ANTIMICROBIAL ACTIVITIES OF *PORTULACA OLERACEA* LINN IN VITRO

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The in vitro antibacterial activities of Pak Bia Yai (Portulacaoleracea Linn.) were tested against Staphylococcus aureus, Escherichiacoli, Salmonella typhimurium, Shigella sonnei and Pseudomonas aeruginosa;using Oxford Cup assay method and dilution technique method. Theantiamoebic activity was tested against Entamoeba histolytica isolatedfrom dysenteric patients from Songklanagarin hospital. The resultsrevealed that the plant could kill both the bacteria and the protozoa. Itis suggested that Pak Bia Yai might be an effective broad-spectrum antimicrobial agent.

KEY WORDS: PAK BIA YAI, Portulaca oleracea Linn, ANTIMICROBIAL ACTIVITY, ANTIBACTERIAL ACTIVITY, ANTIAMOEBIC ACTIVITY

INTRODUCTION

Diarrhoea, amoebic dysentery, abcess and wound ulcers are among themajor health problems in Thailand. Bacterial infection is the most common cause of diarrhoea; the causative agents could be either Staphylococcus aureus, Escherichia coli, Salmonella typhimurium or Shigella sonnei. Nosocomial infection with S. aureus or Pseudomonas aeruginosa may lead to abcess and wound ulcers. Entamoeba histolytica is a protozoan pathogen of worldwide importance known for its ability to produce amoebic dysentery and liver abcess[1]. Each year Thailand has spent a lot of money for anti-microbial drugs for the treatment of these infectious diseases. Yetpatients have to take a risk of side effects or toxicities of the drugs such as antiamoebic agents like emetine (cardiotoxic) and metronidazole (teratogenic and carcinogenic)[2].

Portulaca oleracea Linn. or Pak Bia Yai (Thai name) is a small succulent plant widely distributed throughout Thailand. The plant has been claimed to be effective for treatment of the above mention infections[3,4] However, it is not known whether it can kill the pathogenic organisms. Therefore, this experiment is designed to determine the in vitro antimicrobial activity of the plant preparations against S. aureus, E. coli, S. sonnei, S. typhimurium, P. aeruginosa and E. histolytica.

MATERIALS AND METHODS

Preparation of P. oleracea Linn. extract

The plant used for this experiment was collected from local area in Songkhla province. Crude extracts of the plant were fresh extract (watery juice squeezed from the fresh plant) and boiled extract. The extracts were prepared as follow: roots of the plants were removed and the rest were cleaned with tap water and soaked in potassium permanganate solution for 15 minutes. The plants were then washed with distilled water and left to dry out at room temperature and chopped into small pieces. For the preparation of the fresh extract, the chopped plants were blended and the watery juice was squeezed from them. For the boiled extract, distilled water in the volume of three times of the chopped plant weight was added

and the mixture was boiled until the volume of water decrease to about one-third. The watery juice and the boiled extract were centrifuged for 15 minutes at 3,000 rpm. The supernatant was filtered with filter paper. The concentrations of both fresh and boiled extracts were adjusted with distilled water to 1.5 gm-ml (calculated on the fresh plant weight basis) and then evaporated at 45-50°C until the final concentrations of the fresh and the boiled extracts were 3, 4.5, 6 and 12 gm-ml.

Collection and cultivation of E. histolytica

Strains of E. histolytica were isolated from 5 dysenteric patients from Songklanagarin hospital, Songkhla province, who had blood mucus stool with trophozoites. They were cultivated in the Modified Boeck and Drbohlav's Diphasic medium. Subcultures were done every 48 hours interval.In the medium used for cultivation and drug testing, 0.5 ml of a mixture of streptomycin and penicillin (0.5 mg of each-ml) was added.

Testing procedure for Antiamoebic activity.

