

In-vitro screening of antifungal activity of plants in Malaysia.

Nazmul MHM¹, Salmah I², Syahid A², Mahmood AA²

¹Department of Microbiology, Faculty of Medicine, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia

²Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Abstract

Twelve species of local Malaysian plants and 5 strains of medically important fungi were selected for this study. Antifungal susceptibility test was performed to screen the antifungal activity of these plants against the selected fungi. *Piper betel* produced the best result in antifungal susceptibility testing and showed to possess antifungal property against 4 out of 5 strains of the fungus. Solid Phase extraction (SPE) technique was applied to *Piper betel* to achieve initial separation of active antifungal compound in the form of methanol fractions. These fractions were tested for their antifungal property. *Piper betel* showed the best antifungal activity especially against *Trichophyton rubrum*.

Key words: *In-vitro* screening, antifungal activity, Malaysian plants, Solid phase extraction, *Piper betel*.

Accepted September 23 2010

Introduction

Malaysia has various types of plant species of medicinal importance. There are more than 12,000 species of plants in Malaysia [1]. From the mystical ancient medication technique until today's high technology-oriented therapy, many of these local Malaysian plants have been widely used and scientifically tested as well to explore new therapeutic properties of these plants.

One of the most important studies is to explore antimicrobial compound in the plants. Although many scientific studies have been conducted in order to unleash the mystery behind the medicinal plants, yet gaps remain which need to be completed. It seems that the antifungal activity of certain plant species has not been satisfactorily explored. There are about 100,000 species of fungus present in the environment and more than 100 of them are pathogenic in humans [2].

Materials and Method

The species of the plants used in this study were collected from three different states of Malaysia (Selangor, Melaka, Johor). Most of the species are wild vegetations in Malaysian forests and can be found even on the roadside along the North-South highway whereas the others are found in local nurseries, market places etc. The plants collected were *Averrhoa bilimbi*, *Areca catechu*, *Banksia integrifolia*, *Bixa orellana*, *Cassia alata*, *Garcinia mangostana*, *Gynura procumbens*, *Labisia pumi-*

la, *Piper aduncum*, *Piper betel*, *Piper ribesioides*, *Piper sarmentosum*. Five types of medically important fungi were chosen based on their ability to infect the human. They were *Aspergillus flavus*, *Candida albicans*, *Microsporum canis*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

Fungi were cultured on Sabaroud's Dextrose agar (SDA) and KOH preparation was used for microscopy. Collected plant species were processed for extraction. Plants were dried, powdered, macerated and crude extracts were collected using standard methanol extraction process. Discs were prepared using these crude extracts for antifungal susceptibility test. All the discs prepared from crude plant extracts were tested for their antifungal activity. Standard Disc Diffusion method [3] was used for this purpose.

The plant extract that produced the best antifungal activity against the fungal strains tested was aimed to be fractionated. Solid Phase Extraction (SPE) technique [4] was applied in order to predict the location of the active antifungal compound using methanol.

Result

Antifungal susceptibility test

Antifungal susceptibility test (using the original plant extracts without separation), was carried out using the disc diffusion method. The overall results are summarized in the table below:

Table 1. Results of antifungal susceptibility test of the plant extracts (diameter of clearing zones produced by the plant extracts).

Plants	Diameter of clearing zone (mm)				
	<i>A. flavus</i>	<i>C. albicans</i>	<i>M. canis</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
<i>Averrhoa bilimbi</i>	9.0	6.0	7.0	7.0	7.0
<i>Areca catechu</i>	8.0	7.0	7.0	8.0	9.0
<i>Banksia integrifolia</i>	8.0	7.0	11.0	12.0	15.0
<i>Bixa orellana</i>	9.0	6.0	8.0	9.0	7.0
<i>Cassia alata</i>	9.0	8.0	12.0	10.0	14.0
<i>Garcinia mangostana</i>	9.0	6.0	7.0	9.0	9.0
<i>Gynura procumbens</i>	7.0	6.0	8.0	8.0	9.0
<i>Labisia pumila</i>	7.0	6.0	7.0	8.0	9.0
<i>Piper aduncum</i>	9.0	6.0	8.0	7.0	8.0
<i>Piper betel</i>	11.0	9.0	12.0	12.0	17.0
<i>Piper ribesoides</i>	9.0	7.0	8.0	8.0	9.0
<i>Piper sarmentosum</i>	9.0	8.0	9.0	9.0	9.0
Positive control	12.0	11.0	13.0	14.0	15.0
Negative control	6.0	6.0	6.0	6.0	6.0

***P. betel* extract fractionation and antifungal susceptibility test**

P. betel was separated into different methanol fractions. Methanol fractions were pipetted onto blank discs with the ratio of 100 µl of the fraction per disc. After 24 hours of incubation at 30°C with the discs, clearing zones of 7 mm and 14 mm in diameter were produced by 40% and 50% methanol fractions, respectively. Another series of fractionation was run on the same plant using different concentration of methanol (30%, 70%, 90%) and was tested against *T. rubrum*. 40%, 50% and 60% methanol fractions of *P. betel* were also tested against the other 4 species of fungus. 10 mm diameter of inhibition zone was produced against *Aspergillus flavus*, 10 mm against *Trichophyton mentagrophytes*, 11 mm against *Microsporum canis* and 9 mm on *Candida albicans*.

