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In vitro Antimicrobial Activity of *Vigna radiata* (L) Wilzeck Extracts Against Gram Negative Enteric Bacteria

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Abstract: The rapid spread of multidrug-resistance against conventional antibiotics is a global threat that necessitates the search for alternative therapies from natural sources. In this study, the antibacterial potentials of chloroform and methanol extracts of mung bean sprout (MBS) or *Vigna radiata* (L) Wilzeck extracts were evaluated against *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Salmonella* spp. using agar disk diffusion method and minimum inhibitory concentration (MIC) assessment. Both extracts showed antimicrobial activities against all the tested gram negative bacteria with the exception of *K. pneumoniae* which remained resistant. Chloroform extracts in general exhibited greater antibacterial activity compared to methanol extracts. However, methanol extracts of MBS exhibited significant activity against *P. aeruginosa* compared to chloroform extracts and results were comparable to the standard antimicrobial antibiotic with an average MIC value of 75 mg/mL. Our finding identifies the potential use of MBS as a natural source of antibacterial agent. Further studies are warranted to elucidate the mechanism of action of the extracts and to identify the active components responsible for the antibacterial activity.

Key words: *Vigna radiata* (L) Wilzeck · Medicinal plants · Antibacterial activity · Minimal inhibitory concentration

INTRODUCTION

The unmethodical and indiscriminate use of commercial antimicrobial drugs has lead to the development of multidrug resistance [1], complicating the choice of empirical therapy. The emerging resistance of many gram negative enteric pathogens continues to pose threat, yet such problem is unparallel with the discovery of alternative agents to battle the issue. Recently, much attention has been focused to unravel the medicinal properties of natural products and in tandem, the need to search for effective new antimicrobials continues to escalate [2]. Such interest may pave way to the discovery and invention of novel therapeutic agents that could benefit mankind.

Vigna Radiata (L) R. Wilzeck or Mung bean sprouts (MBS) are edible sprouts that serve as crucial ingredients in many Asian cuisines and are locally known as "yínyá" in Chinese, "moyashi" in Japanese, "taugeh" in Malay and "togue" in Tagalog. The consumption of sprouts has increased its popularity in recent decades due to its availability, easy growing methods and increased consumer preference for healthier food products. Moreover, the high nutritive value and health enhancing benefits of MBS have been well documented [3].

The impact of MBS in the culinary world has been conflicting. Consumption of raw or partially cooked sprouts has been implicated in outbreaks of *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* 0157: H7 in many parts of Asian and European countries [4-6],

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making it a microbial threat and posing food safety concerns. Paradoxically, recent claims on the antimicrobial properties of MBS are stirring global interest in the commodity. There has been limited documentation to date focusing on the antimicrobial nature of MBS, forming the objectives of this study.

MATERIALS AND METHODS

Plant Material: MBS were purchased from local markets in the Selangor state of Malaysia. Sprouts were air dried, ground to fine powder and stored in an airtight container at room temperature until further use. Exposure to light was kept to a minimum to prevent loss of active compounds.

Preparation of Extracts: The powdered samples were subjected to extraction using organic solvents namely methanol and chloroform that were of analytical grade. 50g of powdered sprouts were soaked in 500 mL, of respective solvents for three days, with continuous shaking at 37°C. Solvents were removed by filtration and concentrated under reduce pressure in a rotary evaporator. The extracted compound obtained were preserved aseptically at 4°C and used for the antimicrobial assay.

Test Microorganisms: *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 4157), *Klebsiella pneumoniae* typhimurium (ATCC 4352), *Salmonella typhimurium* (ATCC 14028), *Salmonella paratyphimurium* (ATCC 9150) and *Salmonella enterica* (ATCC 10708) were obtained by the courtesy of Mr. Zed Zakari Abd. Hamid, the Head of Department of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, Malaysia. Bacterial stocks were cultured on nutrient agar at 37°C. for 18 h and validated on the basis of morphological, cultural and biochemical characteristic based on the Bergey's Manual of Systematic Bacteriology [7].

Antibiotic Susceptibility Testing: The antibacterial activities of the chloroform and methanol extracts of MBS were screened using disc diffusion method [8] under strict aseptic conditions. Cultures were grown for 24 h and turbidity was maintained according to the 0.5 MacFarland standards. The extracts were dissolved to a final concentration of 200, 500 and 700 mg/mL and the saturated discs were then placed on top of the bacterial lawn on the surface of the Mueller Hinton agar (MHA) plates. Positive and negative controls used were, gentamicin (10µg/disc), tetracycline (30µg/disc) and 10% (v/v) Dimethyl, Sulfoxide (DMSO; Sigma-Aldrich, USA) respectively. Plates were incubated aerobically overnight at 37°C,. The experiment was performed in triplicates. The diameter of the inhibition zones produced were measured using calipers and classified as "sensitive", "intermediate" or "resistant" based on the standard interpretation chart.

