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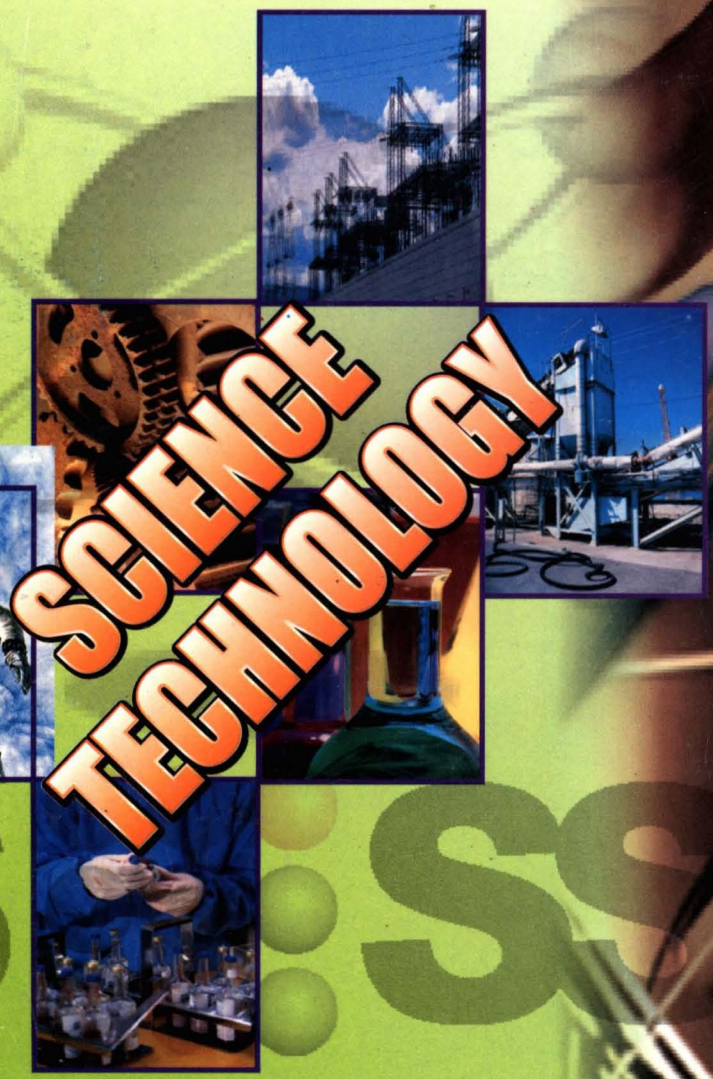

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Antibacillus Activity of Diethyl Ether Extracts of *Chromolaena odorata*

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ABSTRACT

Plants are rich in wide variety of compounds and many of them have been found to have antimicrobial properties. In this study, Chromolaena odorata was chosen due to its history of being used as traditional medicine to treat certain diseases. The leaf and stem samples of the plant were extracted using three different solvents: n-hexane, diethyl ether and methanol. The extraction method was carried out as in Zaidah et al. (2003). The extracts were then tested against eight ATCC target strains using standard disc diffusion assay method. Diethyl ether extracts of both leaf and stem samples showed moderate activity against Bacillus subtilis ATCC 6633 at all tested concentrations. The bioactive substances were observed to be more concentrated in the stem of the plant as the activity at the concentration of 100 µg/ ml was only found from the stem extract and not from the leaf extract. All extracts were found not active against other tested microorganisms.

Keywords: *Chromolaena odorata, antibacillus, bioassay tests*

Introduction

The biodiversity of Malaysian plants offer some 15,000 species of higher plants, but unfortunately less than a 1000 have subjected to chemical and pharmaceutical studies (Goh *et al.* 1993). Some of these plants are medical in nature, having been used for generations to treat various ailments (Salleh & Latiff 2002; Perry & Metzger 1980). The problems with traditional medicines are that, they are not systematic according to their dosages, durations and temperatures (if they need to be immersed).

Screening of both synthetic organic compounds and extracts of natural products has had an impressive history of identifying active agents. For example, among the 50 commercial anticancer drugs available, one third of these drugs are based on natural products.

The genus *Chromolaena* belongs to the family of Compositae, which is the largest family of vascular plants and probably contains 20,000 species. They are distributed over most of the earth and in almost all habitats. To date, there is much work on other species of *Chromolaena* but less work has been done on *C. odorata* (Linn). It was reported to have antifungal agents, e.g. against ringworm, (Burkill 1966), enhance homeostasis and promoting blood coagulation (Akah 1990). The leaves are usually crushed and applied topically on sores and wounds. A number of studies demonstrated that the extract of the plant inhibited the growth of *Pseudomonas aeroginosa, Escherichia coli, Staphylococcus aureus Streptococcus. epidermidis* (Irobi 1992; Nurul Huda 2004) and *Neisseria gonorrhoea* (Caceres 1995).

Among the Malays, the plant is known as "kapal terbang" or "busuk-busuk". This study was carried out to examine antimicrobial property of its leaves and stems.

