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Screening for antimicrobial and antimalarial activities of longan (*Dimocarpus longan* Lour) seeds

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The aims of this study were to evaluate methanolic extract of longan seed for antibacterial, antimalarial (antiplasmodial) activity and conducted with cytotoxicity test by using sulforhodamine B (SRB) assay with Vero cells (African green monkey kidney cell line). The methanolic extract was tested for antimicrobial activity with five strains of pathogenic bacteria including *Staphylococcus aureus, Bacillus cereus, Psuedomonas aeruginosa, Enterococcus faecalis, Escherichia coli* and one strain of pathogenic yeast, *Candida albicans*. The antimicrobial activities of each extract were screened by agar diffusion before conducting with broth macrodilution method for determining minimal inhibitory concentration (MIC). The assessment of antimalarial activities of methanolic extract was determined by microculture radioisotope technique. *Plasmodium falciparum* (K1, multi-drug resistant strain) *in vitro* culture was used for this test. The extract also was screened for cytotoxicity with Vero cells (African green monkey kidney cell line) by SRB colorimetric assay. The results show that longan seed extract (10 mg/ml) inhibited the growth of *S. aureus*, *P. aeruginosa* and *C. albicans* 17, 12 and 11 mm and MIC values were 3.19, 1.59 and 1.59 mg/ml, respectively. The longan seed extract also possessed preferable antimalarial activity (IC_{50} = 2.78 ± 0.44 µg/ml) with no cytotoxic effect to Vero cells.

Key words: Antimalarial, antimicrobial, cytotoxicity, *Dimocarpus longan* Lour, longan.

INTRODUCTION

Longan (*Dimocarpus longan* Lour, syn. *Euphoria longan* Lam.) fruit is one of the popular fruit which is manufactured into canned fruits. The canning industry produces considerable quantities of waste products, in particular Longan seeds. For this reason, longan seeds were met to criteria. Longan fruits make a significant contribution to the Thai diet, especially in Northern Thailand, where the cultivation of longan is favoured over other fruits. In traditional medicine, flesh of longan fruit is administered as a stomachic, febrifuge (antipyretic) or vermifuge (anthelmintic), and is regarded as an antidote for poison. A decoction of the dried flesh is taken as a tonic or as treatment for insomnia or neutrasthenic neurosis.

In the North and the South Vietnam, the "eye" of the longan seed is pressed against a snakebite in the belief that it will absorb the venom. Longan fruits are either consumed fresh or as commercially prepared dried and canned products. The canning industry produces considerable quantities of waste products, in particular longan seeds, which are an exploitable source of natural phenolic antioxidants (Sudjaroen et al., 2012). In comparison to pericarp, longan seeds represent a considerably higher proportion of the by-products arising from fruit processing in Thailand. Except antioxidant activity, it has sparely been reported for other biological effects in terms of health promotion. From this standpoint, it is of interest to evaluate biological properties of the

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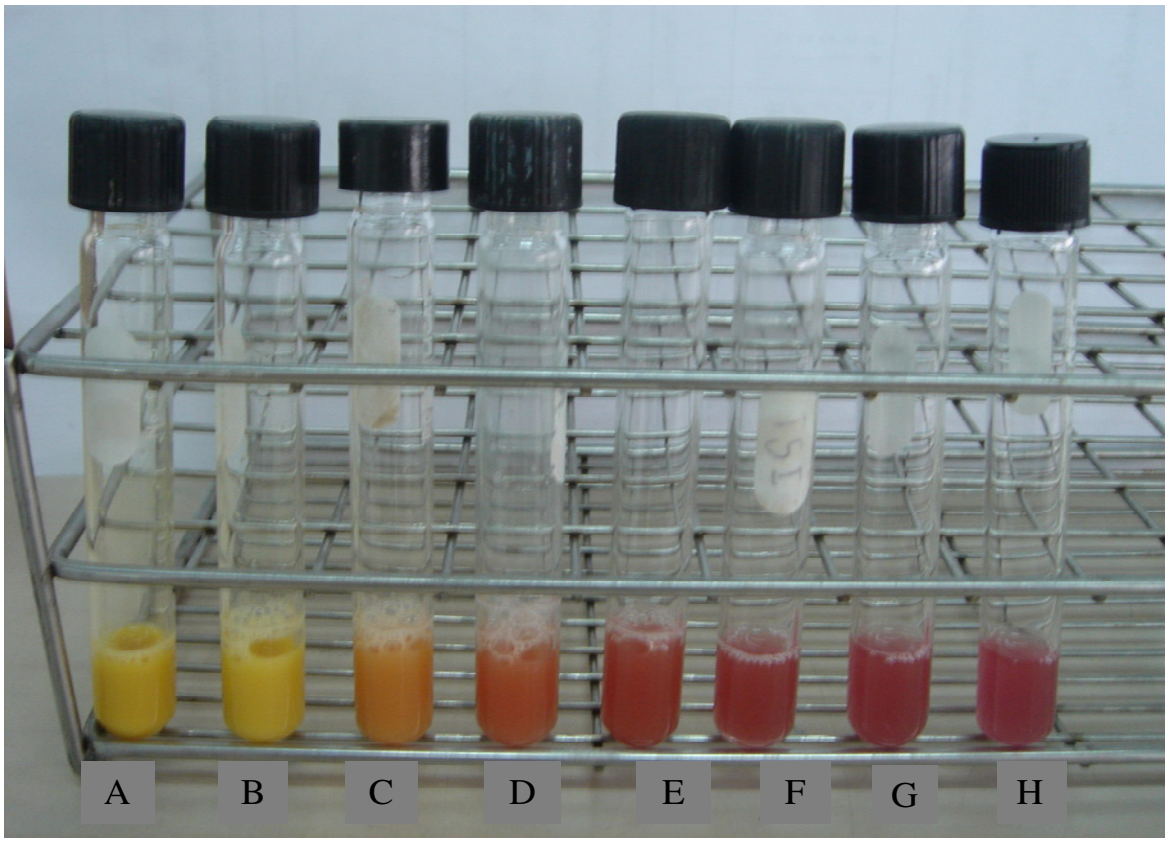

