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Antimicrobial and Cytotoxic Activities of *Hopea utilis* Fruits

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Abstract

Aqueous and ethanolic crude extracts of *Hopea utilis* screened for antibacterial and cytotoxic activities were studied. Antibacterial activity of ethanolic extracts of *H. utilis* were more successful with the pathogens *Salmonella typhi* and *Streptococcus aureus*. The MICs values of ethanolic extract of were *Hopea utilis* active against *Salmonella typhi* (25mg/ml), *Salmonella typhi* (25mg/ml), and *Staphylococcus aureus* (36mg/ml) respectively. The results of both extracts of aqueous and ethanolic of *Hopea utilis* showed the brine shrimp lethality assay LD_{50} values were 1.64µg/ml and 1.34µg/ml.

Key Words: *Hopea utilis;* fruits; antibacterial activity; ethanolic extract.

Introduction

Plants are recognized for their ability to produce a wealth of phytochemicals. Humankind has for centuries used many species to treat several diseases (Cragg *et al*., 1999). Recently, a number of tribal studies concerning the search for new antimicrobial agents from plants and antimicrobial screening of the extracts have been published. A rapid and inexpensive test, brine shrimp (*Artemia salina*) (BST), has been used for screening of biological and cytotoxity activities (De Rosa *et al.,* 1994). The fractions or active compounds in this assay, are further tested in cultured tumoral cells, antimicrobial and antiparasitic assays, generally with good correlation (Sahpaz *et al*., 1994; Colman-Saizarbitoria *et al*., 1995; Siqueira *et al*., 1998).

The Dipterocarpaceae is a plant family of 14 genera 750 species found throughout the tropical and temperate regions of the world. Bandaranayake *et al*., (1977) reported that 44 of the 45 species are endemic. They belong to the genera *Cotylelobium, Hopea, Dipterocarous, Shorea, Stemonoporus, Vateria* and *Vatica*. *Hopea utilis is* a large sized tree distributed in evergreen forest, Southern Western Ghats region, up to sea level 5000 meters. *Hopea utilis* is locally known as ''Karapongu'' in Karaiyar region. Ethnobotanical

information gathered from tribe of Kanis used as fruits was boiled with water and treatment of stomach pain and hypertension. Bioactive constituents of hopeafuran and C-glycosyl resveratrol were isolated from the stem wood (Tanaka *et al*., 2001). In the present study, the *in - vitro* antibacterial and cytotoxic activities of aqueous and ethanol extract of *Hopea utilis* fruits were investigated.

Materials and Methods

Collection of plant materials

The fruits materials of *H. utilis* were collected in the Karaiyar, Tirunelveli District, South India and was identified by Dr. U. Manikandan, SPKCES, Alwarkurichi, South India.

Extract preparation

250gm of powdered materials was individually extracted with 95% ethanol and water at room temperature. The solvent extract was removed by distillation method. The resulting crude extracts were stored at -20°C until assayed.

Antibacterial screening

Test Organisms

Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Vibrio paarahaemolyticus and Salmonella typhi were studied.

Inocula

Inocula for the bioassays were prepared by diluting scraped cell mass in 0.85% NaCl solution, adjusted to McFarland scale 0.5 and confirmed by spectrophotometrical reading at 580nm. Cell suspensions were finally diluted to 10^4 UFC/mL^{-1} for being used.

Disk- diffusion method

In - vitro antibacterial activity of both extract, were studied against four gram - positive and two gram - negative bacterial strains by the standard disc-diffusion method (Barry , 1980; Buer *et al.,* 1966; Berghe and Vlientinck, 1991). Nutrient agar was the bacteriological medium. Both extracts were screened at a concentration of 100µg /ml. Diameters of zones of inhibition produced by the isolated agent were compared with those produced by the standard antibiotic (Kanamycin, 30µg /disc).

