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Full Length Research Paper

Crude methanolic extract activity from rinds and seeds of native durian (*Durio zibethinus)* against *Escherichia coli* and *Staphylococcus aureus*

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This study was conducted to determine the activity of the methanolic crude extracts collected from the seeds and rinds of native durian (*Durio zibethinus)* against hospital isolates of *Escherichia coli* and *Staphylococcus aureus.* The fruit samples were collected from a local durian dealer and subjected to rotary evaporator. Using the Kirby-Bauer disc diffusion technique, the crude extracts with different concentrations (100, 75, 50 and 25%) were tested against the two isolates and after 24 h of incubation, zones of inhibition were measured. Durian seed and rind exhibits greater inhibition on the *E. coli* bacterium with inhibitions greater than chloramphenicol, which is the positive control.

Key words: Antibacterial, durian, disc diffusion, methanolic extracts.

INTRODUCTION

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs (Lalitha, 2004). In the study of the *in vitro* antibacterial activity of polysaccharide gel (PG) extracted from fruit-hulls of durian, results suggest that the PG from fruit-hulls of durian is a potential antibacterial agent (Phaunfoong et al., 2002).

Durian (*Durio zibethinus*), is a tree fruit which grows in a semi-cultivated manner throughout Southeast Asia (Baldry et al., 1972). In the Philippines, the durian-tree grows almost exclusive on Mindanao while in the rest of the country the trees rarely grow. Durian is considered as "King of Tropical Fruit" due to its high nutritional status and with its appearance that resembles the thorny thrones of Asian kings (Subhadrabandhu and Ketsa, 2001). Scientific interest lies in the fact that durian is considered by some botanists to be one of the most primitive of the trees in the tropical rain forest.

According to Faylon (2005), durian fruit, especially its flesh, is said to serve as a medication to eliminate parasitic worms. Moreover, in Malaya, decoction of durian leaves and fruits are applied to swellings and skin diseases while the ash of the burned rind is taken after childbirth. The primary objective of this study was to determine the crude methanolic extract activity from the rinds and seeds of the Philippines' native durian species against the hospital isolates of *Escherichia coli* and *Staphylococcus aureus*.

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MATERIALS AND METHODS

Bacterial sample collection and identification

Bacterial isolates were collected at the General Lluch Memorial Hospital, Palao, Iligan City. Cotton swabbing was employed on a chosen area inside the hospital, and was immediately brought to the laboratory and streaked in two different selective and differential media, Mannitol Salt Agar (MSA) and Eosin Methylene Blue (EMB).

S. aureus is a ubiquitous commensal bacterium on human skins and anterior nares that causes severe infections in humans (Kluytmans et al., 1997). Gram positive and catalase positive cocci occurring in pairs and short chains were subjected to growth on Mannitol salt agar plate, a selective medium developed for the presumptive isolation of *S. aureus* in a single step (Kateete et al., 2010) prepared according to the recommendations of Chapman.

Table 1. Average zones of inhibition of the methanol crude extracts and controls against the test organisms after 24 h of incubation.

Average zone of inhibition (mm)										
Test organism	C	M	Durian Seed				Durian Rind			
			100%	75%	50%	25%	100%	75%	50%	25%
S. aureus	32.17	6.5	20.67	11	27	10.33	12.67	10.67	9.33	8
E. coli	20	6.5	34	27.33	23	18.33	46.67	32.67	27.33	23

*C, chloramphenicol; M, methanol.

For the isolation and identification of *E. coli,* the enriched sample was cultured on selective medium Eosin methylene blue (EMB) agar and incubated at 37°C for 24 h. Morphologically typical colonies (at least 4/plate) producing green metallic sheen were taken into nutrient broth for further identification.

Confirmation of the presumptively identified *S. aureus* and *E. coli* were further identified by the following biochemical tests: lactose fermentation, catalase, oxidase, urease, Simmon's citrate, haemolysis, coagulase, indole production, nitrate reduction, and methyl red - Voges-Proskauer (MR-VP) tests.