Figure 1. Changing of color indicator in broth macrodilution method, the first and second tube was titer positive concentration of extract, which can inhibit bacteria growth (A, B). The red colors mean bacteria still growth (C to H).

methanol extract from longan seeds, such as antibacterial, antimalarial and cytotoxic effects.

MATERIALS AND METHODS

Extraction protocol

Longan seeds were carefully separated from fruits. Air-dried samples were homogenized by blending to a fine homogeneous powder prior to extraction. Air-dried material (5 g) was extracte with hexane in a Soxhlet apparatus (3 h) to remove lipid. The material was dried under a stream of nitrogen and extracted further with methanol (3 h) as modified from the study of Owen et al. (2000).

Antimicrobial tests

Inoculum development

The inoculum was prepared in Tryptic soy broth (TSB) and the number of cfu/ml in the inoculum was determined and standardized. A 0.5 Mc Farland standard is comparable to a bacterial suspension of 10^8 cfu/ml (Clinical and Laboratory Standards Institute, 2010).

Agar diffusion method

The suspension was streaked entirely on Mueller-Hinton agar (MHA) surface and in case of *C. albicans*, Sabouraud dextrose agar (SDA) was used. Holes were made on MHA by sterile Pastuer's pipette. 25 µl of each extract (2.5, 5.0, 7.5 and 10 mg/ml were diluted by 0.025% dimethyl sulfoxide, DMSO in sterile water) was added into the holes of MHA. After incubation for 18 to 24 h at 35°C (in case of *C. albicans* was incubated at 30°C), zones of inhibition was measured in millimeter (mm) and calculated in triplicate from independent experiments. Appropriate antimicrobial drugs were used as positive control and 0.025% DMSO as negative control (Clinical and Laboratory Standards Institute, 2010).

Broth macrodilution method

Extract that showed inhibition zone against pathogens in agar diffusion method were subjected to assay with macrodilution method for MIC determination. The extract (10 mg/ml) was diluted in two fold serial dilution by TSB containing 1% Triphenyltetrazolium chloride (TTC) as growth indicator. A 0.5 Mc Farland standard is comparable to a bacterial suspension of 10^8 cfu/ml for all tests and negative control. The extract control was prepared similarly, without pathogen suspension. The TSB and suspension were used as positive (no pathogen growth) and negative control (pathogen growth), respectively. The inhibition of pathogen was observed by the titer that had yellow color (Figure 1) (Clinical and Laboratory Standards Institute, 2010).

In vitro antimalarial test

P. falciparum (K1, multidrug-resistant strain), were maintained in

Table 1. The IC_{50} of Longan seed extract against *P. falciparum*, K1 Strain (*In vitro*) by microculture radioisotope technique.

Methanol extract	Final concentration (µg/ml)	Count data (Mean ± SD)	IC_{50} (Mean ± SD)
Longan seed	10	9939.67±1309.89	5.63±0.24 µg/ml
	1	20384.33±3182.72	
0.1% DMSO*	-	25524.00±1414.30	-
DHA**	10 nM	183.33±67.28	4.20±0.26 nM
	2 nM	23814.00±3191.96	

*Negative control: 0.1% DMSO, **Positive control: dihydroartemisinine (DHA).

Table 2. Cytotoxic effect of longan seed extract to vero cells in each concentration by sulforhodamine B (SRB) assay[a,b].

Methanol extract	Final concentration (µg/ml)	Growth (%)	Cytotoxicity
Longan seed	50.0	88.81	Non-cytotoxic
	25.0	94.80	Non-cytotoxic
	12.5	102.91	Non-cytotoxic
	6.25	99.02	Non-cytotoxic
	3.125	100.80	Non-cytotoxic
	1.5625	96.91	Non-cytotoxic
	0.7813	97.50	Non-cytotoxic

a: Positive control: ellipticine 0.603 µg/ml; b: negative control: 0.5% DMSO.

continuous culture in human erythrocytes incubated at 37°C in RPMI 1640 medium with human serum, under an atmosphere with 7% CO_2 and low oxygen (1 or 5%). The parasites continued to reproduce in their normal asexual cycle of approximately 48 h (Trager and Jensen, 1976). Antimalarial activity was evaluated against the parasite *P. falciparum*, using the microculture radioisotope technique based on the method described by Desjardins et al. (1979). The extract concentration at 1 and 10 µg/ml were tested in triplicate. The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. The dihydroartemisinin (DHA) was standard compound, and 0.1% DMSO was used as negative control.

