

Antimicrobial Activities of the Ethanol Extracts of Capsicum Fruits with Different Levels of Pungency

*S. Soetarno**, Sukrasno, E. Yulinah and Sylvia
Department of Pharmacy, Faculty of Mathematics and Natural Sciences,
Institut Teknologi Bandung
Jl. Ganesa 10, Bandung, 40132 - Indonesia

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abstract

Ethanol extracts of the fruits of three kinds of Capsicum showed similar potencies in their antimicrobial activities against Gram (+) and Gram (-) bacterias, and fungi, although they contained different level of capsaicin. Bioautographic tests demonstrated that capsaicin was the main antimicrobial component. At least two other non-polar components of ethanol extract also contributed in the antimicrobial activity and very likely that these compounds were responsible for the activity toward Pseudomonas aeruginosa.

KEYWORDS

Antimicrobial activity, ethanol extracts, fruits, Capsicum frutescens L., Capsicum annuum L, Solanaceae.

1. Introduction

Pepper fruits are added at a substantial quantity to produce a characteristic taste of cuisine of West Sumatran (Indonesia). It was observed that cuisine using a large amount of chilli pepper could stand at long period without significant deterioration. Only a special variety of chilli pepper was employed by this ethnic, i.e. *Capsicum annuum* L. var. longum (Solanaceae) which has a curly shape. Chilli tinctur was previously reported to be able to inhibit the growth of *Staphylococcus* sp., *Escherichia coli*, *Bacillus aureus* and *Bacillus subtilis*¹⁾. In Madura island (Indonesia), chilli pepper (*Capsicum frutescens*) is traditionally used to treat ox cuts before race²⁾. In Indonesia, chilli pepper is also traditionally used to treat oral thrush which is usually caused by *Candida albicans*³⁾.

This report presents the results of our study on the activity of the ethanol extracts of *Capsicum frutescens* and two cultivars of *Capsicum annuum*, var. longum. The first pepper fruit was very hot and one of the *C. annuum* was hot while the other one was moderately hot.

2. Materials and Methods

2.1. Plant Materials

Three different pepper fruits were purchased from local market. Fruit-1 was from *Capsicum frutescens* L. (chilli pepper), fruit-2 *C. annuum* L. var. longum (curly pepper), fruit-3 *C. annuum* L. var. longum (sweet pepper). Taxonomic identification of fruits was conducted at The Herbarium Bandungense, Department of Biology, Institut Teknologi Bandung and Horticulture Research Center - Lembang, Indonesian Ministry of Agriculture. Voucher specimens were deposited in the Herbarium of the Department of Pharmacy. The fruits were dried at 60°C in an air oven and then ground to produce pepper powder.

2.2. Extraction

Chilli pepper fruit extracts were prepared by macerating pepper powder in ethanol for 24 h then filtered. The residue was washed twice with fresh ethanol and the filtrates combined with the first filtrate. Combined filtrate was then evaporated to dryness to produce sticky material of pepper extract.

2.3. Microorganisms

The microorganisms used in these tests include :

- Gram (+) bacteria : *Staphylococcus aureus* (ATCC-14154)
Bacillus subtilis (ATCC-6633)
Sarcina lutea (Collection of Microbiology Lab. of Chemical Engineering Department, Institut Teknologi Bandung)
- Gram (-) bacteria : *Escherichia coli* (ATCC-1698)
Pseudomonas aeruginosa (ATCC-23993)
- Yeast : *Candida albicans* (ATCC-14053)
- Dermatophyte : *Microsporum gypseum* (Biofarma-Bandung Collection)
- Mold : *Aspergillus niger* (ATCC-32611)

2.4. Media

Bacteria were grown on nutrient agar (Oxoid). Inoculum for the assay was prepared by suspending bacterial cells 18-24 h old in nutrient broth (Oxoid) to yield 25% transmittance at

580 nm. Fungi were grown on Sabouraud Dextrose Agar (Oxoid) and inoculum for the assay prepared by suspending the 72 h old fungal cells in Sabouraud Dextrose Broth (SDB) to produce 90% transmittance at 530 nm.

2.5. Method of assay

The antimicrobial assay was performed by dispersing 100 μ l of inoculum homogeneously in 15 ml liquified nutrient agar (NA) or Sabouraud Dextrose Agar (SDA) media and then left to solidify on Petri dishes. Six wells of 6.5 mm in diameter were prepared on plate of each dish. Respectively 10 μ l solution of pepper extract in DMSO at the desired concentration was then added into each well. The assay dishes were then left for one hour and subsequently incubated for 24 h at 37°C for bacteria and 22°C for fungi. The diameter of inhibition was then observed and measured. As a comparison, tetracycline hydrochloride was used as standard for antibacteria, nystatine for the activity against *C. albicans* and *M. gypseum*, griseofulvin for *A. niger*.

2.6. Thin Layer Chromatography

Thin layer chromatography was performed on precoated silica gel GF-254 with 10 μ m layer and benzene-acetic acid (9:2) as solvent system. Spot of capsaicin was visualized with diazotised sulfanilamide which was prepared by freshly mixing 5 ml of 1% sulfanilamide in 10% HCl, 5 ml of 5% sodium nitrite and 40 ml of metanol⁴⁾). Intensification of capsaicin spot was performed by spraying with 5% sodium carbonate which yielded red color following mild heating.

2.7. Determination of capsaicin content in the extract

Sample was prepared by dissolving extract in ethanol at concentration of 5 mg/ml and 15 μ l solution was applied on TLC plate. Capsaicin isolated from fruits was used as a standard for the measurement and applied in the range of 5 μ g to 25 μ g in each spot. After development and visualization, the intensity and the area of the spots was measured using Spectrophotometer (Shimadzu CS-910) at 510 nm.

