



Antibacterial activity of the stem bark of *Boswellia dalzielii*

Moses A. Olukemi^{1*}, Yvonne T. Kandakai-Olukemi² and John D. Mawak³

¹Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences; ²Department of Medical Microbiology, Faculty of Medical Sciences, ³Department of Microbiology, Faculty of Natural Sciences, University of Jos, Jos, Nigeria.

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Abstract

Crude extracts of the stem bark of *Boswellia dalzielii* were tested for antibacterial activity. Values for the minimum inhibitory concentration (MIC) for gram-positive bacteria indicated that *Staphylococcus aureus* was the most susceptible, followed by *Staphylococcus aureus* NCTC 6570, *Staphylococcus epidemidis*, *Bacillus cereus* and the least was *Bacillus megaterium*. Similarly, MIC values for gram-negative bacteria showed that inhibitory activity was most pronounced for *Klebsiella pneumoniae* followed by *Escherichia coli*, *Escherichia coli* NCTC 10418, *Proteus mirabilis* and *Pseudomonas aeruginosa*. From the minimum bactericidal concentration (MBC) values, it was found that the bactericidal activity of both gram-positive and gram-negative bacteria followed the exact pattern of the MIC. In general, gram-negative bacteria were more susceptible than gram-negative ones. Data from viable count determination showed that microbial growth was inhibited, in both *Staphylococcus aureus* and *Klebsiella pneumoniae* when various concentrations of the crude extract were added.

Keywords: *Boswellia dalzielii*; Antibacterial activity; Burseraceae

Introduction

Boswellia dalzielii (family Burseraceae), commonly known as frankincense tree; abounds in the Savannah regions of West Africa. The plant has several medicinal uses. The decoction of the stem bark is used to treat rheumatism, septic sores, venereal diseases and gastrointestinal ailments (Burkill 1985; Evans, 1989), Phytochemical studies of the plant revealed the absence of alkaloids (Baoua *et al.*; 1976), while saponins, tannins, flavonoids, cardiac glycosides, steroids, and terpenes were found to be present (Alemika and Oluwole, 1991; Adelakun *et al.*, 2001). The methanolic and aqueous extracts showed

antibacterial and antifungal activities. (Ntiejumokwu and Alemika, 1991; Adelakun *et al.*, 2001). Recent studies of the aqueous extract of the stem bark of *Boswellia dalzielii* showed no antimicrobial activity against all the microbes, used, however, produced some anti-ulcer activity (Nwinyi *et al.*, 2004). In another recent study, incensole was found to be part of the chemical composition of the stem-bark of *Boswellia dalzielii*. Incensole was found to be only moderately active against the microbes used for this study. (Alemika *et al.*, 2004).

This research was done because all the previous antimicrobial investigations on the

* Corresponding author. Tel: 08036276497

stem bark of *Boswellia dalzielii* focused on the water, methanolic and hexane extracts. This was the first time that ethanolic extract was being used.

Experimental

Organisms used. The bacteria that were used for this study were collected from the Jos University Teaching Hospital (JUTH). These organisms are as follows: *Escherichia coli* NCTC 10418, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus* NCTC 6570, *Staphylococcus aureus*; *Staphylococcus epidermidis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Bacillus megaterium*. The pure cultures of all these bacteria were prepared in nutrient broth in test tubes and were kept in the refrigerator at 4°C until they were ready for use.

Plant material. The herb *Boswellia dalzielii* was obtained from Dr. (Mrs.) O. Azija, the herbal healer attached to the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos. This herb was identified as *Boswellia dalzielii* by Mr. Okonkwo, a plant taxonomist attached to the Federal College of Forestry, Jos. The fresh stem bark of *B. dalzielii* was air dried under shade for seven days; thereafter, it was made into powder form using mortar and pestle. 50 grams of this powder was exhaustively extracted in a Soxhlet extractor using 150mls of 70% ethanol. This resulting was evaporated to dryness at 60°C. This extract was stored in the refrigerator at 4°C until it was ready for use.