In vitro cytotoxicity test

African green monkey kidney fibroblast (Vero) cells were performed employing the calorimetric method as described by Skehan et al (1990). The sulforhodamine B (SRB) assay was performed to assess growth inhibition using a colorimetric assay, which estimates cell number indirectly by staining total cellular protein with dye SRB (Skehan et al., 1990). Briefly, 100 µl/each well of cell suspensions ($0.5 - 2.0 \times 10^5$ cells/ml) were seeded in 96-well microtiter plates and incubated at 37°C to allow cell attachment. After 24 h, cells were treated with the extract by adding 100 µl/well of each concentration in triplicate to obtain a final concentration of 50.0, 25.0, 12.5, 6.25, 3.125, 1.5625 and 0.7813 µg/well for the extracts (two-fold serial dilution). The ellipticine 0.603 µg/ml and 0.5% DMSO were used as positive and negative control, respectively.

RESULTS AND DISCUSSION

Longan seed extract (10 mg/ml) inhibited the growth of *S. aureus*, *P. aeruginosa* and *C. albicans* by 17, 12 and 11 mm, and MIC values were 3.19, 1.59 and 1.59 mg/ml for *S. aureus, P. aeruginosa,* and *C. albicans*, respectively. The extract of longan seeds possessed preferably high antimalarial activities with IC_{50} = 5.63 ± 1.24 µg/ml (Table 1). Furthermore, there was no cytotoxic effect of this extract to Vero cells (final concentration = 50 µg/ml), as shown in Table 2. It might imply that the antimicrobial activities of longan seed affected from longan contains significant amount of phenolic compounds (Sudjaroen et al., 2012) which displays multifunctional activities as effective antimicrobial or antioxidants agents (Galal, 2006). The previous study (Sudjaroen et al., 2012) was isolated and characterized polyphenolic fraction (80.90 g/kg dry weight), which was dominated by ellagic acid (25.84 g/kg) and the known ellagitannins corilagin (13.31 g/kg), chebulagic acid (13.06 g/kg), ellagic acid 4-O-a-L-arabinofuranoside (9.93 g/kg), isomallotinic acid (8.56 g/kg) and geraniin (5.79 g/kg).

In case of antiplasmodial or antimalarial activity, the methanolic extact was high and similar to African medicinal plants: *Cassia occidentalis* leaves, *Euphorbia hirta* whole plant, *Garcinia kola* stem bark and *Phyllanthus niruri* whole plant, which had IC_{50} < 3 µg/ml (Tona et al., 2004). Other Thai fruit waste products (methanol extract), such as litchi (*Litchi chinensis* L.) seed and rambutan *(Nephelium lappaceum* L.) seed also possesses anti-malarial activity with 2.70 ± 0.23 and 2.78 ± 0.44 µg/ml, respectively (Sudjaroen, 2008, 2011). Previous study was conducted on longan seed, along

with a wide range of antioxidant activity tests, and the methanol extract of longan seed exhibited strong antioxidant capacities with an IC_{50} of 154 µg/ml for reactive oxygen species, which attacks salicylic acid, and 78 µg/ml for inhibition of xanthine oxidase in the hypoxanthine/xanthine oxidase assay (Sudjaroen et al., 2012). Moreover, longan seed extract presented anti-tyrosinase activity with IC_{50} values of 2.9 to 3.2 mg/ml (Rangkadilok et al., 2007). Therefore, the extracts had less effective antioxidant activity in the 2-deoxyguanosine assay, indicating that gallates, along with ellagic acid and its congeners exert their potential antioxidant effects predominantly by precipitation of proteins such as xanthine oxidase (Sudjaroen et al., 2012).

It is suppose that antimicrobial and antiplasmodial activities of longan seed may also be affected by gallic acid, ellagic acid and ellagitannins, which were precipitate microbial proteins, and caused growth inhibiting or killing effects to microorganisms.

Conclusion

Longan seed is the good source of chemopreventive agents for antimicrobial activity by inhibiting growth of *S. aureus*, *P. aeruginosa* and *C. albicans* and *in vitro* antimalarial activities *to P. falciparum*, with no evidence of cytotoxicity by *in vitro* cytotoxic screening assay with Vero cells. Gallic acid, ellagic acid and ellagitannins may play role of antimicrobial and antimalarial activities by precipitated microbial proteins, which was supported by previous studies that were tested for antioxidant and enzyme inhibitory activities of longan seed. This finding gives an interesting concept to search for more sources of the “low cost” chemopreventive agents in developing countries, especially tropical region which has high range of biodiversity. However, more biological models for confirmation of such activities and test for cytotoxic effect with other cell cultures may need to be conducted in further studies.

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