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An investigative study of dwarf mango (Boribo Muyini) seed kernel extract against inflammatory agent *Staphylococcus aureus*

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Abstract

Alternative herbal medicine has been used to treat various infections from centuries. Natural plants contain Phyto constituents having similar chemical properties as of synthetic antibiotics. Boribo Muyini a typical Tanzanian dwarf breed of *Mangifera indica* have been reported for various medicinal effects like antioxidant, antimicrobial, antihelminthic, antidiabetic and antiallergic etc. *Staphylococcus aureus* belongs to eubacteria group, facultative anaerobic invasive bacterium is a highly evaluated in inducing inflammation in human and highly resistant to antibiotics. Due to its antibiotics resistance, it became vital to evaluate biological properties of medicinal plants in tropical regions. This study was aimed to evaluate the antimicrobial activity of seed kernel on *Staphylococcus aureus* isolated from wound infected patient and diarrhea patient. Cold methanolic extraction was performed to extract bioactive compound from seed kernel. Disk diffusion method was used to assess the antibacterial nature of the extract which where compared with the standard antibiotic vancomycin. Discs prepared in 400µg concentration of methanolic extract of seed kennel was effective against *Staphylococcus aureus* by giving an inhibition zone of 19 mm when compared to standard vancomycin antibiotic disc (30 µg) which gave an inhibition zone of 20mm.

Keywords: Boribo Muyini, Mangifera indica, *Staphylococcus aureus*, Vancomycin, Antimicrobial activity.

INTRODUCTION

Natural plants as herbal remedies are being employed to prevent and cure several illnesses vary in different communities [1]. These herbal plants are largely raw source for the production of modern antibiotics. For many years, medicine had been explored exclusively from leaves, flowers and barks of plants; only recently the synthetic drugs used to treat different infections have same chemical constituents as identified in plants. According to WHO, a medicinal plant could be any plant that contains substances which can be obtained from its different parts and can be applied for beneficial purposes or can be predecessor for the production of useful drugs [2]. A preliminary study showed that the seed represents from 20% to 60% of the whole fruit weight, depending on the mango variety 45% to 75% of the whole seed [3]. Soong *et al.* indicated that mango seed kernel has potent antioxidant activity with relatively high phenolic contents [4]. They referred that mango seed kernel was also shown to be a good source of phytosterols as campesterol, bsitosterol, stigmasterol and also contain tocopherols. Abdalla *et al.* characterized the phenolic compounds in Egyptian mango seed kernels [5].

Continuous spread of infectious diseases is a major apprehension for health institutions, pharmaceutical companies and government think tanks all over the world. Failure of treatment, particularly with the current escalating trends of multi-drug resistance (MDR) to the available modern drugs or antibiotics among emerging and re-emerging bacterial pathogens leads to serious risks [6, 7]. Invasive bacterial infection affects certain populations disproportionately. It is a major public health problem primarily related to health care but no longer confined to intensive care units, acute care hospitals, or any health care institution. *Staphylococcus aureus* is one of the most important invasive infections of human. Invasion is due to its specific virulent factors like haemolysin, leucocidin, lecithinase, catalase etc. *Staphylococcus aureus* invasive bacterium has the ability to invade skin tissues and able to cause wound infections [8]. *Staphylococcus aureaus* is a very significant cause of infections in hospitals, causing superficial skin infections and systemic infections in newborn babies, surgical patients, old and

malnourished persons and patients with diabetes and other chronic diseases [9, 10]. The objective of the present study was to screen methanolic extract of seed kernel form Boribo Muyini mango for antibacterial activity using disk diffusion method against *S. aureus.*

MATERIAL AND METHODS

Bacterial isolates

Antibiotic sensitivity clinical isolates of *S. aureus* were obtained by the Department of Microbiology from Microbiology laboratory at Kisarawe and Amana district hospitals in Tanzania. The isolates were preserved in 16% (v/v) glycerol in brain heart infusion (Oxoid Ltd, UK) and were stored at - 70ºC until use. The bacterial isolates were identified by the standard morphological, cultural and biochemical characteristics including Gram staining, purify cultures, biochemical assays, API 20-E and serological confirmations.