Determination of antibacterial activity. The standard drug used was gentamicin. The stock Concentration of gentamicin was 1000µ/ml. The stock concentration of the crude extract of *Boswellia dalzielii* was 1000mg/ml.

Determination of the minimum inhibitory concentration (MIC) (Scott, 1989). Nine test tubes containing 5mls double strength nutrient broth were selected and labeled 1, 2, 3, 4, 5,

6, 7, 8, and 9. Tube 9 was the control. Using a sterile pipette, 5mls of either stock concentration of drug or crude extract of *B. dalzielii* was added to tube 1. The mixture in tube 1 was shaken properly. 5mls from tube 1 was added into tube 2 which was again shaken properly before 5mls of its contents was added to tube 3. This procedure was carried out until tube 8 was reached. After mixing the discarded aseptically, tube 9, being the control, had no drug or crude extract. 2.5×10^5 cfu/ml of the overnight culture of each of the bacteria used in nutrient broth were placed in each of tubes 1 – 9. The rack was incubated in an incubator for 24 hours at 37°C. After the incubation period, the presence or absence of growth was examined. The minimum inhibitory concentration (MIC) is the smallest concentration of that drug or crude extract that is capable of inhibiting the growth of specific inoculum size of a test organism. This experiment was carried out for all the organisms used in this study.

Determination of the minimum bactericidal concentration (MBC) (Hugo and Russel, 1994). The tubes in each set, which did not show any growth during the MIC determination were used. A loopfull of the content from each of the required MIC tubes was streaked unto drug/crude extract free nutrient agar. The nutrient agar was incubated for another 24 hours at 37°C. After the incubation period, the nutrient agar was examined for growth or lack of growth. The minimum bactericidal concentration (MBC) of a drug/crude extract is the smallest concentration of the drug/crude extract that is capable of killing all the organism present in the specific inoculum size of test organism. Effects of various concentrations of the crude extract of various concentrations of the crude extract of *Boswellia dalzielii* on the label count of *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Four sterile conical flasks were placed on the surface of the bench. The flasks were labeled;

1, 2, 3, and 4. Into each of these flasks was added 50mls sterile nutrient broth. Flask 1, contained the organism only (*S. aureus* v. *K. pneumoniae*) without any crude extract serving as the control. Into flask 2 was placed $\frac{1}{2}$ MIC of the crude extract. Into flask 3 was placed the MIC of the crude extract. Finally, into flask 4, was placed twice the MIC of the crude extract. At time zero, each of the four flasks was inoculated with 2.5×10^5 cfu/ml overnight culture of either *Staphylococcus aureus* or *Klebsiella pneumoniae* in nutrient broth. The flasks were placed on a shaker and shaken at a temperature of 37°C. Every 10 minutes interval, for a total time of one hour, samples were taken and viable count done. 1ml from each of the four flasks was removed and appropriate dilution was done using 9mls. Sterile distilled water in test tubes. After shaking properly, 1ml of the mixture was taken out using a sterile pipette and placed into a sterile Petri-dish. About 20mls of sterile molten nutrient agar was placed into the Petri-dishes. The Petri-dishes were shaken properly, before they were incubated in an incubator for 24 hours at 37°C. After the incubation period, the colonies on each plate were counted in cfu/ml. The graph of log of viable count versus time was plotted for each of these two bacteria. This experiment was

carried for *Staphylococcus aureus* and *Klebsiella pneumoniae* because they were the most active gram-positive and gram-negative bacteria, respectively.