Methanolic crude extraction from seed and rind of native *D. zibethinus*

Fresh native durian (*D. zibethinus)* fruit were collected from a local durian dealer. Seeds and rinds were separated and were thoroughly washed and oven-dried for 72 h at 90°C. The dried samples were grounded and separately placed in a beaker which contains methanol with ratio 1 part of the ground sample and 1 part of methanol. The ground samples were allowed to stand in methanol for at least 72 h for homogenizations. After homogenization, the liquid mixture was filtered and the filtrate was placed in a rotary evaporator to eliminate the solvent methanol. Different concentrations were made with the use of methanol as diluent.

Disk diffusion susceptibility assay

Kirby-Bauer disc diffusion method was used for antimicrobial susceptibility testing as recommended by the NCCLS. Well isolated colonies of *S. aureus* and *E. coli* were selected from an agar plate culture and transferred into a tube with 4 to 5 broth. The broth culture was incubated at 35°C until it achieved the turbidity of the 0.5 McFarland standard (2 to 6 h). Using sterile cotton swabs, *S. auerus* and *E. coli* was transferred from the nutrient broth into the Mueller-Hinton Agar (MHA) by aseptically dipping the swabs into the tubes and streaked on the plate. The discs were dipped on the different concentrations (100, 75, 50 and 25%) of crude extracts and the excess liquid were dabbed away. The treated discs were placed at the center of the divisions on each plate. The MHA plates were stored at 35°C for 24 h and zones of inhibition were measured.

RESULTS AND DISCUSSION

Methanolic crude extract from seed and rind of native durian showed an inhibition to the growth against the tested hospital isolates of *E. coli* and *S. aureus* using the Kirby-Bauer disc diffusion method. The average inhibition zones exhibited by the extracts and the positive and negative controls are shown in Table 1. The data show that the methanol crude extracts of the durian seeds and rinds in four levels of concentrations has an antibacterial activity against the test bacteria.

The inhibition zone was observed on MHA agar with the different concentrations of the crude extracts from durian seed. An increment of inhibition zone diameter was found with respect to increasing concentrations of the extracts as indicated in Table 1. Against *E. coli,* at 100% concentration the average zone inhibited is 34mm, at 75% it is 27 mm, at 50% it is 23 mm, and at 25% it is 12 mm, respectively.

However, inhibition zone against *S. aureus* bacteria at 100% is only 20 mm, at 75% it is 11 mm, at 50% it is 27 mm, and at 25% it is only 10 mm. Similar inhibitory activities were exhibited with the crude extracts of durian rind on the two test organisms.

Figure 1 displays a set of data to easily see where most of the numbers are and show that the gram negative test organism is more susceptible to the extracts than gram positive. Average zone of inhibition of the 100% concentration in seed is 34 and 47 mm for rind, and are greater than the commercially available antibiotic chloramphenicol. Table 2 shows the different average means of the treatment and the controls being compared using pair-wise comparison test to determine the relative order or ranking of a group of items. All the different concentrations of the rinds and seed crude extracts exhibited antibacterial activity.

These results imply that the obtained methanolic crude extracts may contain active components that are responsible for its inhibitory effect against the test organisms. Durian is rich in sulfur compounds (Voon et al., 2007) and has an inhibitory effects from natural products on aldehyde dehydrogenase (ALDH) as proposed by several groups (Veverka et al., 1997; Kitson and Weiner, 1996; Lindros et al., 1995; Brien and Loomis, 1985), but there is yet no study published on the phytochemical components on the durian rind as well as the seed.

This study provides evidence that methanolic crude extracts from seeds and rinds of the native durian (*D. zibethinus)* exhibits an antibacterial activity against two common pathogens. In a study by Pongsamart et al. (2005), it has been found out that durian has a medical use as a film dressing or dressing-patches for healing

Table 2. Pair-wise comparison of the means across treatment and the positive and negative controls.

