*Journal of Advances in Microbiology*

*7(1): 1-8, 2017; Article no.JAMB.37690*
*ISSN: 2456-7116*

# Antibacterial Activity of *Boswellia dalzielii* Leaves Extracts against Some Pathogenic Bacterial Isolates

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both the authors designed the study, conduct the experiment and performed the statistical analysis and wrote the first draft of the manuscript. Author FSN managed the literature searches. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JAMB/2017/37690
Editor(s):
(1) Jayshree Patel, United State Based Pharmaceutical Company, USA.
(2) Pongsak Rattanachaikunsopon, Professor, Department of Biological Science, Faculty of Science, Ubon Ratchathani University, Thailand.
Reviewers:
(1) Mohini Chetan Kuchekar, Pune University, India.
(2) Akpi Uchenna Kalu, University of Nigeria, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/22139

***Original Research Article***

***Received 25th October 2017***
***Accepted 7th November 2017***
***Published 4th December 2017***

## ABSTRACT

**Aim:** The study was conducted to investigate the antibacterial activity of *Boswllia dalzielii* leaves extracts and its major phytochemical constituents.
**Materials and Methods:** The aqueous, methanol and chloroform extracts from the leaves of the plant was tested using agar well diffusion method for their antibacterial activity against some members of Enterobacteriaceae family isolated from diarrheic stool sample (*Escherichia coli, Shigella* spp*, Salmonella typhi* and *Klebsiella* spp).
**Results:** Preliminary phytochemical analyses showed that the leaves extracts contain alkaloids, tannins, terpenoid, Anthraquinone, reducing sugar, amino acid, flavonoid, saponins, glycosides and phenols. The results of antibacterial activity of the leaves extracts shows that the plant extracts were active against the microorganisms tested. The methanol extract showed the highest zones of inhibition against tested organisms compared to aqueous and chloroform extract. Statistical analysis of the result showed that an average zone of inhibition of 15.44 mm, 14.78 mm, 12.92 mm and 11.31 mm for *E. coli*, *Shigella* spp *S. typhi* and *Klebsiella spp,* respectively were found. The

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Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts ranged between 6.25 – 100 mg/ml.
**Conclusion:** The extracts of the plant leaves demonstrated antibacterial activity against microorganisms causing diarrhea stool due to presence of phytochemical constituents hence, the application of the decoction of leaves of the plants in ethno medicine is justified.

*Keywords: Enterobacteriaceae; antibacterial activity; phytochemicals, Boswellia dalzielii.*

## 1. INTRODUCTION

Currently, there has been a lot of attention focused on producing medicines and products that are natural. Several leaves and leaf extracts have been found to have antimicrobial activity against microorganisms [1]. The antimicrobial properties of plants have been investigated by a number of researchers worldwide though thorough biological evaluation of plants extracts is vital to ensure their efficacy and safety. These factors are of importance if plant extracts are to be accepted as valid medical agents for the treatment of infectious diseases especially in light of the emergence of drug-resistant microorganisms [2]. Large populations of the world, especially in developing countries depend on the traditional system of medicine to treat variety of diseases and several hundred genera of plants were utilized traditionally for medicinal purposes [3]. Plants used in traditional medicine may constitute an important source of new biologically active compounds. It is estimated that there are about 2, 500 000 species of higher plants and the majority of these plants have not been studied for their pharmacological activities [4]. The world health organization [5] reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plants extracts. Various specific plants have continued to be an important therapeutic aid for alleviating the ailments of humankind. Therefore, novel antimicrobial agents from different biological sources are continuously sought [6]. Research conducted on medicinal plants have served the dual purposes of bringing up new therapeutic agents and providing useful leads for studies directed towards the synthesis of drugs on the basis of the chemical structures of the natural products. Modern pharmaceutical industries still rely to some extent on the bioactive principle, obtained from plants [7].

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 [8]. The leaves are used in large quantity to make a wash of fever and rheumatism while it is also taken internally for gastro-intestinal troubles [9]. The methanolic and aqueous extracts showed antiberial and antifungal activities [10].

The family Enterobacteriaceae comprises a large group of Gram-negative non-spore forming bacteria typically 1-5 µm in length. Members of the Enterobacteriaceae are widely distributed. Although strains of some species are harmless commensals, such as some strains of *E. coli*, others are important human and animal pathogens, and some are pathogenic to plants and insects. Their ubiquitous distribution means that it is inevitable that some members of the Enterobacteriaceae will enter the food chain [11]. Members of the family are responsible for causing food borne disease and some also cause food spoilage and therefore contribute to substantial economical losses and food wastage [11]. In this study, the phytochemical screening and antibacterial activity of *Boswellia dalzielli* against some members of family Enterobacteriaceae recovered from stool samples were determined. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts was also determined.