Results and Discussion

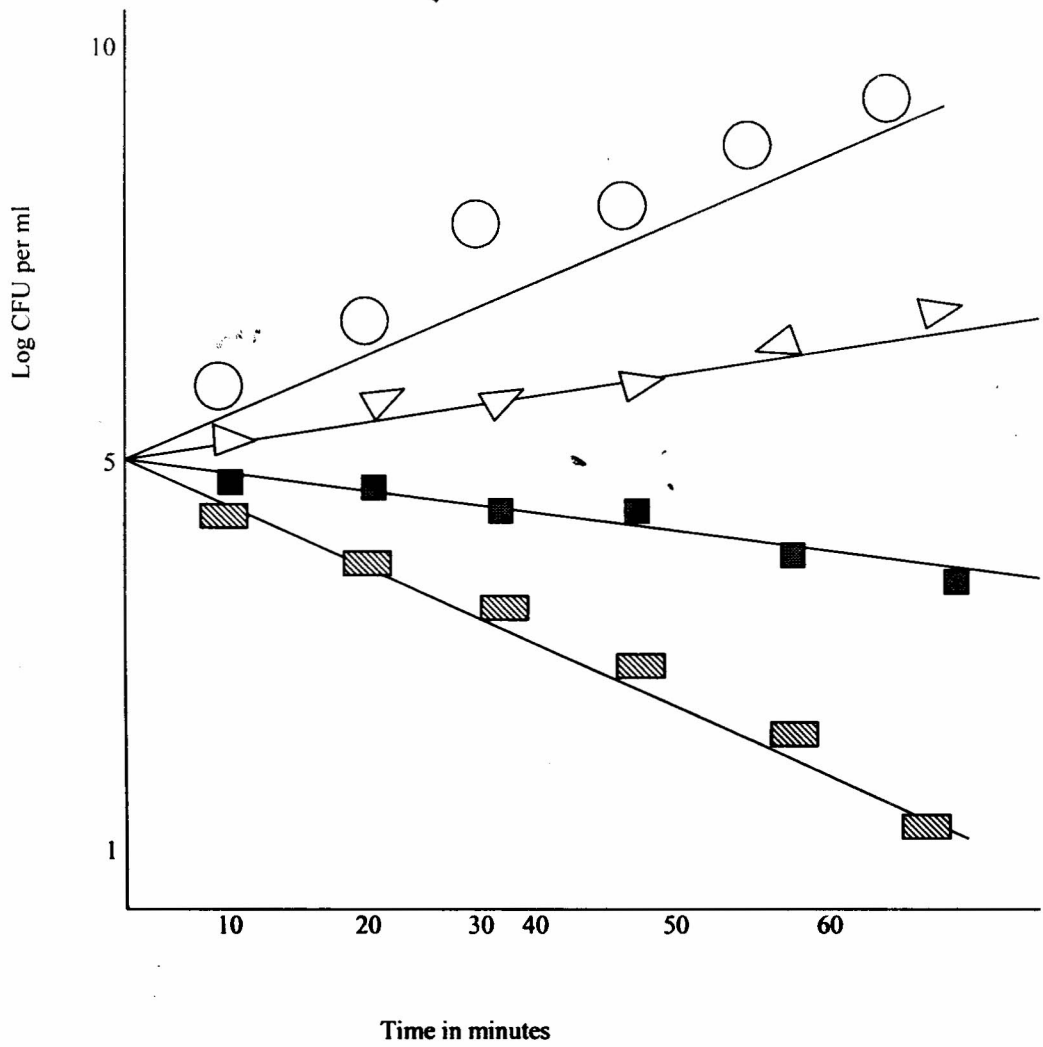
The stem bark of *Boswellia dalzielii* had activity against gram-positive and gram-negative bacteria (Olukemi and Kandakai-Olukemi, 1999). MIC values for gram-negative bacteria (Table 1) showed that it was most active against *Klebsiella pneumoniae*, followed by *Escherichia coli*, *Escherichia coli* NCTC 10418, *Proteus mirabilis*, while *Pseudomonas aeruginosa* was the least. The MBC pattern was the same as the MIC. MIC values for gram-positive bacteria (Table 2) showed that *Staphylococcus aureus* was the most active, followed by *Staphylococcus aureus* NCTC 6570, *Staphylococcus epidermidis*, *Bacillus cereus* while *Bacillus megaterium* was the last. The effects of various concentrations of the crude extract of *Boswellia dalzielii* on the viable count of *Kebsiella pneumoniae* and *Staphylococcus aureus* are shown in Figs. 1 and 2. As the concentration of the crude extract was increased from $\frac{1}{2}$ MIC to MIC and 2MIC, there was a corresponding reduction in the number of viable organisms.