Seed kernel extract

Ripe Boribo Muyini breed of mango were bought from Gongo la Mboto market.

Mango cover and flesh was peeled off under aseptic condition, later the seeds were washed, air dried and the kernels were removed manually from seeds. The kernels were chopped and dried at 50°C [11]. The dried material was ground in a hammer mill into a powdery form. The extracts of mango kernels powder was obtained as described by Bloor [12]. 25 gram of mango kernel powder was extracted with 1litre of methanol: water (60:40 v/v). The mixture was air dried under shade.

Proximate Analysis of mango kernel powder

Mango kernel powder was analyzed for its moisture content, ash, crude fiber, protein and fat, according to the methods described in AOAC [13]. Nitrogen content was estimated by micro Kjeldahl method and converted to protein by using the factor 6.25. For water (WHC) and oil (OHC) holding capacity determinations, Twenty-five milliliters of distilled water or commercial corn oil were added to 0.5 gm of mango kernel powder, shacked vigorously for 1 min and then centrifuged for 15 min at 10,000g. The residue was water or oil per g of dry sample, respectively [14].

Preparation of antibiotic disc

Discs were made from what man filter paper No 1 using a stationery paper punch, then sterilized by ethanol and hot air oven at 100 degrees Celsius at 1 hr. Care was taken to avoid changing of the original disc color. After cooling the different concentrations of methanolic extract solutions of seed kernel (75µg/ml, 150µg/ml, 200µg/ml, 300µg/ml and 400µg/ml) were flooded onto the petriplates containing the sterilized discs for absorption and left to dry under shade at room temperature. The discs were preserved in a sterile screw capped bottle containing silica gel and renamed as Antibiotic disc A, B, C, D and E respectively (Table 2).

Kirby-Bauer disc diffusion method

Methanolic extract was screened for its antibacterial activity against antibiotic sensitive isolates of *S.Aureus* by antibiotic disc diffusion assay. The identified antibiotic sensitivity isolate was sub cultured in peptone broth. 100µl suspension micro-organisms was loaded and evenly spread on Muller Hinton agar plates. Prepared Antibiotic disc of the methanolic extract was placed onto the MH plates opposite standard vancomycin antibiotic disc. Then plates were incubated at 35-37ºC for overnight in upside down position. The diameter of the clear zones was measured in mm with calipers (Sylvac, Fowler, UltraCall11). Based on comparison size of the clear zones, between the prepared Antibiotic disc of the methanolic extract and the standard vancomycin antibiotic disc. Organism can be described as susceptible, intermediate, or resistant to the extract.

RESULTS AND DISCUSSION

The results of proximate analysis of Boribo Muyini mango kernels powders are presented in Table 1. The moisture, ash, protein and WHC were high when compared with the proximity analysis got by Ashoush and Gadallah, were they got moisture (6.57%), ash (1.46%), protein (7.76%) and WHC of 2.08% [15]. OHC was found to be the same of 1.74g oil/g % [4] while fat contents and crude fibers are lower than Ashoush and Gadallah findings. The lower fibers in the seed kernel indicate that the dwarf mango breed has lower water retention when compaired to the higher breeds of mangoes. This observation is agreed with those reported by Abdalla *et al* and Ajila *et al* [5, 16].