## 2. MATERIALS AND METHODS

### 2.1 Isolation and Identification of Test Organisms

The samples (diarrhoeic stool samples) of patients attending Murtala Muhammad Specialist Hospital from September to November, 2015 were collected, labeled and inoculated on the surface of nutrient agar plate and incubated for 24 hours at 37°C for isolation of enteric bacteria. Each colony was isolated in a pure form by sub culturing for further studies and identification. Distinctive morphological properties of each pure

culture such as colony form, elevation of colony and colony margin were observed. Further microbial identification was based on the methods of Jolt et al. [12].

## 2.2 Collection and Identification of Plant Materials

The leaves of *Boswellia dalzielii* were collected at early hours on 23rd March, 2016 at Zango village, Ungogo Local Government Area in Kano state, Nigeria. The identification and authentication of the plant materials was done at Herbarium in the department of Plant Science, Bayero University Kano with the following voucher number BUKHAN 0381, voucher specimens were deposited there for future reference. The parts collected were washed thoroughly with distilled water and air-dried in a shade for two weeks, then cut into pieces and grinded into powder using a sterile pestle and mortar under laboratory condition. The powder was then kept in air tight container for future use.

## 2.3 Extraction of Plant Material

Aqueous (water), methanol and chloroform solvents were used for extraction of the active components of the plant part. For aqueous extraction, water extraction method as described by Asuzu [13] with little modification was used. One hundred gram (100 g) of each of the grounded leaf and stem bark were extracted by successive soaking for 4 days with intermittent shaking using 500 ml of distilled water in a sterile conical flask. The extracts were filtered using Whatman filter paper and the filtrates concentrated in water bath at 70°C. The solid concentrated filtrate (now the extracts) were then stored in universal bottles in the refrigerator at 4°C before use. For methanol and chloroform, 100 g of the powdered plant part was extracted in 500 ml of methanol and chloroform for 2 days with intermittent shaking. The mixture was filtered using Whatman filter paper and the extracts were evaporated to dryness using rotary evaporator at 60°C. The solid residues obtained were reconstituted in 10% DMSO at stock concentration, stored in the refrigerator at 4°C until used.

## 2.4 Phytochemical Screening

Phytochemical screening was done to ascertain the presence of bioactive component present in the leaves of *Boswellia dalzielii.* Presence of Alkaloid, saponin, Glycoside, Tannin, flavonoids, resin, steroid, terpenoid, Anthraquinones, Protein and amino acid were determined using procedure described by Sofowora [14].

## 2.5 Antimicrobial Assay

The agar well diffusion method was used to determine the antibacterial activity of the plant extracts. 0.1 ml of the different standardized organism (0.5 Mac Farland) was inoculated on the surface of Mueller Hinton Agar in a sterile Petri dish and allowed to set and then solidified. A sterile cork borer 6mm was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 50, 100, 150 and 200 mg/ml while the 5th well contained the solution used for the research to serve as control, Ciprofloxacin (Micro lab limited) 125 mg/ml, was used as control in this research. These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each well, were measured to the nearest millimeters [15].

## 2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the extracts was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2ml of 200 mg/ml of the extract into a test tube containing 2 ml of Nutrient broth, thus producing solution containing 100 mg/ml of the extract. The process continue serially up to test tube No. 5, hence producing the following concentrations; 100, 50, 25, 12.5, 6.25 mg/ml. Test tube No. 6 do not contain extracts and serve as Control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37°C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity. The least concentration of the extract where there was no growth in tube was taken as the MIC. From each tube that did not show visible growth in the MIC, 0.01ml was aseptically transferred into extract free Mueller Hinton agar plates. The plates were incubated at 37°C for 24 hour. The MBC was recorded as the lowest concentration (highest dilution) of extract that

had less than 99% growth on nutrient agar plates [16].

### 2.7 Statistical Analysis

The data on the average zone of inhibition produced by the isolates against the extracts used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Screening

Phytochemical constituents of aqueous, methanol and chloroform leaves extract of *Boswellia dalzielii* is presented in Table 1. The result showed that the aqueous, methanol and chloroform leaves extracts contain the following Phytochemicals alkaloid, Anthraquinone, Flavonoid, terpenoid and glycoside, steroid and reducing sugar, while saponin, resin and phenol were absent. The methanol extract has the highest number of phytochemical components.

### 3.2 Antibacterial Activity

The antibacterial activity of different concentration of *Boswellia dalzielii* aqueous, methanol and chloroform leaves extract against test isolates is presented in Table 2. The result showed that higher zone of inhibition was demonstrated by *Shigella* (23 mm) at concentration of 200 mg/ml. Zones of inhibition shown recorded by the control ranges from 19-23 mm.

