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**Original Research Article**

# Phytochemical Screening and *In-Vitro* Antibacterial Potential of *Boswellia dalzielii* (Hutch.) Stem Bark Extracts

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**Abstract:** As part of ongoing research to purify, isolate and characterized antibacterial compounds from the extracts of some Nigerian medicinal plants, stem bark extracts of *Boswellia dalzielii* (Hutch.) were screened for their preliminary phytochemical and antibacterial activity. The preliminary phytochemical screening of the extracts was carried out using standard methods while the antibacterial activity was done using Agar well diffusion method. The results for the phytochemical screening showed the presence of most of the phytochemicals tested. The results for the antibacterial activity showed varying degree of antibacterial activity against the bacterial isolates. However, crude methanolic extract showed relatively high zone of inhibition (mm), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). It was found to inhibit the growth of most of the test bacterial isolates comprising of both Gram-positive and Gram-negative organisms. On the other hand, ethyl acetate and aqueous extracts showed moderate activity against tested isolate, while N-hexane extract showed little or no activity against most of the test isolates. These findings support previous reports on the antimicrobial activity of this plant. The result of the present study signifies the potential of *Boswellia dalzielii* stem bark as a source of therapeutic agents, which may provide leads in the ongoing search for antimicrobial agents from plants.

**Keywords:** Stem bark, *Boswellia dalzielii*, antibacterial activity, phytochemicals and zone of inhibition.

## INTRODUCTION

Pathogenic microorganisms continue to have adverse effects on the quality and safety of life. Synthetic drugs are widely used to treat infections caused by these microorganisms. Unfortunately, they develop resistance to many antimicrobial agents (Anyim *et al.*, 2010). The reason for this high resistance to commonly used antimicrobial agents may not be unconnected with the worldwide and indiscriminate use of these drugs (Mukerjee *et al.*, 2002). In addition, these antimicrobials sometimes cause allergic reaction and immunity suppression. Presently, the herbal drugs play chief role for substitution of synthetic drugs due to fewer side effects and immunity resistance (Bandow *et al.*, 2003; Ohadoma *et al.*, 2014). The use of essential oils and plant extracts are less damaging the human health and environment (Isman, 2000; Misra and Pavolvstaths, 1997). Plants provided an arsenal of chemicals to survive attack by microbial invasion (Martini *et al.*, 2004. Nigeria has rich flora that are used in various fields (medicine, pharmacy, perfumery, cosmetics and food) for their therapeutic and organoleptic properties, though some of them have not been thoroughly studied (Mideko *et al.*, 2017).

*Boswellia dalzielii* Hutch. (Burseraceae) is an aromatic plant commonly known as the frankincense tree that grows up to 13m high and is found mainly in the Sudano-Sahelia Savannah region of West Africa, on rocky, dry and shallow soils (Hassan *et al.*, 2009; Mideko *et al.*, 2017). The tree has a characteristic pale papery bark that is peeling and ragged. The Hausa names include “Ararrabi”, “Basamu” and “Hanu” (Hassan *et al.*, 2009).

Traditionally, a decoction of the bark is drunk as a protection against dysentery, haemorrhage, and angina. The dried and crushed bark is used in combination with other herbs to treat malaria, yellow fever, stomach ailments, and many childhood diseases (Kubmarawa *et al.*, 2013). The bark is also used to treat rheumatism, gastrointestinal disorders, wounds, asthma, pleurisy, appendicitis, dizziness, palpitations, leprosy, diarrhoea and bloating in cattle (Kubmarawa *et al.*,

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2013; Henley-Smith *et al.,* 2013). It has antiseptic, healing, and antifungal potential and is used externally to treat sores, ulcers and dental caries (Ngamo *et al.,* 2007; Zerbo *et al.,* 2013).

As part of an ongoing research to purify, isolate and characterized antibacterial compounds from the extracts of some Nigerian medicinal plants, the stem bark extracts of *Boswellia dalzielii* (Hutch.) was screened for their antibacterial activity.

## MATERIALS AND METHODS

### Materials

### Solvents for extraction

The solvents used were: Butanol (BDH), Distilled water, Ethyl Acetate (BDH), Methanol (BDH) and N-Hexane (BDH).