Table 1: Susceptibility of some Gram-negative bacteria to the ethanolic extract of the stem bark of *Boswellia dalzielii* using nutrient medium.

Organism	Extract		Gentamicin	
	MIC (mg/ml)	MBC (mg/ml)	MIC (μ g/ml)	MBC (μ g/ml)
<i>Pseudomonas aeruginosa</i>	22	44	41	82
<i>Proteus mirabilis</i>	20	40	40	80
<i>Escherichia coli</i> NCTC 10418	18	36	38	76
<i>Escherichia coli</i>	15	30	36	72
<i>Klebsiella pneumoniae</i>	12	24	34	68

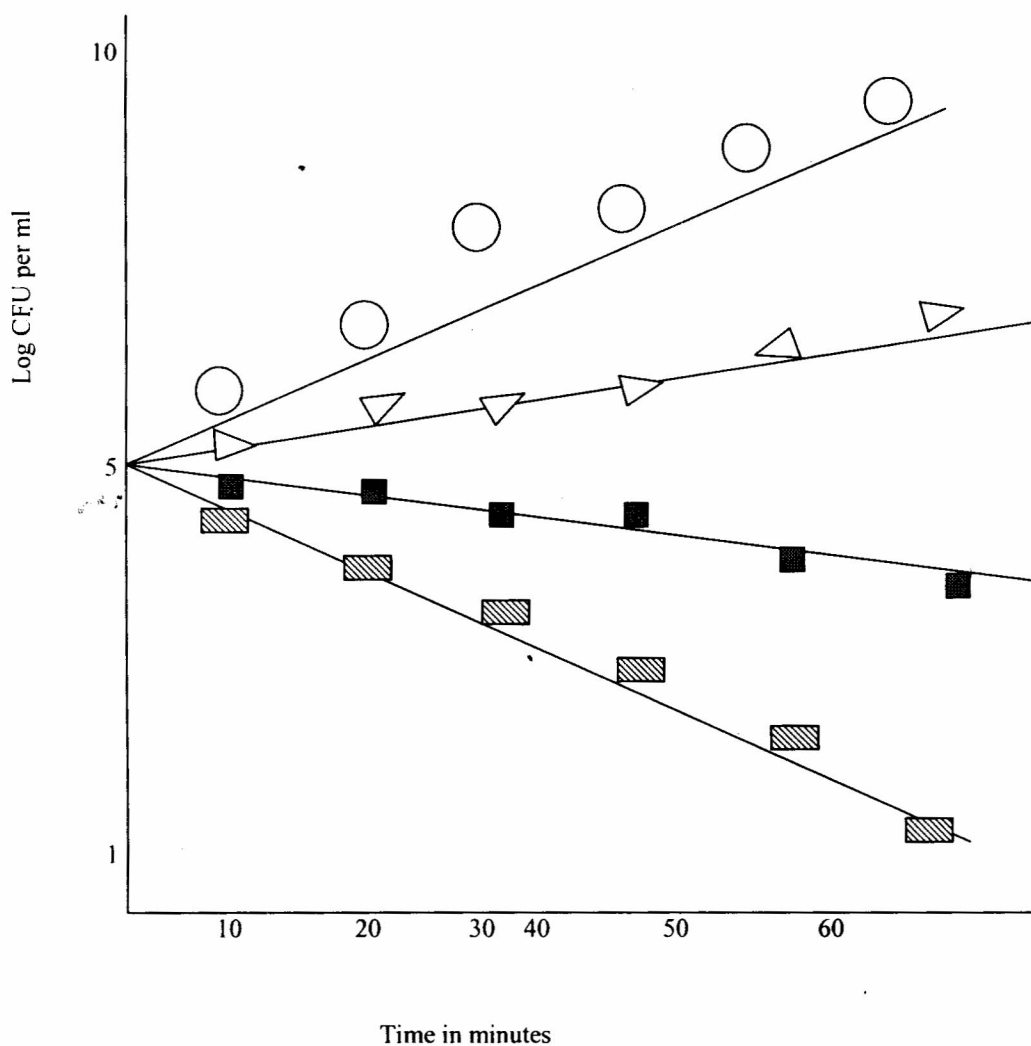
Table 2: Susceptibility of some Gram-positive bacteria to the ethanolic extract of the stem bark of *Boswellia dalzielii* using nutrient medium.