E. histolytica, growing satisfactorily in the medium in association with the normal flora of age from 24-48 hours were utilized. The amoebae were suspended in Phosphate buffer saline to give approximately 10,000 trophozoites per ml. When testing, the various concentrations of drug or plant preparations in volume of 2.0 ml and 5,000 trophozoites were dispensed in 2 ml of new culture medium. The tubes were incubated at 37° C. The tests were designed for recording the results at 24 and 48 hours after innoculation. Each test consisted of 2 subsets with duplications. The results were recorded at 24 and 48 hours after innoculation. Amoebicidal activity was determined by the presence or absence of living amoebae observed directly under the microscope and by subculture. The concentration that gave zero amoebic count and confirmed by subculture was a minimum inhibitory concentration (MIC).

Testing procedure for Antibacterial activity.

The antibacterial activity of the plant extracts were tested on both gram positive and gram negative bacteria. The bacteria used in this experiment were Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Shigella sonnei, Salmonella typhimurium and Pseudomonas aeruginosa.Two techniques were used for the test i.e. agar diffusion and broth dilution technique. The first technique was performed according to Oxford cup assay method[5] and diameter of clear zone was measured. For broth dilution technique method, it followed those described by Barry A.L.[6] and the minimum inhibitory concentration (MIC) was determined.

RESULTS

Antiamoebic activity.

Both extracts of P. oleracea Linn. killed all E. histolytic isolated from 5 patients with MIC of about 2 gm-ml of the fresh extract and 4 gm-ml of the boiled extract. Metronidazole, one of the current drugs use for treatment of amoebic dysentery killed E. histolytica isolated from 5 patients with different MICs (mcg-ml), i.e.1 for E. histolytica isolated from patient No. 1, 5 for those of No. 2 and 3 and 10 for those of No. 4 and 5 (Table 1).

Antibacterial Activity.

The extracts of P. oleracea Linn. prevented the growth of all strains of bacteria under study
with a concentration-related fashion. The acivity of the boiled extract was slightly lower than that of the fresh one (Tabe 2). Experiment with broth dilution technique showed that the extracts could kill all five bacteria with the order of sensitivity as follow: S. aureus〉E. coli〉 S. typhimurium〉 S. sonnei 〉P. aeruginosa. The antibacterial activity of the fresh extract was about 2-time that of the boiled extract (Table 3).

Table 1 Minimum inhibitory concentration (MIC) of metronidazole, the fresh extract and the boiled extracts of P. oleracea Linn. on E. histolytica isolated from 5 patients.

E. histolytic from patient No.	Minimum Inhibitory Concentration (MIC)		
	Metronidazole (mcg/ml)	Fresh extract (gm/ml)	Boiled extract (gm/ml)
1	1	2	3.2
2	5	1.6	4
3	5	1.6	4
4	10	2	4
5	10	2	4

Table 2 Antimicrobial activity of P. oleracea Linn. against S. aureus, E. coli, S. typhimurium, S. sonnei, and P. aeruginosa determined by diameter of clear zone.

Bacteria	Concentration of extracts (gm/ml)	Avge. diameter of clear zone (mm)	
		Fresh extract	Boiled extract
Staphyllococcus	1.5	8.1	8.0
aureus (ATCC 25923)	6.0	17.2	14.1
Escherichia coli	1.5	8.0	7.8
(ATCC 25922)	6.0	15.9	14.1
Salmonella typhimu-	1.5	7.9	7.5
rium	6.0	14.2	12.9
Shigella sonnei	1.5	7.7	7.1
	6.0	13.5	12.5
Pseudomonas aerugi-	1.5	7.6	7.0
nosa	6.0	12.6	10.9

Table 3 Minimum inhibitory concentration (MIC) (mg-ml) of P. oleracea Linn. extracts against Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Shigella sonnei and Pseudomonas aeruginosa.