### MATERIALS FOR ANTIMICROBIAL TEST

- Microbiological media (nutrient broth): Muller Hinton agar.
- Test Organisms: *Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Psedomonas aeruginosa and Proteus spp.*
- Petri Dishes, Sterile Pipette, 6 mm cork borer, Incubator, Autoclave, Dimethylsulphoxide (DMSO) 10%.

## METHODS

### Plant Sample Collection and Identification

Fresh stem bark sample of the plant was separately collected from Aliero Local Government Area of Kebbi State, Nigeria and was identified and authenticated by a Botanist at the Biological Sciences Department, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. The plant was identified as *Boswellia dalzielii* (Hutch.). The sample was shed-dried, ground and kept in air-tight containers till further use.

### Preparation of Plant Extracts

The crude solvents' extracts were prepared by soaking a sample (50g) of stem bark powdered material in 300 ml each of methanol, butanol, water, ethyl acetate and n-hexane for 72 h. The extracts were filtered using clean cloth and Whatman No. 1 filter paper. The filtrate was concentrated in vacuum at 30$^{o}$C and stored in sterile sample containers at 4$^{o}$C until further use.

### Phytochemical Screening

The extracts were screened for the presence of major phytochemicals using standard qualitative methods as described previously (NCCLS, 1990; Njoku *et al.,* 2010; Trease and Evans, 1989). The plant extracts were screened for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids, glycosides and phenols.

### Antibacterial Screening

### Preparation of inoculums of test organisms

0.5 McFarland turbidity standard was used to standardise the organisms. The scale was prepared by adding 0.05 mL of 1 % barium chloride ($BaCl_2$) to 9.95 mL of 1% $H_2SO_4$. Suspensions of the organisms were made in normal saline and compared with 0.5 McFarland turbidity standard by holding the suspension and McFarland turbidity standard in front of a light against a white background with contrasting black lines. The bacterial suspension was diluted with normal saline when the density is higher and additional bacteria were added to the suspension when the density is lower. This continues until the density of the bacterial suspension matched with that of 0.5 McFarland turbidity standard which corresponds to 1.5 x $10^8$ CFU/mL (Vollekova *et al.,* 2001).

### Sensitivity Test of the Crude Extracts

Agar well diffusion method was employed to assay for the antibacterial activity (Mann *et al.,* 2008). The antibacterial activity of crude stem bark extracts of *Boswellia dalzielii* were determined using stock concentration of 100 mg/mL. The standardised inocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (6 mm in diameter), three appropriately labelled wells were bored into each agar plate. A 0.2 mL of the appropriate extract concentrate was placed in each well and then allowed to diffuse into the agar. The plates were later incubated at 37°C for 24 h after which zone of inhibition (diameter) formed was determined as an indication of antibacterial activity. These effects were compared with that of the standard antibiotic amoxicillin at a concentration of 1 mg/ml.

### Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of the extract was carried out on the microorganisms that were sensitive to the extract and was done using broth dilution method (Banso, 2009). Different concentrations of the extract that exhibited antimicrobial activity against the test organisms were prepared in the test tube containing Mueller Hinton Broth (MHB). The organisms were inoculated into each tube containing the diluted extracts. The plates were incubated at 37$^{o}$C for 24 hours. The lowest concentrations of the extract which shows no turbidity was recorded as the minimum inhibitory concentrations.

### Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentrations of the extracts were carried out to check whether the test microbes were killed or only their growth was inhibited. Mueller Hilton agars were prepared according to the manufacturer's instruction, boiled to dissolve and were sterilized at 121$^{o}$C for 15 minutes, the media were cooled to 45$^{o}$C and the medium (20 ml) was poured in to sterile Petri dishes, the plates were covered and

allowed to cool and solidify. The contents of the MIC in the serial dilution was inoculated on to the media, the plates were incubated at 37$^{o}$C for 24 hrs, after which the plate were observed for colonies growth. The MBC was the plate with lowest concentrations of the extract without colony growth (Okoro *et al.*, 2014).

