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# Preliminary investigation on the antibacterial activity of mango (*Mangifera indica* L: Anacardiaceae) seed kernel

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ABSTRACT

**Objective:** To evaluate the antibacterial activity of the methanolic extract of mango (*Mangifera indica* L.) seed kernel. **Methods:** Chokanan mango seed kernel and seed kernels from assorted mango varieties were collected, cleaned, dried and powered. Crude methanolic extracts of mango seed kernel were analyzed for the phytochemical constituents. The free radical scavenging activity was determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Antibacterial activity was evaluated by disc diffusion assay with three medically important bacterial pathogens such as methicillin resistant *Staphylococcus aureus (S. aureus)* (MRSA), *Escherichia coli (E. coli)* and *Vibrio vulnificus (V. vulnificus)*. **Results:** Qualitative phytochemical analysis indicated the presence of important phytochemical compounds such as glycosides, saponins, flavanoids, tannins and alkaloids. There was no significant difference in the phytochemical content between the single and assorted mango seed kernels. However, the free radical scavenging study indicated that the assorted mango kernels showed slightly higher activity than the single species ($P<0.05$). The crude methanolic extract of mango seed kernel at a concentration of 100 mg/mL is found to have potential antimicrobial activity against MRSA and *E. coli* compared to *V. vulnificus*. Study on the antibacterial activity also indicated that there was no significant difference in the antibacterial activity of the single and assorted mango seed kernel extracts. **Conclusions:** The present study conclusively demonstrates the free radical scavenging activity and antibacterial activities of mango seed kernel. In addition, the results also indicated that there is no significant difference in the phytochemical content and biological activity of mango kernels from single and assorted mango varieties.

## 1. Introduction

Despite the availability of a range of synthetic antibiotics, the infectious diseases continue to be the major health problem world wide. The development of widespread antibiotic resistance among the pathogens and undesirable side effects associated with the continued use of synthetic drugs, has stimulated a renewed interest in the alternative therapeutics. Before the advent of modern health care systems, plant based therapeutics were the prime source for treatment of diseases, particularly infectious diseases. Mango (*Mangifera indica* L, Anacardiaceae), considered as "The King of Fruits" is distributed mainly in tropical Asia. The fully ripened fruits are used for table purpose and the unripened fruits are used for making pickles. Despite the fact that mango seed kernel contains 6% protein, 11% fat, 77% carbohydrate, 2% crude fiber and 2% ash[1], the seeds are considered to be a waste. Among the rural folks the mango seed kernels are used traditionally as antimicrobials against gastric pathogens especially for the infants. In the food processing industry, a considerable quantity of seeds are discarded as waste, after the extraction of mango

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pulp. Though several investigations have been done on the medicinal properties of mango fruit flesh and peel[2], to our understanding, very limited work has been carried out on the mango seed kernel. Hence this present study aimed to evaluate the antimicrobial potential of the methanolic extract of mango seed kernel on medically important human bacterial pathogens. Phytochemical constituents and free radical scavenging ability of the mango seed kernel were also evaluated.

## 2. Materials and methods

### 2.1. Chemicals and plant materials

All the chemicals and reagents used in this study were ACS grade. Mango seed kernels were divided into two groups. One from a mango variety known as Chokanan and the other from assorted mango varieties. The samples were collected in Sungai Petani, Kedah Darul Aman, Malaysia, during April-June 2009. The plant materials were identified and authenticated by a botanist from the Faculty of Applied Sciences, AIMST University, Kedah Darul Aman, Malaysia.

### 2.2. Preparation of plant extracts

The collected plant material was washed under running tap water to remove sand, dirt and other contaminating foreign particles. The plant material was then oven dried at 60 ℃ for 4 days. The seed kernels were powdered using a heavy duty blender. The powder was extracted with methanol according to the maceration method and the extracts were filtered by Whatman No.1 filter paper. The filtrate was concentrated in a rotary evaporator at 40 ℃. The concentrated extract was oven dried at 40 ℃ for 4 days and then freeze dried for 48 h. The freeze dried extracts were stored at -20 ℃ until use.