	MIC (mg-ml) against				
Bacteria P. oleracea Linn.	S. aureus	E. coli	S. typhimurium	S. sonnei	P. aeruginosa
Fresh extract	6	12	94	94	375
Boiled extract	12	23	187	187	750

DISCUSSION AND CONCLUSION

The results revealed that both plant extracts were effectively killed both protozoa and bacteria. The minimum inhibitory concentration of metronidazole obtained from this study was higher than those reported by other investigators[7-9]. This might be due to the medium used for the test which was egg-slant, compare to those monophasic all liquid medium in other experiments. It was possible that the egg-slant medium might adsorb the drug in a large proportion. In term of sensitivity of the organism to the action of the test agents, if the assumption is that E. histolytica isolated from different patients are of different strains. It appeared that some strains of E. histolytica were less sensitive to the inhibitory action of metrdinazole i.e. those isolated from patient No. 4 and 5. However, the sensitivity of all strains to the extracts of P. oleracea Linn. were the same. For the antibacterial action, the results showed that both plant extracts could kill both gram positive (+) and gram negative (—) bacteria. S. aureus was the most sensitive to the action of the plant extracts and P. aeruginosa was the least sensitive. The activity against gram negative (—) bacteria was also reported by other investigators.[10] Although there were many reports on the chemical constituents isolated from P. oleracea Linn.[11-15]and also reports on clinical uses of the plant against infections[4], the chemicals responsible for the antibacterial activity have not been identified. Yet our results suggest that the whole plant of P. oleracea Linn . might be a potential broad-spectrum antimicrobial agent.

The activity of the fresh extract of P. oleracea Linn. in all of the tests was higher than that of the boiled extract, suggesting that the active ingredients might be partly destroyed by the heat.

The results of this experiment confirm the possibility of usingP. oleracea Linn. for the treatment of amoebic dysentery, diarrhoea, abcess and wound ulcers.

REFERENCES

1. Brown, H.M. and Neva, F.A. Basic clinical parasitoloty. 5th ed. Appleton Century - Crofts,New York, 1983 ; p 339.
2. Goldman, P. Drug therapy, Metronidazole. N Eng J Med 1980 ; 303 :1212-1218.
3. Kirtikar, K.R.; Basee, B.D. Indian medicinal plants. Vol I. Latit Mohon Basee M.B., Allahabad, 1980 ; pp 241-243.
4. Jaidee, S.การใช้สมุนไพร เล่ม 1 (Use of medicinal plants, Vol. I). Sarnmaulchone Company, Bangkok, 1979 ; pp 93-100.

5. National Committee of Clinical Laboratory Standards NCCLS Approved standard ASM-2, performance standards for antimicrobic disc susceptibility test, 2nd ed. Pensylvania. 1978.
6. Barry, A.L. Broth dilutions techniques, In the antimicrobic susceptilibity tests: Principles and practices, Barry, A.L. editor, p. 92, Lea and Febiges, Philadelphia. 1976 .
7. Vinayak, V.K. and Prakash, O. A comparative evaluation of metronidazole and other amoebicidal drugs on the strains of Entamoeba histolytica. Indian J Med Res 1969 ; 57:841-847.
8. Gorveeda, L.M. A study of the effect of Flagyl upon Entamoeba histolytica in culture. Trop Dis Bull 1965 ; 62:1115.
9. Woolfe, G. Chemotherapy of amoebiasis. Experimental Chemotherapy. Academic Press, New York, 1963 ; 1:419-420.
10. India Council of Scientific and Industrial Research (New Delhi) (1948): The wealth of India. B.N. Sastri chief editor. Sree Saraswaty Press, Culcatta.
11. Ilarionov, I. and Kolev, D. Preliminary phytochemical and pharmacological studies of the native wild prostrate form of Portulaca oleracea species. Chemical Abstracts. 1966 ; 65:17557f.
12. Tulloch, A.P. Leaf wax of Portulaca oleracea . Chemical Abstracts. 1977 ; 82: 28596r.
13. Tashbekov, I. Chemical composition of wild Portulaca oleracea. Chemical Abstracts. 1977 ; 87:2384y.
14. Yasuye, M. and Honda, Y. Component of Portulaceae plants. Chemical Abstracts. 1951 ; 45:823a.
15. Handa, K.L.; Vishwa, Pual, V. and Chandhari, S.S. Examination of the fixed oil of Portulaca oleracea seeds. Chemical Abstracts. 1957 ; 51: 8456h.