## RESULT AND DISCUSSION

**Table 1: Phytochemical constituents present in crude stem bark extracts of *Boswellia dalzielii.***

| Phytochemical Constituent | Methanol | Ethyl acetate | Aqueous | N-hexane |
|---|---|---|---|---|
| **Flavonoids** | + | + | + | + |
| **Tannins** | + | + | N.D | + |
| **Saponins** | + | + | + | + |
| **Glycosides** | + | + | + | N.D |
| **Alkaloids** | + | + | N.D | + |
| **Steroids** | N.D | N.D | + | N.D |
| **Terpenoids** | + | N.D | + | + |
| **Phenols** | + | + | + | N.D |

Keys: + = present N.D = not detected

**Table 2: Weight (g) recovered and yield (%) of crude stem bark extracts of *Boswellia dalzielii*.**

| Extract | Weight (g) | Percentage yield (%) |
|---|---|---|
| **Methanol** | 9.28 | 18.56 |
| **Ethyl acetate** | 7.29 | 14.58 |
| **Aqueous** | 4.00 | 8.00 |
| **N-hexane** | 10.20 | 20.40 |

**Table 3: Antibacterial activity of crude stem bark extracts of *Boswellia dalzielii.***

| Bacterial isolates | Zone of Inhibition (mm)* | | | | |
|---|---|---|---|---|---|
| | Methanol | Ethyl acetate | Aqueous | N-hexane | Amox. |
| ***E. coli*** | 20.67±2.31 | 14.67±2.31 | 10.33±1.53 | 4.33±0.58 | 28.67±1.15 |
| ***K. pneumoniae*** | 23.33±2.08 | 13.33±3.21 | 12.67±2.31 | 0 ±0.00 | 26.67±0.58 |
| ***Proteus spp.*** | 19.00±2.00 | 8.00±1.00 | 13.67±2.08 | 6.00 ±1.00 | 30.67±1.15 |
| ***P. aeruginosa*** | 18.67±0.58 | 14.00±2.65 | 14.33±1.52 | 0 ±0.00 | 25.37±1.53 |
| ***S. aureus*** | 18.33±2.08 | 15.67±3.06 | 11.33±2.08 | 7.00±2.00 | 23.00±1.00 |
| ***S. typhi*** | 21.33±2.08 | 11.00±3.61 | 7.33±2.08 | 0 ±0.00 | 28.00±2.00 |
| ***S. pneumonia*** | 17.67±1.53 | 12.33±0.58 | 9.00±1.73 | 0 ±0.00 | 21.67±1.53 |

*values are mean and standard deviation of three (3) replicates, 0 ±0.00 = No activity
Amox. = Amoxicillin as positive control

### Minimum Inhibitory Concentration (MIC)

**Table 4: The minimum inhibitory concentrations (mg/ml) of the crude stem bark extracts**

| Bacterial isolates | Methanol | Ethyl acetate | Aqueous | Amoxicillin |
|---|---|---|---|---|
| ***E. coli*** | 1.56 | 3.12 | 3.12 | 0.13 |
| ***K. pneumoniae*** | 6.25 | 12.5 | 12.5 | 0.50 |
| ***Proteus spp.*** | 3.12 | 3.12 | 6.25 | 0.13 |
| ***P. aeruginosa*** | 3.12 | 6.25 | 3.12 | 0.25 |
| ***S. aureus*** | 1.56 | 1.56 | 3.12 | 0.13 |
| ***S. typhi*** | 6.25 | 6.25 | 6.25 | 0.50 |
| ***S. pneumonia*** | 3.12 | 3.12 | 6.25 | 0.50 |

### Minimum Bactericidal Concentration (MBC)

**Table 5: The minimum bactericidal concentration (mg/ml) of the stem bark extracts**

| Bacterial isolates | Methanol | Ethyl acetate | Aqueous | Amoxicillin |
|---|---|---|---|---|
| ***E. coli*** | 100 | 100 | N.D | 1.00 |
| ***K. pneumoniae*** | N.D | N.D | N.D | 1.00 |
| ***Proteus spp.*** | 100 | 100 | 100 | 1.00 |
| ***P. aeruginosa*** | 100 | N.D | N.D | 0.50 |
| ***S. aureus*** | 50 | N.D | 100 | 0.50 |
| ***S. typhi*** | 100 | N.D | N.D | 1.00 |
| ***S. pneumonia*** | N.D | 100 | N.D | 0.50 |

