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GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS AND ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *Boswellia dalzielii* ON CLINICAL ISOLATES OF *Escherichia coli* AND *Salmonella* spp.

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ABSTRACT

Humans have used medicinal plants for thousands of years as natural remedies to prevent, treat and manage various health conditions. The fragrant Boswellia dalzielii plant, is primarily found on rocky, arid, and shallow soils in the West African Savannah region. Antibacterial activity of aqueous and ethanolic extracts of Boswellia dalzielii stem bark were tested against clinical isolates of E.coli and S.Typhi. Phytochemical tests were conducted to identify the classes of compounds present, the idendity, characteristic structures the various compounds were identified using Gas chromatography-Mass Spectrometric analysis (GC-MS) at the department of Chemistry, ABU, Zaria. Antibacterial activity of the extracts were assessed using agar well diffusion method. Flavonoids, saponins, alkaloids and phenols were detected in both ethanolic and aqueous extracts, while GC-MS analysis revealed presence of n-hexadecanoic acid, 9-octadecanoic acid and a trace amount of some lipophilic acids. Antibacterial activity of the extract against the bacterial isolates (Salmonella spp. and E. Coli) revealed varied degrees of antibacterial activity. In contrast to aqueous extracts, the ethanolic extract had a comparatively higher zone of inhibition. , the zone of inhibition ranged from 8.00 mm to 16.0 mm, which was much greater than when utilizing an aqueous extract (6.00 mm to 14.0 mm). Both the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations were also recorded. The findings of this study suggested that Boswellia dalzielii stem bark would serve as an important source of medicinal compound which could be harnessed for antibacterial activity.

Keywords: Antibacterial Activity, Aqueous, Boswellia dalzielii, Ethanolic Extracts

INTRODUCTION

Humans have long used medicinal plants, and their accomplishments are largely responsible for the development of modern medicine. It is necessary to evaluate the advantages and disadvantages of some common alternative and traditional medicines used in disease treatment because orthodox pharmaceuticals are currently unable to meet the World Health Organization's dream and goal of providing general health benefits (Abdulhamid, 2019). In Africa, microbial infections pose a significant threat to public health. Despite the fact that modern medicine uses a large variety of synthetic medications to treat microbiological infections, poverty has led many populations in most African countries to turn to herbal therapy (Ahmad, 2009). Herbal medications are the main source of healthcare in almost 80% of underdeveloped nations (Anyim *et al.*, 2010).

Herbal medications are typically composed of numerous component combinations that work together to produce the desired effects. However, due to the presence of naturally occurring radioactive materials (NORMs), these products have a number of drawbacks and inadequate safety data. The World Nuclear Association and the International Food Safety Authorities Network (Balarabe *et al*., 2023). State that naturally occurring radionuclides are frequently discovered in medicinal plants. The fact that medicinal plants are inexpensive, generally available, and extensively disseminated and may be collected for the dealing with various forms of disorders is the basis for the usage of herbal drugs in Nigeria today, particularly in rural areas.

Many herbal remedies, both registered and unregistered, are freely offered by license practitioners in Nigeria. As per the Centers for Disease Control and Prevention (CDC, 2003). Serious hepatic toxicity was documented in 1999 after the use of herbal remedies containing lava lactone, necessitating liver transplantation for multiple individuals. However, a study conducted by Emmanuel *et al.,* (2015) highlighted the negative effect of indiscriminate consumption of herbal medicines especially from unregistered dealers. This therefore calls for more research to be conducted so as to determine the safety of herbal formulations made up from numerous plants (Hassan *et al*., 2009).

The widespread and careless use and misuse of antimicrobial agents is increasingly plunging the public to an even greater risk of antimicrobial resistance. This further stretches the already limited health care facilities leading to high morbidity and mortality rates (WHO, 2024).

Though some of the indigenous flora have not been completely explored, several researches describe how they are employed in different industries (medicine, pharmacy, fragrance, cosmetics, and food) for their medicinal and organoleptic properties (Croxen *et al*., 2013). Boswellii dalzeilii is commonly referred to as the frankincense tree. It grows up to 13 metres tall and is primarily found on rocky, arid, and shallow soils in some parts of Savannah region of West Africa. The tree's distinctive pale, papery bark is ragged and flaking. Ararrabi, Basamu, and Hanu are among the Hausa names (Yakubu *et al*., 2022). Boswellia dalzielii (Hutch.) is known by folk medicine as a plant with great antimicrobial activity, the leaves, roots, and stem bark harbour a great amount of phyto-constituents although a lot needs to be done to substantiate and standardise its usage (Olaleye *et al*.,2007).

MATERIALS AND METHODS

Sampling Area

This study was focuses on the activities of antibacterial in aqueous and ethanolic extracts of *Salmonella* species and *Escherichia coli* from the general hospital Dutsin-Ma, Katsina state.

Study area

The area of study of this work was carried out in Dutsin-Ma local government of Katsina State Nigeria. The area is situated on 12°17.00N' through 12°17.84 and 007°26'E of latitude and longitude respectively.

Sampling Site and Sample Collection

General hospital of Dutsin-Ma was utilized in the collection of the samples. *Boswellia dalzielii* (Frankincense tree) was collected from Farmers in this area and was stored at room temperature.

Collection of clinical isolates

The Clinical isolates were collected from General Hospital Dutsin-Ma, Katsina State. The isolates were later sub-cultured on agar slants and kept at a temperature of 4°C until when needed.