Determination of Minimum Inhibitory Concentration (MIC): MIC for the most sensitive organism was determined by microdilution method, in accordance to the National Committee for Clinical Laboratory Standards (NCCLS) [9]. Two-fold serial dilutions of the stock extract solution were prepared. One hundred microlitre of each extract dilution and an equal amount of standardized inoculums were dispensed in triplicates into 96, well microtiter plate. Two control tubes containing the growth medium and extract-free bacterial suspensions were maintained for each test batch. The microtitre plate was incubated overnight at 37°C and observed for turbidity. The MIC was defined as the lowest concentration of the extract that inhibited the growth of microorganisms and produced no visible microbial growth.

RESULTS

Antimicrobial Susceptibility and MIC Assessment: Qualitative results were obtained and appraised observationally. Our findings revealed the inhibitory effect of MBS on gram negative bacteria showing visible zones of inhibition on the surface of MHA plates (Fig. 1). The average diameter of the zones of inhibition exhibited by the extracts against the test bacteria species was calculated and ranged between 6 -18 mm for chloroform extracts and 6 -20 mm for chloroform extracts (Fig. 2,). The antibacterial activity of the extracts increased linearly with the increase in concentration of the extracts. In general, the chloroform extracts of the MBS revealed greater antibacterial action in contrast to the methanol extracts. This study found that 700 mg/mL chloroform extracts produced a zone of inhibition of 18 mm against *E. coli*, compared to a zone of inhibition of 10 mm with methanol extracts. On the other hand, methanol extract showed remarkable antimicrobial properties against *P. aeruginosa* as opposed to the chloroform extracts, even at low concentrations with a zone of inhibition of 20 mm at 700 mg/mL and an average MIC value of 75 mg/mL,.

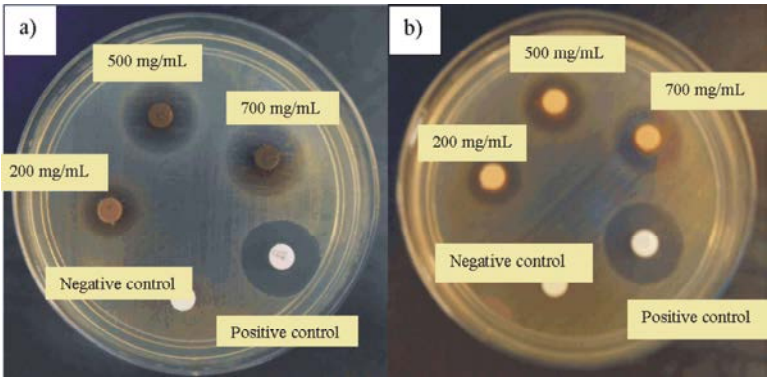

Fig. 1: Zones, of inhibition on MHA of (a) methanol extract of MBS against *P.aeruginosa* ; b) Chloroform extract of MBS against *E.coli.* Gentamicin and tetracycline were used as positive control and 10% (w/v) DMSO as the negative control.