The result of the phytochemical analysis of the crude stem bark extracts of *Boswellia dalzielii* is presented in Table 1. The result reveals the presence of flavonoids, tannins, saponins, glycosides, alkaloids and terpenoids in the crude stem bark extracts of *Boswellia dalzielii*. Steroids were only detected aqueous extract of the plant. Several other studies have reported similar phytochemicals from this plant (Hassan *et al.,* 2009; Mideko *et al.,* 2007); these support the data reported in this research. These compounds are known to be biologically active (Cowan 1999; Satyajit *et al.,* 2006) and thus may contribute to the observed antibacterial activities in these plants.

Phytochemicals exert antimicrobial activity through different mechanisms. For instance, flavonoids possess a wide range of biological activities which include antimicrobial, anti-inflammatory, analgesic, anti-allergic effects, cytostatic and antioxidant properties (Eloff, 2001). The antibacterial activity of flavonoids had been shown to be a result of their ability to form complexes with bacterial cell walls (Ververidis *et al.,* 2007). Tannins act by iron deprivation, hydrogen bonding or specific interaction with proteins such as enzymes, cell envelopes and complex formation with polysaccharides (Satyajit *et al.,* 2006; Scalbert *et al.,* 2005). Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Scalbert *et al.,* 2005); thus exhibiting antimicrobial activity. Saponins are known to produce inhibitory effects on inflammatory processes (Cowan, 1999). They were also reported to possess antibacterial property. Alkaloids are another kind of phytochemicals detected in extract of this plant. Alkaloids have been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Elmahmood *et al.,* 2008). Taken together, these facts support the utilization of this plant in various African communities in the preparation of local medications for the treatment of diseases.

The amount (weight in grams) and the percentage (%) yield of the four extracts are presented on table 2. Of all the four solvent extracts, aqueous extract has the highest percentage yield (20.40%), followed by methanol extract (18.56%), ethyl acetate extract (14.58%) and lastly N-hexane extract (8.00%). The amount of extract recovered and consequently the percentage yield depends largely on the fibre content of the plant/sample being extracted. High fibre contents gave a very low percentage yield. On the other hand, low fibre content gave a very high percentage yield (Ukwuani and Igbokwu, 2015).

The antibacterial activities of the solvent extracts against test isolates showed different degrees of activity. Out of the four stem bark extracts of *Boswellia dalzielii*, methanolic extract showed highest zone of inhibition against most of the test organisms. Ethyl acetate and aqueous extracts showed moderate activity while N-hexane extract showed little or no activity against test organisms (Table 3). This suggests methanol will be good solvent for the isolation and purification of the active principles present in the stem bark of *Boswellia dalzielii*.

The minimum inhibitory concentration (MIC) was determined for the crude methanolic, ethyl acetate and aqueous stem bark extracts of *Boswellia dalzielii*. From the MIC values exhibited by crude methanolic, ethyl acetate and aqueous extracts against each test bacterium showed that MIC values of crude methanolic extract against test bacteria were smaller than they were for ethyl acetate and aqueous extracts. This suggests that the test bacteria are more sensitive to crude methanolic extract than the ethyl acetate and aqueous extracts. An exception was observed for *S. typhi* which has the same MIC values for the three crude extracts (Table 4).

Table 5 shows the minimum bactericidal concentrations (MBC) exhibited by the crude methanolic, ethyl acetate and aqueous extracts against the susceptible test isolates. The MBC exhibited by the crude methanolic extract against the test isolates ranged between 50 mg/mL and 100 mg/mL. Also, the ethyl acetate and aqueous extracts showed an MBC ranging between 50 mg/mL and 100 mg/mL. Thus, the MBC exhibited by both extract and the two fractions followed the same pattern.

## CONCLUSION

The result of the present study signifies the potential of *Boswellia dalzielii* stem bark as a source of therapeutic agents, which may provide leads in the ongoing search for antibacterial agents from plants. Further, the activity exhibited by the extracts against tested bacteria species that are associated with various infectious diseases, may provide scientific justification for the ethnomedicinal uses of the plant.

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