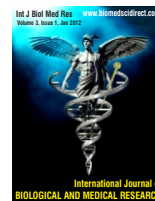


Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com



Original Article

Synergistic Activity of Chloroform Extract of *Durio zibethinus* Wood Bark With Penicillin G Against *Staphylococcus aureus*.

Sivananthan Manoharan

Department of Biomedical Science, Faculty of Biomedicine and Health, ASIA Metropolitan University
G-8, Jalan Kemacahaya 11, Taman Kemacahaya, Batu 9, 43200 Cheras, Selangor, Malaysia

ARTICLE INFO

Keywords:

DMSO
Durio zibethinus
Kirby Baur technique
Penicillin G
Staphylococcus aureus ATCC 33862.

ABSTRACT

Aim: This study was conducted to determine the synergistic activity of chloroform extract of *Durio zibethinus* wood bark with Penicillin G against *Staphylococcus aureus*. **Method:** Extract of the plant obtained after maceration of plant powder into chloroform, then was filtered using filter paper and finally evaporated. The powder form was freshly reconstituted in dimethyl sulfoxide (DMSO) and tested against *Staphylococcus aureus* ATCC 33862 using Kirby Baur technique and the plates were incubated at 37 °C. The zone of inhibition was measured after 24 hours and recorded in millimeters. The synergistic study was conducted using the *Durio zibethinus* wood bark extract in combination with penicillin G with the proportion of 1:1 in homogenous condition and incubated at 37 °C for 24 hours. The zone of inhibition was measured and recorded. **Result:** The inhibition zone of *Durio zibethinus* wood bark extract against *Staphylococcus aureus* ATCC 33862 was 10 mm and the Penicillin G was 12 mm. The combination of both *Durio zibethinus* wood bark extract and Penicillin G was 14 mm. **Conclusion:** *Staphylococcus aureus* have created resistance to various antibiotics. Chloroform extract of *Durio zibethinus* bark exhibit mild synergistic activity against *Staphylococcus aureus* ATCC 33862. Although the effect was mild but it is possible that *Durio zibethinus* could reverse such resistance and thus potentiate the effect of common penicillins against resistant *Staphylococcus aureus*.

© Copyright 2010 BioMedSciDirect Publications IJBMR -ISSN: 0976:6685. All rights reserved.

1. Introduction

Microorganisms have created resistance to various antibiotics and this had developed immense clinical difficulty in the curing of contagious illness. The enlarge in resistance of microbes due to indiscriminate utilize of commercial antimicrobial medicines supported scientists to investigate for modern antimicrobial substances from several sources including medicinal plants [1].

Medicinal herbs are widely used with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. It has been recorded that 80% of the world's population has fidelity in traditional medicine, particularly plant based drugs for their primary healthcare [2].

Durian (*Durio zibethinus* Murr) is a popular and expensive tropical fruit widely grown in South-East Asia. Durian is entitled "King of Tropical Fruit" due to the superlative flesh, which is highly nutritional and its appearance which resembles the thorny thrones of Asian kings [3]. Among exotic fruits durian is less known [4].

The fruit of durian is a huge 5 shouldered, spiny, loculicidal capsule. Each fruit weight from 2 to 4 kg. It has a thick, tough rind covered with pyramidal shape pointed spines. The unique character of fruit that drops upon reaching maturity or from 16 weeks of flower opening, requires that they be gathered and marketed without delay. Durian fruit are known to deteriorate within 36 to 72 hours from fruit drop. This indicates that the fruit have very short shelf life [5].

Durian grows well in humid conditions (75-80% humidity) with a rain fall between 1,600 and 4,000 ml a year, and average temperature between 24 and 30°C. Durian prefers a loamy soil type with a pH between 5.0 and 6.5. It requires a tropical climate and

* Corresponding Author : **Sivananthan Manoharan**
Faculty of Biomedicine and Health,
ASIA Metropolitan University,
G-8, Jalan Kemacahaya 11, Taman Kemacahaya, Batu 9,
43200 Cheras, Selangor, Malaysia. PIN- 43200
Phone: +600169534735
E mail: siva8905@gmail.com

will not grow well in areas over 3000 feet altitude. There are about 200 different varieties of durian in Malaysia, but only few varieties are favored and grown commercially [6].

