Journal of Medicinal Plants Research Vol. 3(8), pp. 596-599, August, 2009
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2009 Academic Journals

Full Length Research Paper

Comparison of antitussive, expectorant and antiasthmatic activities of different extracts from *Ficus microcarpa*

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Accepted 30 July, 2009

The antitussive, expectorant and antiasthmatic activities of different extracts from *Ficus microcarpa* were compared. The water fraction was purified by a ceramic membrane to give membrane fraction. The evaporated water extract was extracted with MeOH, EtOAc, n-BuOH, respectively to obtain responding fractions. The animal experimental results showed the membrane fraction had remarkable antitussive and expectorant activities. The water and MeOH fractions can obviously prolong latency time of cavy and were regarded as effective antiasthmatic fractions.

Key words: *Ficus microcarpa*, antitussive, antiasthmaic, expectorant, extract.

INTRODUCTION

Ficus microcarpa belongs to the genus Moraceae, which is also called Wannianqing in Chinese and its aerial root and leaves which have been used as traditional folk medicine for flu, malaria, acute enteritis, tonsillitis, bronchitis, and rheumatism. *F. microcarpa* is a popular ornamental tree grown widely in many tropical regions of the world, such as India, Southern China, Ryukyu Islands, Australia and Okinawa Islands (Ao et al., 2008). The extracts from *F. microcarpa* were reported to have antioxidant (Rahman et al., 1994), antibacterial (Ao et al., 2008), anticancer (Chiang et al., 2005), and hypoglycemic activities (Mandal et al., 1997). Flavonoids, triterpenoids, acyclic compounds and steroid are the main components found in the leaves of *F. microcarpa* (Li and Kuo, 1997; 1998; 2000; Chiang and Kuo, 2002; 2003; Chiang et al., 2005; Xu et al., 2009). The aim of the present study was to investigate the antitussive, antiasthmatic and expectorant activities of different extracts from *F. microcarpa.*

MATERIALS AND METHODS

Chemicals and materials

Leaves of *F. microcarpa* were collected on the campus of Guangxi University, Nan-ning, Guangxi, in August 2007. The plant was identified by College of Pharmacy, Guangxi traditional Chinese Medical University. All reagents and solvents were of analytical reagent grade and used without further purification unless otherwise noted. All aqueous solutions were prepared using deionized water. Mice and cavy were provided by Guangxi Medical University.

Preparation of extracts

One kilogram of raw material was extracted three times with 10000 ml H_2O for 24 h at room temperature. And then 2000 ml water extract was evaporated in vacuum at 60℃ to give a black residue (water fraction) and 8000 ml water extract was then divided into two parts. One part was purified by a ceramic membrane and concentrated under reduced pressure on a rotary evaporator to give a black residue (membrane fraction). The other part was evaporated in vacuum at 60℃ and then extracted with MeOH, EtOAc, and n-BuOH, respectively. The solvents in MeOH extract, EtOAc extract and n-BuOH extract were removed under reduced pressure and the residues were dried in vacuum at 60℃ to obtained MeOH fraction, EtOAc fraction and n-BuOH fraction. The extraction process is shown in Figure 1.

Antitussive activity of extracts

Sixty male mice and sixty female mice were divided into twelve groups (n = 10). The control group (n = 10) received saline and other groups (n = 10) received single dose of extract (100 and 200 mg/kg) through the stomach for three days respectively. Pentoxyverine citrate (Ke Bi Qing, 50 mg/kg) was used as positive control. One hour after the last drug administration, the mice (n = 10) were placed in a

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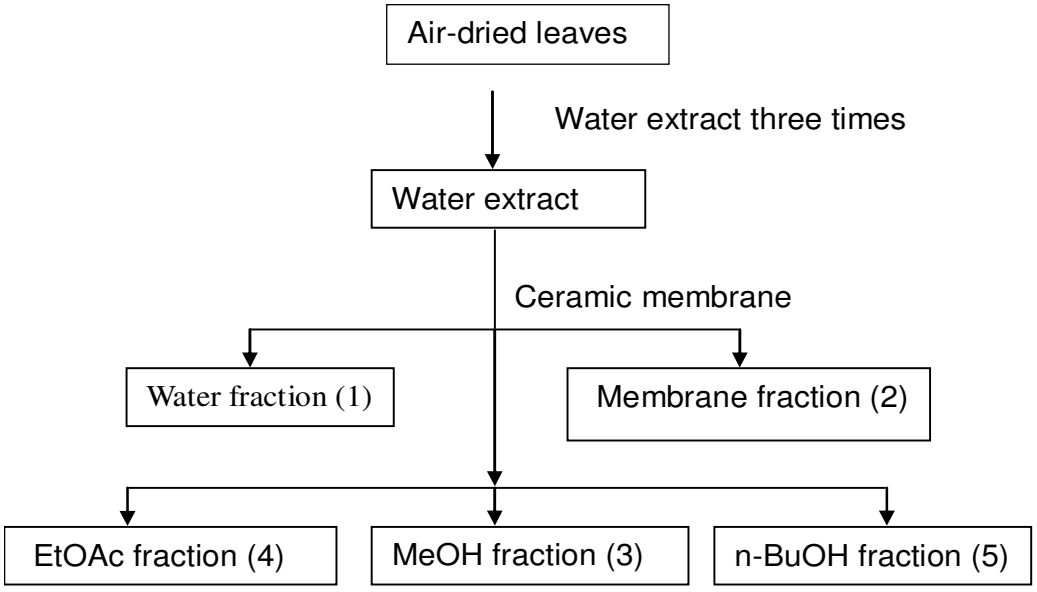