**Table 1. Phytochemical constituent of aqueous, methanolic and chloroform leaves extracts of *Boswellia dalzielii***

| S/N | Phytochemicals | Aqueous extract | Methanolic extract | Chloroform extract |
|---|---|---|---|---|
| 1 | Alkaloid | + | + | + |
| 2 | Flavonoid | + | + | - |
| 3 | Glycoside | + | + | - |
| 4 | Resin | - | - | - |
| 5 | Saponin | - | - | - |
| 6 | Steroid | - | + | + |
| 7 | Tannin | + | + | + |
| 8 | Phenol | - | - | - |
| 9 | Terpenoid | - | - | + |
| 10 | Anthraquinone | + | + | - |

*Key: + = Presence of phytochemical. - = Absent of phytochemical*

**Table 2. Antibacterial activity of the extracts**

| Extracts | Conc. (mg/ml) | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| ALE | 50 | 11±0.27 | 12±0.00 | 09±0.18 | 11±0.27 |
| | 100 | 12±0.27 | 12±0.18 | 10±0.27 | 13±0.18 |
| | 150 | 15±0.18 | 16±0.18 | 13 ±0.27 | 17±0.27 |
| | 200 | 16±0.27 | 19±0.27 | 18±0.27 | 21±0.00 |
| MLE | 50 | 11±0.00 | 10±0.27 | 11±0.27 | 12±0.27 |
| | 100 | 12±0.18 | 13±0.18 | 12±0.00 | 13±0.27 |
| | 150 | 17±0.18 | 20±0.27 | 16 ±0.00 | 19±0.00 |
| | 200 | 20±0.27 | 21±0.27 | 19±0.00 | 23±0.18 |
| | 50 | 09±0.27 | 10±0.00 | 08±0.27 | 11±0.00 |
| CLE | 100 | 10±0.18 | 12±0.00 | 09±0.27 | 11±0.27 |
| | 150 | 11±0.27 | 15±0.27 | 10±0.18 | 14±0.27 |
| | 200 | 14±0.00 | 17±0.27 | 10±0.18 | 16±0.27 |
| Control | 125 | 22 | 23 | 19 | 21 |

*Key: ASE = Aqueous stem bark extract, MSE = Methanolic stem bark extract, CSE = Chloroform stem bark extract, ALE = Aqueous leaves extract, MLE = Methanolic leaves extract, CLE = Chloroform leaves extract*

### 3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration of aqueous leaves extract of *Boswellia dalzielii* is represented in Table 3 which shows dilutions of various concentrations of the extract of aqueous leaves against test isolates. Lower MIC was recorded in *E. coli* and *Shigella* (25 mg/ml) while highest MIC was recorded in *S. typhi* and *Klebsiella* (50 mg/ml).

Table 4 present the minimum bactericidal concentration (MBC) of the plant extracts. The result shows that the extract of the plant can kill the some test isolates at concentration of 25 – 100 mg/ml. However, the MBC of some isolates was unable to be found.

Phytochemical analysis of the three extracts of leaves of *B. dalzielii* revealed that the plant is very rich in secondary metabolite compounds of alkaloid, saponin, tannin, Anthraquinone, Flavonoid, phenols, terpenoid and glycoside. On the other hand steroid and reducing sugar were absent. The aqueous and methanolic extract has the highest number of phytochemicals according to this research while chloroform extract has the least number of Phytochemical extracted. This indicated most of the phytochemical constituents of have medium or high polarity as most are detected by water and methanol. The result of phytochemical analysis of this work is inconformity with several researchers who worked on antibacterial activity of the plant. The phytochemical studies of the plant revealed the absence of alkaloids [17], while saponins, tannins, flaonoids, cardiac glycosides, steroids, and terpenes were found to be present [18,19]. The methanolic and aqueous extracts showed antibacterial and antifungal activities [20].

**Table 3. Minimum inhibitory concentration (MIC) of the extracts against the test isolates**

| Extracts | Isolates/ Minimum inhibitory concentration (mg/ml) | | | |
|---|---|---|---|---|
| | ***E. coli*** | ***Klebsiella*** | ***S. typhi*** | ***Shigella*** |
| ASE | 12.5 | 12.5 | 50 | 12.5 |
| MSE | 50 | 50 | 50 | 25 |
| CSE | 100 | 50 | 100 | 50 |
| ALE | 25 | 50 | 50 | 25 |
| MLE | 12.5 | 12.5 | 25 | 12.5 |
| CLE | 50 | 50 | NF | 50 |

*Key: ASE = Aqueous stem bark extract, MSE = Methanolic stem bark extract, CSE = Chloroform stem bark extract, ALE = Aqueous leaves extract, MLE = Methanolic leaves extract, CLE = Chloroform leaves extract, NF = Not found*