Determination of MIC and MBC

Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) were determined for the extracts detailed methods (Suffredini *et al*., 2004)

Cytotoxic activity

The cytotoxic effect of both, aqueous and ethanolic extract was evaluated by LC_{50} of brine shrimp lethality test were followed by Mayer *et al*., 1982 and Persoone, 1980. Both aqueous and ethanolic extract were dissolved in dimethylsulphoxide (DMSO) separately and five graded doses 5, 10, 20, 40 and 80 mg ml/L were used for 5ml sea water containing 10 brine shrimp nauplii in each group. Mortality was recorded 12h and dead animals were removed immediately. LC_{50} values, upper and lower confidence limits and slope values were calculated using the POLO computer software (Russell *et al*., 1979). The regression coefficient between exposure time and different values of LD_{50} was determined by probit analysis (Finney, 1947). All computations were performed with a computer with a capacity of 28GB in the hard drive, 256 MB in RAM and working with a Microsoft Window XP^{TM} system at a speed of 1.6GHz.

RESULTS

Many microorganisms which cause damage to human health exhibit drug resistance due to inadequate use of antibiotics. Thus, there is a need for the discovery of new substances from natural resources, including plants (Sartoratto *et al*., 2004). In the present study, the antibacterial activity of aqueous and ethanolic extract of *H. utilis* is shown in Table 1. Antibacterial activity of ethanolic extract of *H. utilis* was more activity observed both pathogen of *Salmonella typhi* (30mm) and *Streptococcus aureus* (29mm). The MICs values of ethanol extract active against *Salmonella typhi* 25mg/ml, and *Staphylococcus aureus* 36mg/ml, respectively.

The cytotoxicity of the compound was bioassayed against brine shrimp nauplii and the results were shown in Table 2. The 50% mortality of log-dose concentrations (LD_{50}) of the aqueous extract was 1.62 µg/ml. Figure- 1 shown the 95% confidence regression value Y=3.67+1.44X and significant level of ($P < 0.05$) were observed in aqueous extract of *H. utilis*. While the more cytotoxic activity was observed in 95% ethanol extract observed seen in Table-2 and Fig.2. The present study was agree to previous workers studied in bioactive compounds of Kolavenic acid and *Clerodane diterpine* (Islam *et al.*, 2001); Isoflavone (Shah Alam Bhuyan *et al.*, 2003); Triterpenoid (Rahman *et al*., 2002) and galic acid (Saker *et al.*, 1998). The antibacterial and cytotoxic activity of extract of *H. utilis* bioassay-guided fractionation procedure to characterize and isolate the bioactive principle is under way in our laboratory.

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Table 1. Antibacterial activity of ethanolic and aqueous extract of *H. utilis* fruits.

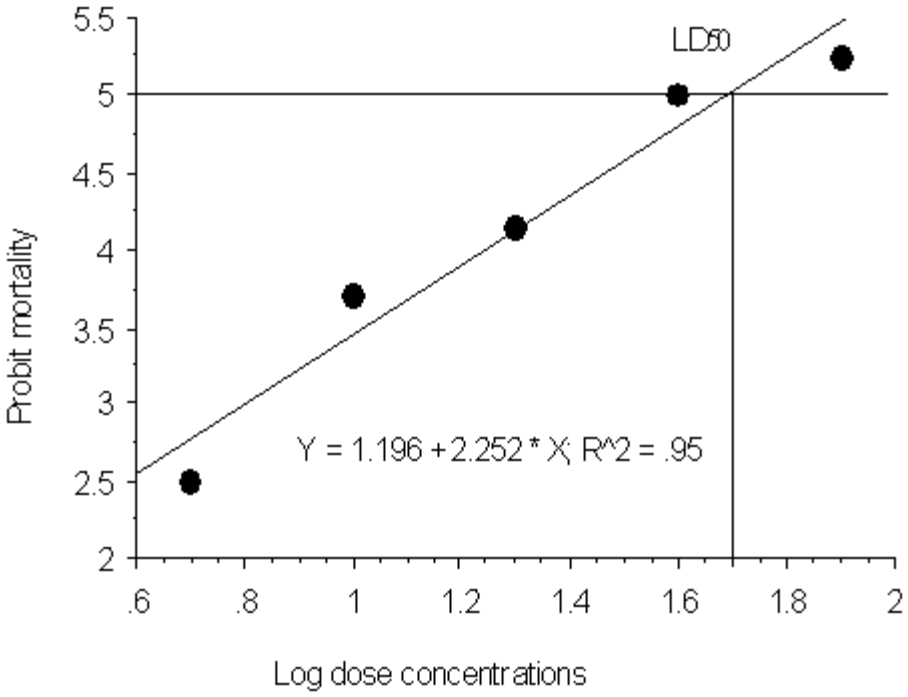
	Diameter of the Zone of the inhibition (mm)		95% ethanol extract	