Durian is rich in carbohydrate, protein, fat, phosphorus, iron and vitamin A. Durian is usually used for fresh consumption. The edible portion (aril) of durian has a very strong odor [3]. Most of the photochemicals are an integral part of the durian fruit and also being used in medicinal formulations. A number of health protective effects of phenolic compounds have been reported due to their antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antimicrobial, and other biological possessions. Currently durian fruit is popular in daily utilization because of their first-class flavor and health-promoting compounds, such as flavonoids, phenolics and carotenoid contents [6]. Durian 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 [7].

Toxicity study of *Durio zibethinus* was conducted previously. Polysaccharide gel which is extracted from the durian was used in determining the toxicity study in mice and rats. A high oral dose (2g/ kg) did not induced severe toxicity in male mice and rats. No toxic effect were observed in subacute treatment in male mice and subchronic studies in male and female confirm the consumptive safety of polysaccharides gel [8].

Staphylococcus aureus is one of the gram-positive microorganisms that have been shown to exhibit resistance to a wide range of commonly available antibiotics, especially the penicillins. Therefore, penicillins are often administered in combination with other antibiotics in the treatment of resistant (or suspected resistant) bacterial infections [9]. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment [10]. Therefore, the present study was conducted to investigate the synergistic activity of chloroform extract of *Durio zibethinus* with penicillin G.

2. Material and methods

Plant material:

The bark of the *Durio zibethinus* was obtained from durian orchard in Sungai Klau, small village in Raub, Pahang, Malaysia. The bark then wash thoroughly under running tap water and dried under shade. They were then finely ground to a powder in an electric blender [11].

Preparation of crude extract:

The solvents used for the extraction procedure in the present study was chloroform. About 18 g of dried durian bark powder were extracted using 180 ml of the extraction solvent with continuous

shaking on a rotary shaker at 150–180 rev/min for 48 hours [11]. The filtrates was concentrated using a rota-vapour, at 40°C and then in water bath until the powder was form [12]. The percentage of yield was 0.6%.

Bacterial strains:

Bacterial strain used in the study was *Staphylococcus aureus* ATCC 33862

Antimicrobial activity:

The media used were Muller Hinton agar [13]. 10 mg of extracts were freshly reconstituted with dimethyl sulphoxide (DMSO). Antimicrobial activity was determined by the well diffusion method. Wells (8 mm diameter) were cut into the agar. 200µl of the plant extracts were tested in a concentration of 10mg/ml and 200µl of penicillin G were tested in a concentration of 6.25µg/ml separately. The agar were seeded with 24h culture of the microorganism. Incubation was performed at 37°C for 24 hours for bacterial strain. Microbial growth was determined by measuring the diameter of zone of inhibition in millimeters [14]. The work was done in triplicate [1].

Synergistic activity:

The synergistic activity study was calculated by means of cup plate method (Kirby and Bauer technique). Chloroform plant extract of *D.zibethinus* 10mg/ml was used in combination with penicillin G 6.25 µg/ml in propotion of 1:1 (100 µl : 100 µl). The combination were in homogenous condition. The plates then incubated at the standard conditions for 24 hours at 37°C and the zone diameters was measured in the second day. The work was done in triplicate.

3.Result and Discussion

From the table 1, it was clearly stated that the inhibition zone of chloroform extract of *D.zibethinus* against *Staphylococcus aureus* was 10 mm. The inhibition of *Staphylococcus aureus* by penicillin G was 12 mm. In synergistic activity, the inhibition of *Staphylococcus aureus* by combination of chloroform extract of *D.zibethinus* and penicillin G was 14 mm. Mild synergistic effect was produced.