**Plate 1. Zone of inhibition shown by aqueous extract**

**Table 4. Minimum bactericidal concentration (MBC) of the extracts against the test isolates**

| Extracts | Isolates/ Minimum bactericidal concentration (mg/ml) | | | |
|---|---|---|---|---|
| | ***E. coli*** | ***Klebsiella*** | ***S. typhi*** | ***Shigella*** |
| ASE | 100 | 50 | 100 | 100 |
| MSE | 50 | 50 | 100 | 50 |
| CSE | 100 | NF | NF | 100 |
| ALE | 50 | 100 | 100 | 50 |
| MLE | 50 | 50 | 100 | 50 |
| CLE | NF | NF | NF | 50 |

*Key: ASE = Aqueous stem bark extract, MSE = Methanolic stem bark extract, CSE = Chloroform stem bark extract, ALE = Aqueous leaves extract, MLE = Methanolic leaves extract, CLE = Chloroform leaves extract, NF = Not found*

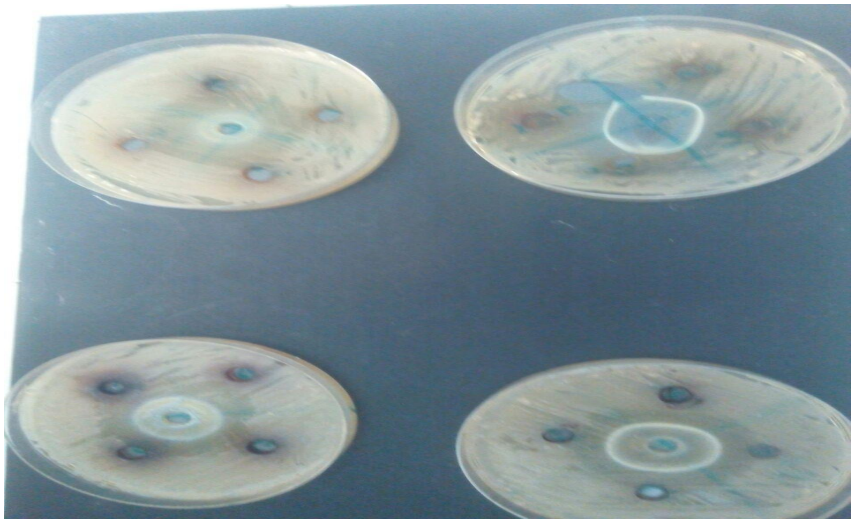

**Plate 2. Zone of inhibition shown by methanol extract**

The result of the antimicrobial activities of the extracts by agar well diffusion showed that the extracts produced higher zones of inhibition all the test organisms even at the lowest concentration however, few resistance was observed at 50 mg/ml. Methanolic extract is the first interm of activity with average zone of inhibition of 15.5 mm, followed by aqueous extracts (14.06 mm) while the chloroform extracts recorded the lowest zones of inhibition (11.62 mm), thus the bioactivity of the extracts followed the sequence methanol extracts > aqueous extracts > chloroform extracts. Higher zone of inhibition of methanol and aqueous extracts is due to better solubility of the phytochemical constituents of the plant parts. However, there is no significant different on the activity of different extracts on the tested isolates but significant different exist between the activity of the extracts and that of the standard antibiotic used in the experiment. Similarly, the result of minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the extract showed that methanol extracts had the lowest MIC and MBC ranges 12.5 – 25 mg/ml then aqueous extract (12.5 – 50 mg/ml) while chloroform extract recorded the highest MIC value (50 mg/ml to above) while the MBC values of most isolates were not found due to lower antibacterial activity. The result showed that the leaves extract of the plant have strong activity against the isolates used in this study. Thus, the extracts have spectrum of activity and this is inconformity with the finding of Ntiejumokwu and Alemika, [20] who reported that the extracts of *B. dalzielii* have a broad spectrum of activity against both gram positive and gram negative bacteria. The fact that the extracts are active against some members of Enrerobacteriaceae confirmed the ethnobotanical usage of the plant in treating gastroenteritis particularly those caused by the organisms.

## 4. CONCLUSION

In this study, methanol was found to be the solvent of choice for extracting bioactive phytochemical from leaves and stem bark of *B. dalzielii*. The active phytochemical components found in this study include alkaloid, saponin, tannin, Anthraquinone, Flavonoid, phenols, terpenoid and glycoside. The study confirmed that the leaves and stem bark extract of *B. dalzielii* used were active against microorganisms causing diarrhea stool and this provide scientific support to the traditional usage of the plant parts in treating many diseases involving the organisms.

## ETHICAL APPROVAL

Ethical approval (issue number HMB/GEN/488/ VOL. 1) was obtained from Kano State Hospital Management Board based on the consent of Murtala Muhammad Specialist Hospital ethical committee.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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