### 2.3. Phytochemical analysis

Methanolic seed kernel extract was used for preliminary qualitative screening of phytochemicals as per standard biochemical procedures. The crude extract was diluted with methanol to the concentration of 200 mg/mL. The qualitative phytochemical analyses of crude extract mango seed kernel was conducted to determine the presence of reducing sugars (glycosides), saponins, anthraquinone derivatives, flavanoids, tannins and alkaloids[3].

### 2.4. Measurement of DPPH free radical scavenging activity

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical[4]. The mango seed kernel extract was added to a 0.004 % MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min. The commercial antioxidant butylated hydroxytoluene (BHT) was used for comparison as a positive control. The percent inhibition activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where, $A_0$ was the absorbance of the control reaction and $A_1$ was the absorbance in the presence of the sample.

### 2.5. Antibacterial activity

Three human pathogenic bacteria namely methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli (E. coli)* and *Vibrio vulnificus (V. vulnificus)* were used in this study were obtained from Department of Microbiology & Parasitology, Health Campus, Unversiti Sains Malaysia (USM) Kubang Kerian, 16150, Kelantan, Malaysia. The bacterial strains were grown in 50 mL of nutrient broth at 37 ℃ and maintained in nutrient agar slant at 4 ℃. The disc diffusion technique was used for antimicrobial test. An overnight suspension culture of the three bacterial strains was spread on the Mueller-Hinton agar (MHA) media. Sterile discs were prepared and placed on the culture spread agar media. The discs were impregnated with the methanolic extract of mango seed kernel in the range of different concentrations. Methanol was included as a negative control and the commercial antibiotic chloramphenicol was included as a positive control to determine the sensitivity of the bacterial strains. The inoculated plates were incubated at 37 ℃ for 24 h. The antibacterial activity was evaluated by measuring diameter of the inhibition zone around the disc.

### 2.6. Statistical analysis

Experimental data were analysed using one -way analysis of variance (ANOVA) using SPSS version 11 and the significant difference among the treatment means were compared using Duncan's multiple range test (DMRT).

## 3. Results

The phytochemical analysis of the crude extract of mango seed kernel showed the presence of glycosides, saponins, flavanoids, tannins and alkaloids (Table 1).

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of biomolecules present in the plant extracts[5]. Both single and mixed mango seed kernel methanolic extracts were used in increasing concentration to assess the free radical scavenging activity. BHT was used as standard. The results of DPPH free radical scavenging activity of both the crude extracts of mango seed kernel and BHT showed significant free radical scavenging effect on the

DPPH radical and the effects increased with the increasing concentration. At 1 mg/mL and 5 mg/mL concentrations the scavenging effect on the DPPH radical, the crude methanolic extract of mixed mango seed kernel showed higher activity compared to the crude methanolic extract of single mango seed kernel and BHT. However, the free radical scavenging activity of BHT exhibited higher activity at 7 mg/mL and 10 mg/mL, compared to the mango seed kernel extracts (Figure 1). Comparing the two types of extracts, though the differences are insignificant, there was a slight increase in the free radical scavenging activity of the crude methanolic extract of mixed mango seed kernel. This may be attributed to the minor variations in the amount of secondary metabolites present in different varieties of mango cultivars.

The results of antibacterial activity studies showed that crude methanolic extract of single mango seed kernel exhibited higher antibacterial activity against methicillin resistant *S. aureus* followed by *E. coli* and *V. vulnificus*. Similarly when comparing the antibacterial activity of the crude methanolic extract of mixed mango seed kernels, methicillin resistant *S. aureus* is highly sensitive followed by *E. coli* and *V. vulnificus*.

The antibacterial activity of crude methanolic extracts of mango seed kernel is significantly higher compared to the commercial antibiotic chloramphenicol. However, chloramphenicol exhibited a higher bactericidal activity against *V. vulnificus* compared to the mango seed kernel extract (Table 2)

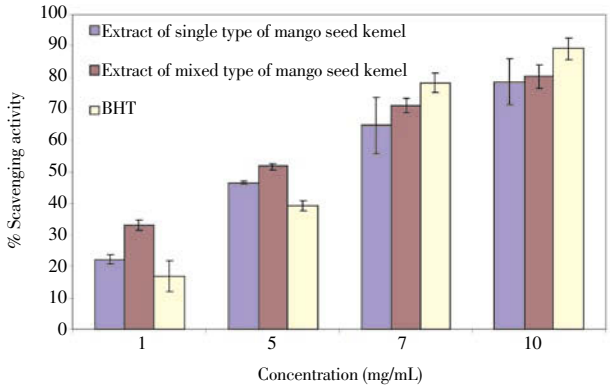


Figure 1. DPPH free radical scavenging activity of methanolic extract of mango seed kernel. Values are expressed as mean±SD.