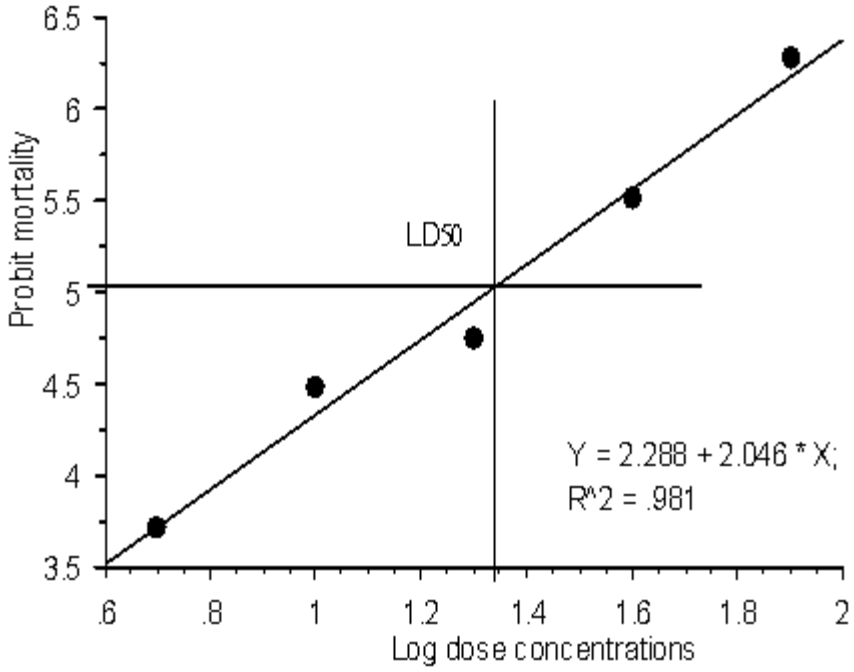
Test organisms	Aqueous	95% ethanol	MIC (mg/ml^{-1})	MBC (mg/ml^{-1})
Gram –positive				
Bacillus subtilis	13	24		
Staphylococcus aureus	15	29	36	58
Streptococcus pyogenes	8	16		
Gram –negative				
Salmonella typhi	15	30	25	34
Pseudomonas aeruginosa	13	24		
Vibrio parahaemolyticus	16	25		

Table 2. Antibacterial activity of ethanolic and aqueous extract of *H. utilis* fruits.

Concentrations (mg/ml)	**% Mortality**	**LD_{50} Values mg/l^{-1}**	**Regression Equations**	**95% Confidential limit**	**X^2 value**
Aqueous extract					
5	0				
10	10				
20	20	1.623	y = 2.2521x + 1.196	1.42-1.84	0.183
40	50				
80	60				
Ethanol					

extract					
5	10				
10	30				
20	40	1.349839	y = 2.0462x + 2.288	1.21 - 1.49	0.616
40	70				
80	90				




6.5
6
5.5
5
4.5
4
3.5
Probit mortality
LD50
Y = 2.288 + 2.046 * X;
R^2 = .981
.6
.8
1
1.2
1.4
1.6
1.8
2
Log dose concentrations