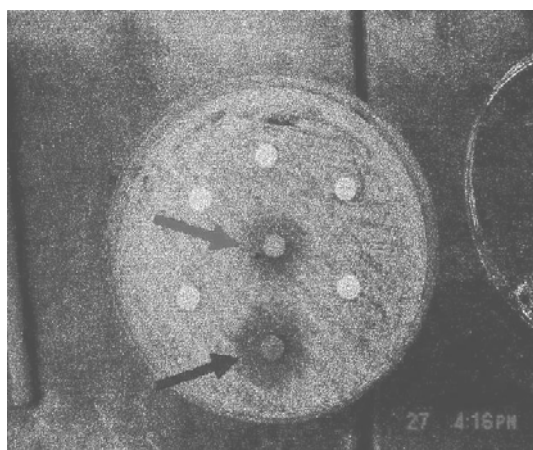


Figure 1. Inhibition zone produced by *P. betel*. Arrows showing zones developed by 40% (upper) and 50% (lower) methanol fractions on *T. rubrum*.

Discussion

The antifungal-related study on plant species is quite rare in Malaysia. It is important to study every aspect and every detail of the plant species known to have medicinal values. In this study, antifungal property of the plants was examined. For this purpose, Sabaroud's Dextrose Agar (SDA) was used for culture and susceptibility test. Production of inhibition or clearing zone was carefully observed from time to time. A clearing zone of 10 mm was chosen as the cut off point to indicate the presence of significant antifungal property of the plant fractions. Different size of zones of inhibition was observed when different extracts were assayed. Safety and sterility measures throughout the experiments were maintained to obtain significant results, to avoid contamination and infection as well.

For antifungal susceptibility test (using the original plant extracts without separation) only 3 (25%) among the 12 local plant species had shown positive activity towards the fungi or had the ability to suppress fungal growth. They were *Piper betel* (Sireh Melayu), *Banksia integrifolia* (Berus Botol) and *Cassia alata* (Gelenggang). The rest had low activity against the fungi, or did not show the ability to stop fungal growth. *Piper betel* was reported to have antibacterial activity [5] but its antifungal activity has never been explored before us. However, *P. betel* extract was also found to reduce acid producing properties of certain bacteria [6]. In this study, *P. betel* showed antifungal activity towards the maximum number of fungi tested. *P. betel* produced a diameter of clearing zone of 11 mm against *A. flavus*, 12 mm against *M. canis*, and 12 mm against *T. mentagrophytes* and 17 mm against *T. rubrum*. *P. betel*'s antifungal activity against

C. albicans was the least (9mm) compared to other fungi tested.

P. betel was selected for fractionation and was separated into different methanol fractions as it showed significant antifungal value in the preliminary antifungal susceptibility test. After 24 hours incubation of its 50% methanol fraction at 30°C, the result showed a clearing zone of 14 mm in diameter (7 mm larger than the 40% fraction's inhibition zone, 3 mm less than the inhibition zone produced by the original extract) against *T. rubrum*. The results suggest that 50% methanol fraction of *P betel* has a significant antifungal property.

Conclusion

In conclusion, the Disc Diffusion method can be used to screen antifungal activity of the plant methanol extracts and Solid Phase Extraction can be applied to fractionate and isolate the active antifungal principles of the plants. *Piper betel* has shown the highest antifungal activity among all the plant fractions so far tested, especially on *Trichophyton rubrum*. The antifungal activity was found to be highest in the 50% methanol fraction of *P. betel*.

References

1. Burkill IH, Hanif M. Malay Village Medicine. Book, 1966. The Gardens Buletin Straits Settlement.
2. Keeler RF, Tu AT. Toxicology of Plant and Fungal Compounds in: Handbook of Natural Toxins, Vol. 6, 1991. Marcel Dekker, Inc. NY 665 pp.
3. Koneman WE. Introduction to Diagnostic Microbiology. 2000.
4. Duke JA. Handbook of phytochemical constituents of GRAS herbs and other economic plants, 1992. Boca Raton, FL, CRC Press.
5. Fathilah AR, Rofinah YO, Zubaidah HR. The effect of *Piper betel* and *Psidium guajava* extracts on the cell surface hydrophobicity of selected early settlers of dental plaque. J Oral Sci 2006; 48: 71-75.
6. Nalina T, Rahim ZHA. The crude aqueous extract of *Piper betel* and its antibacterial effect towards *Streptococcus* mutants. Am J Biotech Biochem 2007; 3: 10-15.

Correspondence to:

Nazmul MHM
Department. of Microbiology
Faculty of Medicine
Universiti Teknologi MARA
Shah Alam, 40450 Selangor
Malaysia

