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POTENTIAL USE OF RHIZOME CRUDE EXTRACT OF *Curcuma petiolata* ROXB AS AN ANTI AGENT FOR GRAM NEGATIVE PATHOGENIC BACTERIA

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ABSTRACT

The main objective of this study was to investigate the potency of rhizome crude extract of *Curcuma petiolata* Roxb. in inhibiting *in vitro* the growth of several species of gram negative pathogenic bacteria, by applying the method of Kirby and Bauwer (1966) with small modification. Three species of gram negative pathogenic bacteria (*Vibrio cholerae*, *Escherichia coli*, and *Salmonella thypi*) were exposed in the assay with the crude extract of rhizome of the *C. petiolata* Roxb. at various concentrations.

The results showed that all tested bacteria were inhibited by the rhizome crude extract of the plant at the concentration of 10% or more. No inhibition was observed in the control treatment, indicating that the inhibition zone occurred in all treatments must be due to the action of active compounds contained in the extract. *V. cholerae* was found to be the most resistant species to the extract when compared to *E. coli* or *S. thypi*.

Key words: Curcuma petiolata Roxb., *Vibrio cholerae, Salmonella thypi, Escherichia coli.*

INTISARI

Tujuan dari penelitian ini adalah untuk mempelajari potensi ekstrak kasar rimpang temu putri (*Curcuma petiolata* Roxb. dalam menghambat pertumbuhan in vitro tiga species bakteri pathogen gram negatif (*Vibrio cholerae*, *Salmonella thypi*, dan *Escherichia coli*). *Assay* mikrobiologi menggunakan metoda Kirby dan Bauwer (1966) dengan sedikit modifikasi.

Hasil penelitian menunjukkan bahwa semua bakteri uji terhambat pertumbuhannya *in vitro* setelah diperlakukan dengan ekstrak pada konsentrasi 10% atau lebih. Tidak terbentuknya zone hambatan pada kontrol menunjukkan bahwa terbentuknya zone hambatan pada perlakuan ekstrak pasti disebabkan oleh senyawa-senyawa aktif yang terkandung di dalam ekstrak kasar rimpang temu putri. Dalam bioassay ini, *V. cholerae* menunjukkan sifat yang paling resistan terhadap ekstrak rimpang temu putri, jika dibandingkan dengan bakteri uji yang lain (*E. coli* atau *S. thypi*).

Kata kunci: *Curcuma petiolata* Roxb., *Vibrio cholerae, Salmonella thypi, Escherichia coli.*

INTRODUCTION

C. petiolata Roxb. is one of many tropical plants rarely found in Bali, but it has been used as an alternative traditional medicine (Kriswiyanti, 2001). Puspawati *et al.*, (1998) reported that the crude rhizome extract of this plant has been widely used in Bali to cure diseases mainly caused by *V. cholerae* (the causative agent of cholerae). According to Sirat *et al.*, (1984) and Ichiro *et al.*, (1995) the crude rhizome extract of this plant contains several active compounds, such as curcumenon, dehydrocurdion, 1,3 hidroxygermakron, and zedoarol, which were believed to play important roles in inhibiting the growth of *V. cholerae*. The crude rhizome extract of this plant has also been used to cure several other diseases, such as fever, stomach disconfort, and irritation (Rifai *et al.*, 1992). Although there were many findings on the potential of this rhizome extract to cure several diseases, study on the *in vitro* bioassay of this rhizome extract on gram negative pathogenic bacteria still need to be conducted in order to investigate the minimal concentration of this extract required to control gram negative pathogenic bacteria.

Based on the above background *in vitro* bioassays of rhizome crude extract of *C. petiolata* Roxb on three gram negative pathogenic bacteria (*V. cholerae*, *E. coli*, and *S. thypi*) were conducted with the following objectives:

a. to investigate the minimal rhizome crude extract of *C. petiolata* Roxb. needed to inhibit the growth of tested bacteria *in vitro*.
b. to compare the sensitivity of the tested bacteria to the extract, exposed at various concentrations.

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MATERIALS AND METHOD

Extraction

Some 100g of rhizome of *C. petiolata* Roxb. was macerated in 1000 mL methanol, incubated at ambient temperature for 72 hours, and evaporated in a rotary evaporator at 40 °C to remove the solvent. This crude extract was then assumed as 100% concentration. Prior to use in the bioassays, this crude extract was diluted with methanol to achieve concentrations of 10, 20, 30, 40, and 50%.

Preparation of bacterial suspensions

Suspensions of tested bacteria were prepared by inoculating a loopful of pure culture of each bacterial species into different McCartney bottles, each containing 10 mL sterile Nutrient Broth. All inoculated medium were incubated at 37 °C for 24 hours to achieve an approximate cell density of 10^8 cells/mL.

