium graminearum Schwabe*, as the MIC data is 7.81.

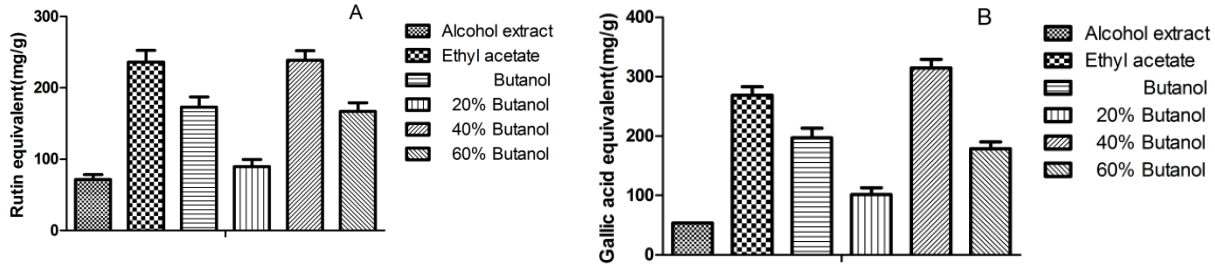

Figure 3 : The contents of TP and TF

TABLE 1 : The MIC data of the six parts of the ethanol extract of *Zanthoxylum avicennae*

phytopathogen	The MIC data of the six parts of the ethanol extract of *Zanthoxylum avicennae*					
	95% EtoH fraction	EtoAc fraction	n-BuOH fraction	20% n-BuOH fraction	40% n-BuOH fraction	60% n-BuOH fraction
E.t	250	15.63	125	125	7.81	—
F.g	250	62.5	15.625	—	3.91	125
F.s	—	7.81	31.25	62.5	3.91	125
B.c	250	31.25	—	250	62.5	—
R.s	—	62.5	—	—	31.25	125
F.o	250	15.63	31.25	62.5	3.91	62.5
S.s	250	62.5	125	125	15.63	125

That the content of total flavonoids and total phenolic and antifungal activity of the six parts of the ethanol extract of *Zanthoxylum avicennae* is shown in TABLE 2. Between different parts of the active ingredient content and antifungal activity has been significantly correlated. Inhibitory effect was significantly positively compared to the total flavonoids content, as correlation indexes were greater than 0.8, expect Fusarium graminearum. Inhibitory effect was significantly positive correlation compared to the total phenolic content, as correlation indexes were greater than 0.8, expect Fusarium graminearum and Fusarium graminearum Schwabe.

TABLE 2 : Correlation index between active ingredient content and antifungal activity

Correlation index	*E.t*	*F.g*	*F.s*	*B.c*	*R.s*	*F.o*	*S.s*
The total flavonoids	0.924*	0.875	0.916*	0.992**	0.997**	0.897*	0.872*
The total phenolic	0.940*	0.875	0.856	0.973*	0.993**	0.892*	0.923**

***Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level.**

CONCLUSION

This study found that 40% n-BuOH parts has the highest content of total flavonoids and phenolic acids, and the strongest antifungal activity than the other six parts of the ethanol extract of *Zanthoxylum avicennae*, which is enriched in contents of total flavonoids and phenolic acids. The 40% n-BuOH parts can be as a valid part of the *Zanthoxylum avicennae* extracting flavonoids and phenolic acids. It is found that the inhibitory effect has significantly positive correlation comparing to the content of total flavonoids and total phenolic, indicating that total flavonoids and total phenolic acids are the main antifungal ingredient in ethanol extract of *Zanthoxylum avicennae.*

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