2.8. Bioautography

To improve the separation and increase the loading capacity of the plate, the extract was firstly fractionated into aqueous and dichlormethane fractions at pH 3 and the later fractions collected and dried with sodium sulfate. Dichlormethane fraction was evaporated to dryness at reduced pressure and redissolved in ethanol and applied on silica gel as a strip. TLC chromatogram was dried using spray drier to eliminate solvent remaining on the plate and then laid on the surface of NA/SDA media freshly inoculated with the assay microbes. The plate was left for 15-30 min to facilitate diffusion of substances in the plate to the gel and then removed. Subsequently the assay gel was incubated at 37°C (bacteria) or 22°C (fungi) for 18-24 h (bacteria) or 24-48 h (fungi).

3. Results and Discussion

In order to provide background information on the pepper fruits under our study, following is a brief description of the three fruits. The first pepper was identified as one cultivar of *C. frutescens*, since there are different type of fruits based on the color, size, form and pungency. The other two fruits were all from *C. annum* var longum, but they are very different in shape and also pungency. Table 1 gives more detail description of the *Capsicum* fruits used in our study.

All fruit extracts were active against most Gram (+) and Gram (-) bacteria tested. Differ from fruit-1 and fruit-3 extracts, fruit-2 extract was inactive against *P. aeruginosa*. Against fungi tested, i.e. *C. albicans*, *M. gypseum* and *A. niger*, the three fruit extracts were only active to the first microorganism. These results suggest that all kinds of *Capsicum* fruits tested are useful as antibacterial and anticandidal agents and not necessarily the most pungent pepper as in the traditional use^{2,3}). Consistent with their pungency, the sequence of capsaicin level in the three *Capsicum* fruit extracts was fruit-1 > fruit-2 > fruit-3 (see Table 1).

Based on their minimum inhibition concentrations (MIC), the activity of fruit-1 and fruit-3 extracts to *P. aeruginosa* compared to the other bacteria tested and to *C. albicans* was the lowest, while fruit-2 extract was inactive. Although fruit-3 contained the lowest level of capsaicin, its extract exhibited the lowest MIC against *S. lutea* (see Table 1). Inactivity of fruit-2 against *P. aeruginosa*, and the similarity in the activity of the three fruit extracts to the other microorganisms suggest that capsaicin may not be the only compound responsible for the antimicrobial activity.

Bioautography of chromatogram of the three extracts which was conducted by contacting the chromatogram with the assay gel for 15-30 min followed by incubation and observation the inhibition area showed that capsaicin was the main compound for antimicrobial activity. Bioautographic tests against *S. aureus* and *B. subtilis* gave the same pattern of results, while against *C. albicans*, good result was not obtained although several repetition had been made. Apart from capsaicin, in extract of fruit-1 another component having hRx 123.3 (relative to capsaicin) also showed antimicrobial activity. In fruit-2 capsaicin was the only antimicrobial compound, while in fruit-3, two other active components i.e. spots with hRx 153.8 and 165.4 were also active. The higher Rf value of the other active components than capsaicin in the TLC system used suggest that these compounds are less polar than capsaicin. The bioautographic data also suggest that these compounds may be responsible for the activity toward *P. aeruginosa* since fruit-2 extract which contained capsaicin as the main active component was inactive. Clearly further tests are needed to confirm this possibility. The similarity in the antimicrobial activity of the hottest and the least hot pepper fruit extracts tested suggests the presence of synergism between capsaicin and the other components in the pepper fruit extracts.

Compared to the activity of the standard antibiotics, the activity of pepper fruit extracts was still much lower. Relative activity fruit-1 extract compared to tetracycline HCl for example only approximately 10^{-5} times and variation existed depended on the bacterial test used. The relative activity of *Capsicum* fruit extracts to nystatine was in the order of 10^{-3} time magnitude which was higher than to tetracycline. These results support the traditional use of *Capsicum* fruits to treat oral thrush caused by *C. albicans*³⁾.

Table 1. Botanical description of the three *Capsicum* fruits, antimicrobial activity and bioautographic data of their ethanol extracts

Criteria evaluated	Fruit-1	Fruit-2	Fruit-3
Species	<i>C. frutescens</i>	<i>C. annuum</i>	<i>C. annuum</i>
Local name	cabe rawit	cabe keriting	cabe besar
Length/diameter (cm)	3.5-5.0/1.0-1.2	6.5-10.0/0.5-1.0	13.0-15.0/2.0-2.5
Color	red (mature) green (raw)	red (mature) green (raw)	red (mature) green (raw)
Shape	stright	curly	stright
Pungency and capsaisin content	very hot	hot	moderately hot
In extract	8.49	4.28	2.18
MIC {% ($\mu\text{g}/\text{well}$)}			
<i>S. aureus</i>	0.40 (80)	0.40 (80)	0.40 (80)
<i>E. coli</i>	0.06 (12)	0.08 (16)	0.06 (12)
<i>P. aeruginosa</i>	5.00 (1.000)	-	5.00 (1.000)
<i>B. subtilis</i>	0.40 (80)	0.20 (40)	0.60 (120)
<i>S. lutea</i>	0.10 (20)	0.10 (20)	0.08 (16)
<i>C. albicans</i>	0.06 (12)	0.04 (8)	0.06 (12)
HRx of active bioautographic spots to <i>B. subtilis</i> and <i>S. aureus</i>	100 (capsaicin) 123.3	100 (capsaicin)	100 (capsaicin) 153.8 165.4

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