Figure 1. The chart of extraction process.

glass chamber and exposed to 20% ammonia. The cough frequency (NE/3 min) and the cough latency period were evaluated for these five fractions.

Antiasthmatic activity of extracts

Cavies (180 - 220 g) were divided into twelve groups (n = 10). The control group (n = 10) received saline and other groups (n = 10) received single dose of extract (100 and 200 mg/kg) through the stomach for three days respectively. Hexadecadrol (5 mg/kg) was used as positive control. One hour after the last drug administration, the cavies were placed in a glass chamber and exposed to 2% acetylcholine and 0.1% histamine phosphate (1:1, v/v). The asthmatic latency period was measured. If the asthmatic latency period was longer than 6 min, it was recorded 6 min.

Expectorant activity of extracts

Mice (18 - 22 g) were divided into twelve groups (n =10). The control group received saline and other groups (n = 10) received single dose of extract (100 and 200 mg/kg) through the stomach for three days respectively. Ammonia chloride solution (1 mg/kg) was used as positive control. One hour after the last drug administration, 5% of phenol red in saline (500 mg/kg) were inject into the abdomen of mice. And then the mice were killed after 30 min. The windpipe of the dead mice were peel off and then put into 3.0 ml of saline. 0.3 ml NaOH (1 mol/L) was added and mixed for 30 min. The optical density (OD) values were measured on a 722 spectrophotometer with the wavelength of 546 nm. The excretion of phenol red was determined according to the standard curve.

Statistics

Data were shown as the mean ± SD for the experiments and statistical significance was analyzed by the Student's unpaired t-test. It was considered as significant difference when $P < 0.05$.

RESULT AND DISCUSSION

Antitussive activity of different extracts

As shown in Table 1, the positive control group (Ke Bi Qing, 50 mg/kg) showed remarkable antitussive effect on the cough latency period and the cough frequency. The membrane fraction group (200 mg/kg) can obviously prolong the cough latency period and reduce the cough frequency of mice. And compared with the result of normal reference, there is also obvious difference. The n-BuOH fraction only reduced the cough frequency of mice.

Anti-asthmatic activity of different extracts

As shown in Table 2, the positive control group (hexadecadrol, 5 mg/kg) prolonged remarkably the latency period of cavy. The water fraction and MeOH fraction groups can obviously prolong the latency period of cavy. The water fraction (200 mg/kg) group has similar effect with the positive control group. The MeOH fraction (100 and 200 mg/kg) groups have stronger effect than the positive control group of cavy.

Expectorant activity of different extracts

Expectorant effect of different extracts mice was evaluated by the phenol red excretion quantities of mice. OD values of phenol red standard solution (0.01 - 10.00 mg/l) were measured with the wavelength of 546 nm. From the results shown in Table 3, it can be seen that there were a good

Table 1. Antitussive effect of extracts from *F. microcarpa* (*P < 0.05).

Group	Dose (mg/kg)	n	Latency (s)	Frequency of cough
Saline	200	10	27.60 ± 3.31	37.0 ± 5.5
Ke Bi Qing	50	10	32.20 ± 2.20*	23.5 ± 2.3*
Water fraction	100	10	27.90 ± 6.57	36.2 ± 3.5
	200	10	24.20 ± 4.87	33.0 ± 3.8
n-BuOH fraction	100	10	24.70 ± 5.33	30.2 ± 6.6*
	200	10	26.20 ± 5.33	33.4 ± 8.5
EtOAc fraction	100	10	23.60 ± 6.87	37.3 ± 3.1
	200	10	24.10 ± 4.43	34.0 ± 8.0
MeOH fraction	100	10	22.50 ± 4.55	39.0 ± 6.1
	200	10	29.30 ± 5.17	33.7 ± 3.8
Membrane fraction	100	10	23.10 ± 9.60	36.2 ± 4.6
	200	10	31.90 ± 5.04*	31.9 ± 4.8*

Table 2. Antiasthmatic effect of extracts from *F. microcarpa* (P < 0.05).