In vitro bioassay

The method of Kirby and Bauwer (1966) with small modification was applied in the bioassay. Some 20 µL rhizome crude extract of *C. petiolata* Roxb. previously prepared was deposited in each filter paper disk and air dried at ambient temperature. In the meantime, bacterial lawns of tested bacteria were prepared by evenly spreading 100 µL of tested bacterial suspension on the surface of each nutrient agar plate. The crude extract-deposited filter paper disks were then placed at equidistance on the surface of bacterial lawns prepared above and incubated at 37 °C for 24 hours. Filter paper disks deposited with solvent only (solvent in the absence of extract) served as controls. The assay was repeated three times (triplicates per treatment). Positive results were indicated by the formation of inhibition zones around the disks. Measurement of inhibition zones was made from three different angle and averaged.

Data analysis

Analysis os variance (ANOVA) of data obtained from this study was carried out using SPSS software for Windows. The significant differences between means were further tested using Duncan test at p of <0.05, following ANOVA.

Results and discussion

The relative inhibition zones produced by rhizome crude extract of *C. petiolata* Roxb. applied at various concentrations on the three tested bacterial lawns are presented in Table 1.

The rhizome crude extract of *C. petiolata* Roxb. was found to be very effective to inhibit the growth of all tested bacteria *in vitro*, with the the diameter of inhibitions varied according to the concentration of the extract applied and on bacterial species tested. In all cases, the diameter of inhibition zones was proportionaly related to the concentration of the extract exposed (Table 1). When compared to controls (solvent only), all treatments (extract at various concentrations) were found to be statistically significant ($p<0.05$) in term of diameter of inhibition. All tested bacteria were found to be inhibited by the extract at the concentration of 10% or more, alhough in general the diameter of inhibitions produced was not statistically significant ($p>0.05$) among other (Table 1). Based on inhibition zones produced by various concentrations of the extract, in all cases *V. cholerae* was found to be the most resistant species when compared to *E. coli* or *S. thypi* (Table 1). If this extract is to be used to cure certain diseases, especially those caused by infection of any one of the tested bacterial species, it is recommended to apply minimal concentration (at least 10%) in order to minimize the possible negative side effects of the extract.

As no inhibition zones was observed in all controls, in all cases where growth inhibition occurred, these inhibition zones must be due to the action of active compounds contained in the plant crude extract. Although it was not determined in this study, some researchers reported that crude extract of *C. petiolata* Roxb (Wijayakusuma, 2002) and related plants, such as *C. domestica* (Herdianto, 2000; Kadir, 2001) normally contain curcumin, curcuminoid, essential oil, desmetoxy curcumin, bidesmetoxy curcumin. The present of these compounds in the crude

Table 1: Relative inhibition zones on bacterial lawns following application of rhizome crude extract of *C. petiolata* Roxb at various concentrations

Extract concentration (%)	Relative inhibition zones (Cm)**		
	E. coli	V. cholerae	*S. thypi*
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
10	1.00 ± 0.65^{b}	0.88 ± 0.59^{b}	1.08 ± 0.67^{b}
20	1.03 ± 0.66^{b}	0.98 ± 0.62^{b}	1.63 ± 0.07^{cd}
30	1.23 ± 0.89^{bc}	1.18 ± 0.86^{bc}	2.02 ± 0.28^{de}
40	1.36 ± 0.03^{bcd}	1.45 ± 0.06^{bcd}	2.07 ± 0.31^{de}
50	1.75 ± 0.20^{cd}	1.70 ± 0.25^{cde}	2.47 ± 0.58^{e}

** each value in Table 1 ± standard error is an average of triplicates. Values followed by the same letter(s) are not significantly different at $p<0.05$ according to Duncan test following ANOVA.

extract individually or collectively might play significant roles in the growth inhibition of tested bacteria in the present study. According to Doerge (1982), Pelczar and Chan (1986), and Girindra (1993) toxic compounds including active compounds extracted from plants may attack enzyme systems of living cells and may result in growth interference on the exposed cells. In the case of bacterial species, the respiratory-related enzymes located in their plasma membrane may first be attacked as soon as they are in contact with active or toxic compounds. The effect of this can be fatal as the cells may lose their ability to generate their cellular energy (Lechninger, 1982; Voet and Voet, 1990; and Creager *at al.*, 1990). Depends on the level of toxicity of the compounds exposed, the effect may either be lethal or bacteriostatic (temporary inhibit the growth of the exposed bacterial cells) (Creager *et al.*, 1990). In the present study, it was not known whether the effect of the extract on the tested bacteria was lethal or bacteriostatic. Therefore further investigation on this aspect need to be addressed in the future.

CONCLUSION

It was clearly demonstrated in this study that the rhizome crude extract of *C. petiolata* Roxb has a great potential to inhibit the growth of three gram negative pathogenic bacteria (*E. coli, V. cholerae,* and *S. thypi*) *in vitro*. The degree of inhibition varied according to the crude extract concentration and bacterial species. All tested bacteria were inhibited by the extract at the concentration of 10% or more. In all treatments, *V. cholerae* was found to be more tolerant than others to the extract of *C. petiolata* Roxb., when applied at various concentrations.

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