Solvent used for the extraction purpose in this study was chloroform [11]. In previous studies, ethanolic extract of *D. zibethinus* leaves exhibited no inhibitory effect on *Staphylococcus aureus*. According to the researchers, no previous report has been found on the antimicrobial activity of this plant [15]. Methanolic crude extract from seed and rind of native durian showed an inhibition to the growth of *Staphylococcus aureus* [7].

The antimicrobial activities of the plant extracted in different solvents varied greatly because there are many factors influence the active compounds present in the plant. The polarity of the extracting solvent are different and greatly influenced the antimicrobial properties. This may be due to the better solubility of the active compounds in organic solvents [14].

Table 1: Synergistic activity of chloroform extract of *Durio zibethinus*

Microorganism	Inhibition zone (mm)		
	E	P	EP
<i>Staphylococcus aureus</i> ATCC 33862	10	12	14
DMSO	NA	NA	NA

E= Chloroform extract of *D.zibethinus*, P= penicillin G, EP= Chloroform extract of *D.zibethinus* + penicillin G, NA= No inhibition

Durian fruit contain a considerable amount of flavonoids [6]. Flavonoids exhibit a broad spectrum of biological activity including antiviral activity and research on synergism is very limited [10].

In wood bark extract of *Durio zibethinus*, two new triterpenoids, namely, methyl 27-O-trans-caffeoylcyclicodiscate and methyl 27-O-cis-caffeoylcyclicodiscate, a new phenolic, 1,2-diarylpropane-3-ol, and seven known compounds, fraxidin, eucryphin, boehmanan, threo-carolignan E, (-)-(3R,4 S)-4-hydroxymellein, methyl protocatechuic acid, and (+)-(R)-de-O-methylsiodiolin contents were present [16]. From the table 1, combination of wood bark extract of *D.zibethinus* and penicillin G produce synergism activity against *S. aureus*. Penicillin G act on cell wall. Penicillin G, inhibits the third and final stage involved in the synthesis of peptidoglycan, which is a heteropolymeric component of the cell wall, which provides a rigid mechanical stability by virtue of its highly cross-linked lattice work structure. This cross linking is accomplished by a transpeptidation reaction that occurs outside the cell membrane [9]. Activity of one or more compound that were present in the wood bark extract of *D. zibethinus* which may having antibacterial properties against *S. aureus* and combination with Penicillin G produces synergism. The compounds that were present in the wood bark extract of *D.zibethinus* that were lead to the inhibition of *S. aureus* are still unclear but these double attack of *D. zibethinus* and Penicillin G on different target sites of the bacteria could lead to synergistic effect [9]. Further studies on the activities and the target site on the *S. aureus* possess by the compounds in wood bark extract need to be conducted so that the synergism activity can be understood better.

Most *Staphylococci* isolated from individuals outside the hospital are resistant to penicillin G due to beta lactamases, which inactivate the drug [9]. Although the synergism activity in the present finding was mild but it is possible that *D. zibethinus* could reverse such resistance and thus potentiate the effect of common penicillins against resistant *S.aureus*.

4. Conclusion

This study provides evidence that chloroform crude extracts from bark of the native durian (*D. zibethinus*) exhibits an antibacterial activity against *Staphylococcus aureus* and the combination of chloroform crude extracts from bark of the native durian (*D. zibethinus*) and penicillin G were exhibits mild synergistic activity against *Staphylococcus aureus*.

Acknowledgements

I would like to thank all the lab technicians that were involved in my research for providing necessary equipment and chemicals to conduct the research. I also would like to thank Mr. Kaviyarasu Elangkovan, Department of Economics, Faculty of Economics and Management, University Putra Malaysia (UPM) for his contribution in this research.