Bacteria		100%	75%	50%	25%	M	C
	100%		0.0170	0.0004	0.0002	0.0002	0.0002
	75%			0.1707	0.0018	0.0002	0.0170
E. coli (seed)	50%				0.1250	0.0002	0.7268
	25%					0.0003	0.7268
	M						0.0002
	100%		0.0002	0.0002	0.0002	0.0002	0.0002
	75%			0.7752	0.2688	0.0030	0.0002
S. aureus (seed)	50%				0.9152	0.0253	0.0002
	25%					0.1286	0.0002
	M						0.0002
	100%		0.0002	0.0002	0.0002	0.0002	0.0002
	75%			0.0676	0.0011	0.0002	0.0002
E. coli (rind)	50%				0.1753	0.0002	0.0048
	25%					0.0002	0.3110
	M						0.0002
	100%		0.1096	0.0045	0.0004	0.0001644	0.0002
	75%			0.4352	0.0222	0.0008	0.0002
S. aureus (rind)	50%				0.4352	0.01481	0.0002
	25%					0.3211	0.0002
	M						0.0002

** Values in bold are significant P-values.

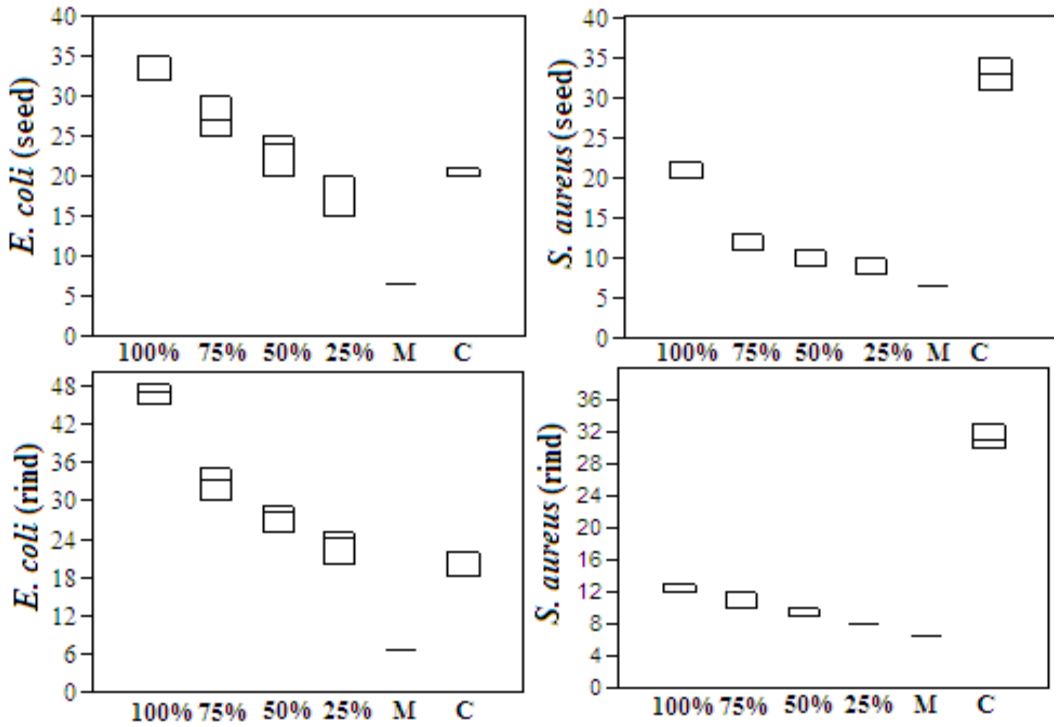

Figure 1. Box-and-whisker plots comparing the diameter of the zones of inhibition across treatments and (+/-) controls.

wounds because of its antimicrobial activity and film forming property. Such information should be useful to health care professionals in considering durian as a source of antibacterial agent.

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