Materials and Methods

Plant materials

The *C. odorata* plant samples were collected from different sites in the state of Selangor. Following identification, the plant was cut and separated into parts; leaves and stems. They were then oven-dried at 40°C for two days.

Preparation of extract

The dried plant material was immersed in hexane in a conical flask and left at room temperature for three days. The solvent was drained into another flask by filtration and evaporated using evaporator (Buchi rotavapor, Switzerland) at 60°C. The same dried plant material was then soaked with diethyl ether, followed by methanol. After the evaporation process, the sediment was kept at 4°C until further use.

Tester strains

All strains were purchased from American Type Culture Collection (ATCC) and kept at the Department of Microbiology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam. The following cultures were used: *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Streptococcus pyogenes* ATCC 19615, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. All bacteria were cultured overnight with shaking at room temperature in nutrient broth. For yeast and mould, potato dextrose broth was used.

Bioassay test

a) Preparation of bioassay plate

100 mililiter of molten nutrient agar (NA) was inoculated with one ml of 10^6 cfu/ ml bacterial broth culture, mixed well and poured into sterile Petri dishes. They were allowed to set at room temperature. The same procedure was carried out using yeast broth culture into potato dextrose agar (PDA).

Mould assay medium was prepared using spores harvested from 3 to 5 days cultures. 0.85% of sterile saline was used to harvest the spores. The turbidity of the spore suspension was adjusted to 75 – 80% transmission at 530 nm to obtain 10^6 spores/ ml. One ml of the suspension was added to 100 ml of sterile molten PDA. The medium was poured into Petri dishes and were allowed to solidify at room temperature.

b) Antimicrobial activity tests

The standard Kirby-Baeur method was followed to determine the growth inhibition of selected microorganisms by the plant extracts. The crude extracts were dissolved in acetone at the concentration of 100, 200 and 500 mg/ml. Sterile, 6 mm diameter filter paper disc were impregnated with the extract, gently tapped to remove excess liquid, and positioned on bioassay plates. Acetone was used as negative control. As positive controls, discs of erythromycin and cyclohexamide were used to inhibit bacteria and fungi respectively.

c) Collection of data

All plates were observed for zones of inhibition, and the diameter of these zones was measured in milimetres. Results obtained in this study are presented in Table 1. Each test was carried out in triplicates.

Results

Diethyl ether extracts of both stems and leaves of the plant were found active against *B.subtilis* (refer to table 1). However, the extracts were not active against other tested strains. Both hexane and methanolic extracts were also found to be non active.

Table 1: Average of diameter of inhibition zones (mm) of diethyl ether extracts against *B. subtilis*

Plant parts	Concentration of the plant extracts (µg/ml)		
	500	200	100
Leaf	12	9	na
Stem	15	12	8
Erythromycin	15.5		

Note: the size of the discs is 6mm.

Discussion and Conclusion

The plant was found to have antibacillus agent which is more concentrated in its stem compare to the leaf. The value of the activity measured was no significant difference between the tested extracts and the positive control, erythro-

mycin. It showed that the *C. odorata* is a potent candidate to be investigated further to obtain a novel antibiotic especially in the treatment of bacilli-causing diseases.

At higher concentrations, the plant extract was reported active against *Staphylococcus aureus* and *S. epidermidis* (Nurul Huda 2004). In this study, it did not show any activity against other tested strains including two fungal strains, *A. niger* and *C. albicans*, eventhough Salleh & Latiff (2002) reported that the plant was observed to have antifungal agents.

As a conclusion, the extract of *C. odorata* can be considered as a potential bactericidal compound against bacilli.

References

Akah, P. A. (1990). Mechanism of hemostatic activity of *Eupatorium odoratum*. *Int J Crude Drug Res.* 28: 235-253.

Burkill, I. H. (1966). A dictionary of the economic product of the Malay Peninsular. Volume 1 and Volume 2.

Caceres, A. (1995). Antigonorrhoneal activity of plant used in Guatemala for the treatment of sexually transmitted diseases. *J Ethanopharma.* 48: 85-88

Irobi, O. N. (1992). Activities of *Chromolaena odorata* (Compositae) leaf extract against *Pseumodonas aeroginosa* and *Streptococcus faecalis*. *J Ethanopharma.* 37: 81-83

Nurul Huda, A. K., Mamat, A. S., Effendy, A. W. M., Hussin, Z. M. and Sayed Hasan, M. Z. (2004). The antimicrobial effect of *Chromolaena odorata* extract on Gram-positive bacteria. Proceedings of The 11th International Conference Of The Association Of Institutions For Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress, 23-27 August 2004.

Salleh, M. K. and Latiff, A. (2002). Tumbuhan ubatan Malaysia. Bangi: Universiti Kebangsaan Malaysia.

Zaidah Z. A., Rohana, M. N., Zainon A. R. and Zainon, M. N. (2003). *The Treasures of Sarawak Rain Forest.* Proceedings of the International Conference on Biotourism 2003, Miri Sarawak.

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