**Table 1**
The phytochemical substances in the crude methonolic mango seed kernel extract.

| Phytochemicals | Observation | Result |
|---|---|---|
| Benedict's test(to determine reducing sugars) | Extract change into dark brick red colour | Presence of glycosides |
| Frothing test for saponins | Formation of frothing | Presence of saponins |
| Borntrager's test(anthraquinone derivatives) | Extract became greenish | Absence of anthraquinone derivatives |
| Flavonoid test | Development of yellow colour | Flavonoids present |
| Ferric Chloride test for tannins | Extract change into dark blue | Presence of tannins |
| Test for alkaloids | formation of white precipitate in extract | Presence of alkaloids |

**Table 2**
Antibacterial activity of crude methanolic extract of mango seed kernel by disc diffusion assay.

| Samples | Zone of inhibition (mm) | | |
|---|---|---|---|
| | MRSA | *E. coli* | *V. vulnificus* |
| Single mango species methanolic extract (100 mg/mL) | 21.25 ± 1.28$^{*\triangle}$ | 17.70 ± 0.75$^{*\triangle}$ | 007.80± 0. 29$^{*}$ |
| Mixed mango species methanolic extract (100 mg/mL) | 21.40 ± 0.78$^{*\triangle}$ | 18.60 ± 0.43$^{*\triangle}$ | 13.10 ± 2.11$^{*}$ |
| Chloramphenicol disc | 9.18±0.60 | 15.00 ±0.50 | 20.30± 0.29 |
| Methanol | – | – | – |

–=no zone of inhibition, Values expressed as mean±SD, *$P$<0.05 presence of significant differenre when comparing with Chloramphenicol, $^{\triangle}P$<0.05 presence of significant differenre when comparing with *V. vulnificus*.

## 4. Discussion

Mango plant has been the focus of intense research in searching of a variety of biomolecules from different parts such as stem bark, leaves and pulp. However, not many literatures are available on the medicinal properties of mango seed kernel. Mango seed kernel is one of the by-products of food processing industry and is not commercially exploited, but are discarded as waste. Mango seed represents from 20% to 60% of the whole fruit weight, depending on the mango variety and the kernel

inside represents from 45% to 75% of the whole seed[6]. Thus we evaluated the phytochemical content, free radical scavenging activity and antibacterial potential of the mango seed kernel.

In general fruits and vegetables contain many antioxidant compounds, including phenolic compounds and carotenoids, anthocyanins and tocopherols[7]. Mango seed kernels contain various phenolic compounds and can be a source of natural antioxidants. Mango seed kernel meal was found to contain tannic acid, gallic acid and epicatechin[1]. Individual phenolic compounds that exhibit antioxidant activity in fruits cannot be identified and measured by HPLC methods [8]. Thus a qualitative analysis was performed to determine the phytochemical constituents in the methanolic extract of mango seed kernel. The phytochemical studies indicated the presence of an array of phytochemicals including saponins, flavanoid, glycosides, tannins and alkaloids. The present study has demonstrated a correlative relationship between the phytochemicals in the methanolic seed kernel extract and its free radical scavenging activity.

Natural antioxidants are preferred in cosmetic and food processing industry. The limitation of using a plant extract as a natural antioxidant is the availability and cost[6]. However, the availability and cost factor appear to be negligible when it is possible to generate a plant based natural antioxidants from a waste material such as mango seed kernel.

The present study also demonstrated a comparable antibacterial potential of the methanolic extract of mango seed kernels. Dietary doses of mango seed kernel stimulated the immunity and rendered *L. rohita* more resistant to *Aeromonas hydrophila*[9]. The possible mechanism of disease control may be through microbicidal as well as activation of immune system. Further studies in this area has already been initiated using mixed mango seed kernels with different solvents, to elucidate the horizon of antibacterial activity and the structural information of the biomolecules involved.

## Conflict of interest statement

We declare that we have no conflict of interest.

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