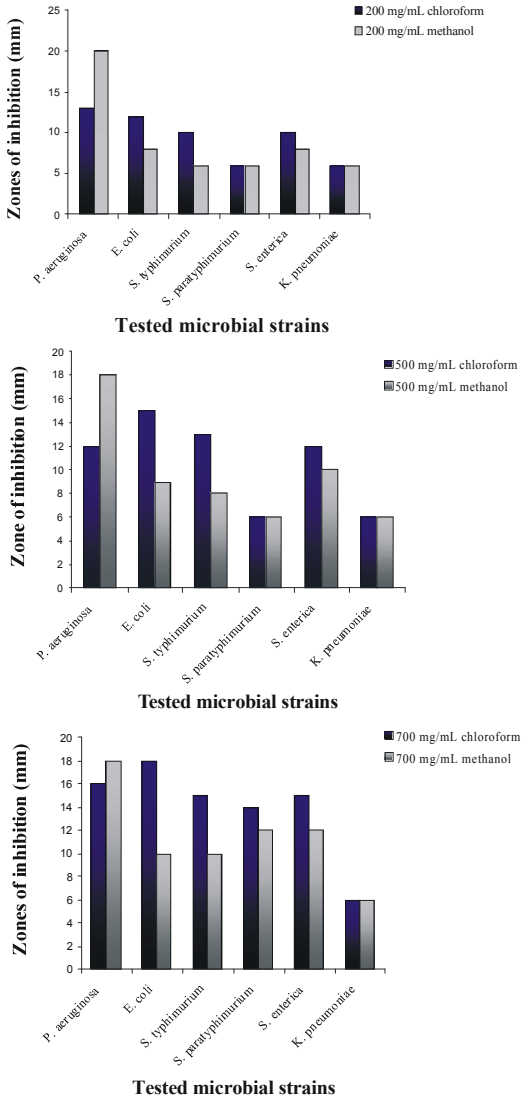

Fig. 2: Antibacterial activity of chloroform and methanol extracts of MBS

DISCUSSION

The evolution and dissemination of multidrug-resistant (MDR) bacteria continues to exhaust the supply of antibiotics for effective chemotherapy worldwide and Malaysia is of no exception [10, 11]. Extended spectrum beta lactamase producing bacteria (ESBL) are particularly notorious and has increased significantly during the last decades [12], urging, the discovery of alternative treatments. Recently, much attention has been directed towards the actions of biologically active compounds isolated from natural sources. A large, body of evidence has accumulated to demonstrate the promising potential use of plants to challenge resistant pathogens, providing alternative therapy to commercial antibiotics. Studies into the antimicrobial activities and potential use of natural products have regained importance for pharmacological research and could pave way towards the discovery of novel chemotherapeutic agents [13] assisting strategies to mitigate the health needs in developing countries. Empirical.

This study, was conceived to explore the antibacterial properties of MBS using simple, rapid and cost-effective methods of antibacterial screening through disc diffusion assay. Positive findings will be of great benefit since MBS are cheap, widely accessible and have significant importance in the international trade. Previous work has shed some light into the therapeutic value of MBS. The study conducted by Randhir *et al*., 2004, revealed its antibacterial activities against *H. pylori* [14] and more recently, Hafidh *et al*., 2011, provided additional evidence of both antimicrobial and antifungal activities from MBS against MDR bacteria including gram positive organisms using methanol as the main solvent [15]. Our study compared the antimicrobial activities of MBS against

selective gram negative bacteria using both polar and non polar solvents, variations of which may result in differences in the properties of the extracts as well as the type of biologically active compound isolated [16].

In the present investigation, chloroform and methanol extracts of MBS were screened for their antibacterial activities against six gram negative enteric bacteria, selected on the basis of their clinical and pharmaceutical importance. Results of the antimicrobial assay revealed broad spectrum activity against all tested bacteria with exception of *K. pneumoniae* that showed negligible inhibitory activity. Chloroform extracts demonstrated higher antimicrobial activities than the methanol extracts, particularly against *E. coli,* although methanol extracts deemed most active against *P. aeruginosa.* The zone of inhibition produced by chloroform extracts were almost comparable to that produced by the commercially available antibiotics used. Our results are in agreement with findings by Hafidh *et al*., 2011, whereby methanol extracts at 500 mg/ml were found to exert greater antibacterial effect than streptomycin [15]. Furthermore, our preliminary findings into the ultrastructural effects of both chloroform and methanol extracts against *E.coli* and *P. aeruginosa* using scanning electron microscopy revealed significant changes on the surface of the infected bacteria including clumping, deformation and exterior destruction (data not shown). Such changes were absent in chloroform and methanol extract-free cells, providing additional evidence of the antibacterial activity of MBS.

Factors responsible for this susceptibility are unknown, but could be due to the presence of secondary metabolites which is soluble in both methanol and chloroform extracts. Further efforts should be targeted towards the discovery of the active components in MBS that is responsible for its antimicrobial properties. Although the mechanism of action has not been fully elucidated, previous studies have demonstrated the antimicrobial properties of MBS methanol extracts are due to the actions of secondary metabolites such as phenolic antioxidants and flavonoids [15, 17]. The latter are found copiously in plants and has also been shown to have antiviral, anticarcinogenic, antiproliferative and anti-inflammatory actions [18]. The curative potential and diverse health promoting effects of flavonoids, including that in MBS merits further investigation.

In conclusion, the antibacterial activities of MBS highlighted in this study indicate the potential use of this plant as a natural alternative agent that could complement antimicrobial therapy. Detailed scientific scrutiny on the pharmacological active compounds responsible for the antimicrobial activities are warranted to further evaluate the clinical, pharmaceutical and therapeutic potentials of MBS.

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