Results of antibacterial properties of methanolic extract of Boribo Muyini mango seed kernel against *S. aureus* were measured as zone of inhibition in mm. It is demonstrated that zone size was directly proportional to the increase in concentration. 400µg of the extract showed the largest zone of inhibition (19 mm) when compaired to the standard vacomycin antibiotic which had 17mm (fig. 1, fig. 2 and table 3). The control antibiotic disc showed no zone of inhibition (Fig. 1). The present study can be compared with a Maisuthisakul and Gordon study on inhibitory activity of mango seed kernel that showed zones of 3.0, 5.0, 7.0, 12.0, 14.0 mm at concentrations of 70, 140, 250, 320 and 410 µg/ml respectively [3]. It represents that extract from Boribo Muyini dwarf mango breed of Tanzania showed more activity and exhibited wide zones (mm) at nearly the same concentrations as in Maisuthisakul and Gordon study; from this study we got an inhibition zone of 19mm at 400µg/ml while Maisuthisakul and Gordon had 14mm at 410µg/ml. The likely explanation might be due to the difference in species of mango, climatic and growth conditions and methodology employed. This study also demonstrates that methanolic extract of mango seed kernel exerted highest activity against *S. aureus* when compaired to acetonic extracts of mango seed kernel employed by Maisuthisakul and Gordon.

Table 1: Proximate composition (g/100g dry sample), WHC, OHC of Boribo Muyini seed Kernel powder.

Characteristics	Seed kernel powder
Moisture (%)	6.52 ± 0.31
Ash (%)	1.42 ± 0.06
Fat (%)	8.15 ± 0.06
Protein (%)	7.80 ± 0.30
Crude fiber (%)	0.15 ± 0.07
WHC (g H_2O/g)	2.10 ± 0.12
OHC (g oil/g)	1.74 ± 0.10

Data are the mean ± SD, N =3

Table 2: Different concentrations of Methanolic Extract of Boribo Muyini mango seed kernel in double distilled water.

Sample	**A**	**B**	**C**	**D**	**E**
Concentration (µg/ml)	75	150	200	300	400
Antibiotic disc name	Disc A	Disc B	Disc C	Disc D	Disc E

Table 3: Zones of inhibition of different concentrated samples and standard antibiotic against on *Staphylococcus aureus*.

Disc /standard antibiotic	**Disc A/ Vancomycin disc**	**Disc B/ Vancomycin disc**	**Disc C/ Vancomycin disc**	**Disc D/ Vancomycin disc**	**Disc E/ Vancomycin disc**
Zone of inhibition	2/17	4/17	6/17	12/17	19/17

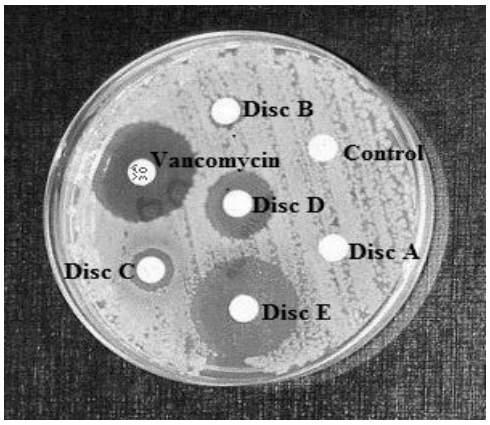

Figure 1: Zones of inhibition

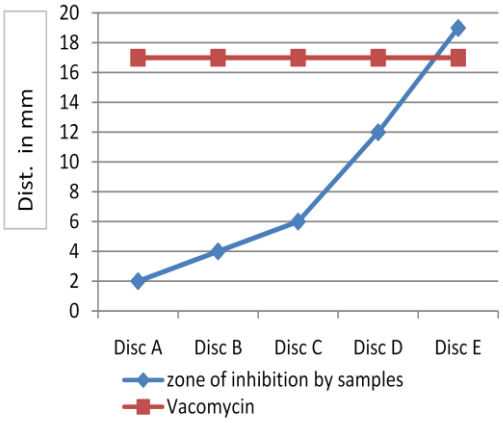

Figure 2: Graph of zone of inhibitions

CONCLUSION

Demonstration of antimicrobial activity of methanolic extract of Boribo Muyini mango seed kernel against inflammatory agent *S. aureus* is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents. The results achieved by present study indicate that leaves of mango tree in tropical countries have the potential to be used as antibacterial agent. The research must go on beyond what is done so far, so that we may utilize our natural sources to treat any ailment associated with *S. aureus.*

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