Group	Dose (mg/kg)	n	Latency before drug (s)	Latency after drug (s)
Saline		10	83.50 ± 22.00	76.4 ± 23.42
hexadecadrol	5	10	78.00 ± 22.30	141.10 ± 83.45*
Water fraction	100	10	80.70 ± 18.05	121.90 ± 64.09*
	200	10	81.30 ± 19.39	140.50 ± 70.09*
n-BuOH fraction	100	10	80.20 ± 21.20	106.70 ± 46.64
	200	10	81.40 ± 21.28	100.80 ± 47.84
EtOAc fraction	100	10	82.50 ± 19.35	84.60 ± 21.26
	200	10	82.10 ± 19.26	105.10 ± 38.87
MeOH fraction	100	10	83.50 ± 26.78	154.70 ± 109.41*
	200	10	82.00 ± 20.56	172.30 ± 116.99*
Membrane fraction	100	10	83.20 ± 21.80	84.40 ± 35.29
	200	10	80.40 ± 24.26	86.70 ± 25.35

Table 3. The expectorant effect of extracts from *F. microcarpa* (*P < 0.05).

Group	Dose (mg/kg)	OD value	Excretion (mg/l)
Saline		0.04 ± 0.02	0.62 ± 0.37
Ammonium chloride	1	0.07 ± 0.02*	1.11 ± 0.66*
Water fraction	100	0.03 ± 0.02	0.49 ± 0.32
	200	0.04±0.02	0.59 ± 0.31
n-BuOH fraction	100	0.05 ± 0.04	0.80 ± 0.69
	200	0.05 ± 0.02	0.78 ± 0.28
EtOAc fraction	100	0.05 ± 0.03	0.82 ± 0.54
	200	0.03 ± 0.01	0.54 ± 0.21
MeOH fraction	100	0.03 ± 0.02	0.47 ± 0.32
	200	0.03 ± 0.01	0.51 ± 0.31
Membrane fraction	100	0.06 ± 0.03	0.91 ± 0.55
	200	0.06 ± 0.02*	1.01 ± 0.39*

line relation between OD value and concentration of phenol red solution. Figure 2 showed the standard curve.

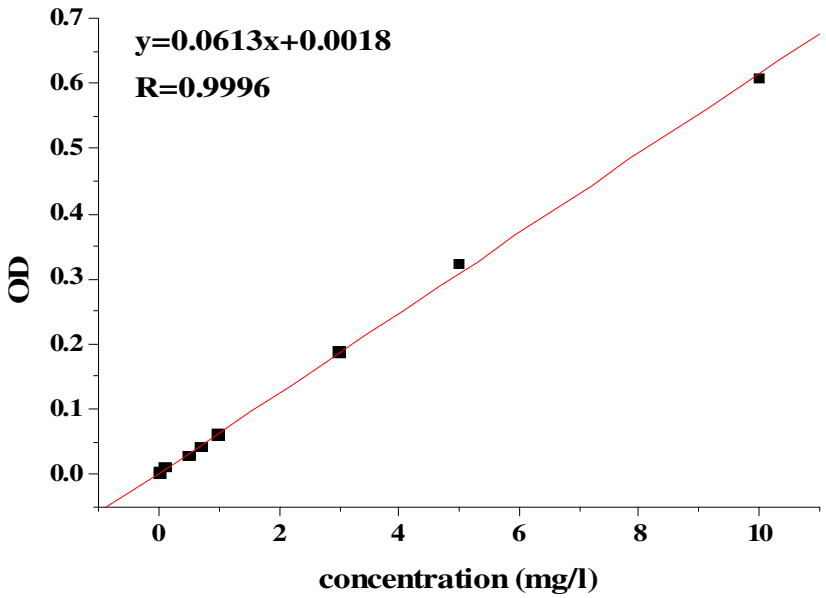

Figure 2. The standard curve of phenol red solution determined at 546 nm.

As shown in Table 3, the positive control group (ammonium chloride, 1 mg/kg) improved remarkably the excretion of phenol red in mice. Among these five extracts from *F. microcarpa*, only the membrane fraction group (100 and 200 mg/kg) can obviously increase the excretion of phenol red in mice.

Conclusion

In summary, the results obtained in this study clearly demonstrate that the extracts/fractions of *F. microcarpa* showed different activities such as antitussive, anti-asthmatic and expectorant activities. The animal experimental results showed the membrane filter fraction had remarkable antitussive and expectorant activities. The water and methanol fractions can obviously prolong latency time of cavy and were regarded as effective antiasthmatic fractions. The results obtained in the study will provide basic data for further development and utilization of *F .microcarpa.*

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support by the Returned Science Foundation of Guangxi Province (SFG0731001), the department of education of Guangxi Province (200710MS050), Graduate innovation Fund of Guangxi Province; Science and Technology research development of Guangxi Province (815005-1-3): Science and Technology research development of Nanning city (200801034C).

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