Organism	Extract		Gentamicin	
	MIC (mg/ml)	MBC (mg/ml)	MIC (μ g/ml)	MBC (μ g/ml)
<i>Bacillus megaterium</i>	10	20	30	60
<i>Bacillus cereus</i>	6	16	28	56
<i>Staphylococcus epidermidis</i>	6	12	26	52
<i>Staphylococcus aureus</i> NCTC 6570	4	8	24	48
<i>Staphylococcus aureus</i>	2	4	22	44



○—○ control;
 ◁—◁ 6mg/ml. ($1/2$ MIC); ■—■ 12mg/ml. (MIC) and ▨—▨ 24mg/ml. (2MIC).

Fig. 1: Effect of various concentrations of crude extract of *B. dalzielii* on the viable count of *Klebsiella pneumoniae*



○—○ control;
 ◁—▷ 1mg/ml. ($1/2$ MIC); ■—■ 2mg/ml. (MIC); and ▨—▨ 4mg/ml. (2MIC).

Fig. 2: Effect of various concentrations of crude extract of *B. dalzielii* on the viable count of *Staphylococcus aureus*

Discussion

MIC and MBC tests showed that the ethanolic extract of the stem bark of *Boswellia dalzielii* (Tables 1 and 2). The results of this study showed that the crude extract of the stem bark of *Boswellia dalzielii* has activity against some gram-positive and gram-negative bacteria (broad spectrum of activity) (Ntiejumokwu and Alemika, 1991). For gram-negative bacteria (Table 1), *Klebsiella*

pneumoniae was the most sensitive while *Pseudomonas aeruginosa* was the least. For gram-positive (Table 2), *Staphylococcus aureus* was the most sensitive while *Bacillus megaterium* was the least.

In general, this herb was more active with gram-positive bacteria than gram-negative ones (Tables 1 and 2). This is due to the complex nature of gram-negative cell wall which makes entry of drugs and other

chemotherapeutic agents extremely difficult (Olukemi et al. 1997). Other results obtained from this study showed that both *Pseudomonas aeruginosa* and *Bacillus megaterium* possess some resistance properties. This observation is not surprising since it is widely known that *Pseudomonas aeruginosa* has been showing resistance to drugs. It must be noted that *Bacillus megaterium* is a spore former. (Montefiore et al., 1989). The effects of different concentrations of the crude extract of *Boswellia dalzielii* on *Klebsiella pneumoniae* and *Staphylococcus aureus* are shown in figs. 1 and 2. In both cases, when compared with the control, there was a corresponding decrease in the number of viable bacteria as the concentration increased (Olukemi et al., 2002). There was a correlation between antibacterial activity and the use of the stem bark of *Boswellia dalzielii* by herbal practitioners in Jos. They use it to treat gastroenteritis (Nwinyi et al., 2004).

Staphylococcus aureus is widely known to cause food poisoning while *Klebsiella pneumoniae*, can occasionally cause diarrhoea in infants, and children. (Thomas, 1988). In addition, the stem bark was also found to contain anti-ulcer activity (Nwinyi et al. 2004, Alemika and Oluwole, 1991).

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