5. References

- [1] Alagesaboopathi C, Kalaiselvi N. Antimicrobial activities of the root, stem and leaf extracts of *Argemone mexicana* L. Int. J. Biosci 2012; 2(5): 61-68.
- [2] Alireza V, Mihdzar AK, Soon GT, Daryush T, Mohd PA, Sonia N. Nain-e-Havandi *Andrographis paniculata* present yesterday, absent today: a plenary review on underutilized herb of Iran's pharmaceutical plants. Int. J. Mol. Cell. Bio 2011;39(5): 5409-5424.
- [3] Norjana I, Noor AAA. Quality attributes of durian (*Durio zibethinus* Murr) juice after pectinase enzyme treatment. Int. Food. Res. J 2011; 18(3): 1117-1122.
- [4] Leontowicz H, Leontowicz M, Haruenkit R, Poovarodom S, Jastrzebski Z, Drzewiecki J, Ayala ALM, Jesion I, Trakhtenberg S, Gorinstein S. Durian (*Durio zibethinus* Murr.) cultivars as nutritional supplementation to rat's diets. Food. Chem. Toxicol 2008; 46: 581-589.
- [5] Pascua OC, Cantila MS. Maturity indices of durian (*Durio Zibethinus* Murray). Phillip J. Crop. Sci 1992; 17(3): 119-124.
- [6] Ashraf MA, Maah MJ, Yusoff I. Estimation of Antioxidant Phytochemicals in Four Different Varieties of Durian (*Durio Zibethinus* Murray) Fruit. Int. Con. Biosci, Biochem. Bioinfo 2011; 5: 131-135.
- [7] Duazo NO, Bautista JR, Teves FG. Crude methanolic extract activity from rinds and seeds of native durian (*Durio zibethinus*) against *Escherichia coli* and *Staphylococcus aureus*. Afr. J. Microbiol. Res 2012; 6(35): 6483-6486.
- [8] Lipipun V, Nantawanit N, Pongsamart S. Antimicrobial activity (in vitro) of polysaccharide gel from durian fruit-hulls. Songklanakarin J. Sci. Technol 2002; 24(1): 31-38.
- [9] Esimone CO, Iroha IR, Ibezim EC, Okeh CO, Okpana EM. In vitro evaluation of the interaction between tea extracts and penicillin G against *staphylococcus aureus*. Afr. J. Biotech 2006; 5(11): 1082-1086.
- [10] Kumar AS, Venkateshwaran K, Vanitha J, Saravanan VS, Ganesh M, Vasudevan M, Sivakumar T. Synergistic activity of methanolic extract of *Thespesia populnea* (Malvaceae) flowers with oxytetracycline. Bangladesh J Pharmacol 2009; 4: 13-16.
- [11] Soma R, Kiranmayee R, Bhuvaneshwari CH, Archana G, Lakshmi NM. Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. World J. Microbiol. Biotech 2009; 26(1): 85-91.
- [12] Rusmiati. The effects of durian wood skin methanol extract (*Durio zibethinus* Murr) of Ovarium and Uterus Microanatomy Structure Female Mice (*Mus musculus* L). Sains dan Terapan Kimia 2010; 4(2): 108-118.
- [13] Al-Haddad AM. Urinary tract infection among pregnant women in Al-Mukalla district, Yemen. East. Mediter. Health. J 2005; 11(3): 505-510.
- [14] Katakya A, Handique PJ. Antibiotic Activity and phytochemical estimation of micropropagated *Andrographis paniculata* (Burm.f) Nees. Asian J. Sci. Technol 2010; 5: 91-94.
- [15] Aziz ASM, Low CN, Chai LC, Razak ASSN, Selamat J, Son R, Sarker MZI, Khatib A. Screening of selected Malaysian plants against several food borne pathogen bacteria. Int. Food. Res. J 2011; 18(3): 1195-1201.
- [16] Lim TK. *Durio zibethinus*. Edible medicinal and non medicinal plant 2012; 1: 569-583