Biochemical characterisation of the Clinical Isolates

All biochemical tests were conducted according to the principles highlighted in (Chessbrough, 2006).

Gram Staining

Gram staining was conducted and the slides were viewed under the oil immersion objectives to determine the gram reaction of the isolates.

Indole Test

A sterile test tube was filled with 4 ml of tryptophan broth, the isolated bacterial colonies were inoculated, and the broth culture was cultured for 24 hours at 37 °C. After that, 0.5 ml of Kovac's reagent was added, and the presence or absence of rings was monitored.

MR/VP Test

The Methyl red-Vogesproskauer (MRVP) broth was inoculated with pure cultures of bacteria, and the mixture was incubated at 37℃.The results were read after 24 hours of incubation.

Triple Sugar Iron Agar (TSI) Test

Using a straight wire, a pure colony was picked and the butt of the TSI agar was stabbed, the slant was thereafter streaked. Incubation was done for 24 hours at 37℃. Gas production, visible colour change at the butt and slants were observed and results recorded.

Urease Test

The urea agar slant was inoculated with a pure colony of the test organism and incubated at 37℃ for 24 hours.

Plant Extracts Preparation

The crude extracts were made by dropping a sample of 40g of powdered stem-bark for 72 hours in 400 ml of ethanol and water each. Whatman No. 1 filter paper and a clean towel were used to filter the extracts. While the filtered ethanolic solution was placed inside a rotary evaporator until the ethanol was removed leaving the crude extracts of *boswellia dalzielii*, the filtered aqueous solution was placed inside a water-bath until the water was completely evaporated. For the purposes of the experiment, the crude extract was allowed to cool and solidify at room temperature.

Phytochemical Screening B*oswellia dalzielii (*frankincense tree*) E*xtract

The plant extracts were examined for the presence of chemicals as provided in the below subheadings.

Saponins test

A test tube containing 1 cm^3 of crude extract and distilled water was shaken vigorously for 30 seconds. When heated, frothing developed and remained, indicating the presence of saponins.

Tannins test

Three drops of ferric chloride solution were added to a portion of the extract. Condensed tannins are represented by a greenish-black precipitate, whereas hydrolyzable tannins produce a blue or brownish-blue precipitate.

Alkaloids test

To a portion of the extract, few drops of Dragendorff's reagent were added. A reddish-brown precipitate indicates the presence of alkaloids.

Flavonoids test

The presence of flavonoids is indicated by the extract's yellow colour, which was obtained after a small amount of the extract was treated with a few drops of 10% sodium hydroxide

Steroid tests

2ml of acetic anhydride was added to 0.5g of the extract and 2ml of sulphuric acid was added by the side of the test tube a color change was observed from violet or blue-green which showed the presence of steroids

Glycoside test

2.0 ml of acetic acid and 2 ml of chloroform was added with whole aqueous fruit extract and ethanolic extract separately in test tubes. The mixture was allowed to cool then few drops of H_2SO, concentrated will be added. Green color shows the entity of aglycone, steroidal part of glycosides.

Phenol test

To 2 ml of extract, 3 ml of ethanol and a pinch of ferric chloride are added. A greenish yellow color appears which indicates the presence of Phenols.

Gas Chromatography Mass Spectrometry (GCMS) Conditions

The mass spectrometer is linked to the Agilent GC/MS. The compound will be separated using an HP-5MS 30 m x 0.25 mm, 0.25 mm film thickness, for nine minutes at 59 °C, followed by one minute at 230 °C, with a one-minute pause and a three-degree Celsius temperature increase every minute The injector temperature will be 245 degrees Celsius, and the carrier helium gas flow rate will be one milliliter per minute. The analyzer and ion source temperatures of the MS will be 70 e V and 260 °C, respectively.

Preparation of Mcfarland Standard

To manufacture the 1% v/v solution of sulphuric acid, 1 milliliter of concentrated H2SO4 will be added to 99 milliliters of distilled water to create barium sulphate. Additionally, 0.5g of dehydrated barium chloride will be dissolved to create a one percent (1%) weight per volume solution of barium chloride. To create a 1.0% w/v barium

sulphate suspension, the solution will be mixed with 99.4 milliliters of sulfuric acid solution. The sterile suspension will be placed into separate test tubes and each will contain 0.1 ml of an overnight broth culture of Salmonella species and Escherichia coli. This functions as the reference inoculant for the antibacterial experimentation

Antibacterial activity of *Boswellia dalzielii* extract

Antibacterial activity of the extract was conducted following the method of Kubmarawa *et al* (2013).DMSO was used to dissolve the concentrated extracts in order to create stock solutions with known and variable concentrations.

Agar well Diffusion Method

Agar well diffusion method was used to screen the antibacterial activity. Of the plant. Standardized bacterial inoculums was poured onto Muller Hinton agar plates in an amount of 0.1 ml. Using a sterile glass spreader the inoculums were equally distributed around the plate. The prepared concentrations were poured unto each well that was dug using a sterile cork borer (6mm). The wells were labelled according to the concentrations prepared (100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively). The plates were allowed to stand for 30minutes before incubating to allow suffient extract diffusion into the agar. Incubation was done at 37℃ for 24 hours and the plates were observed for zones of growth inhibition.

Minimum Inhibitory Concentrations (MIC) Determination

To determine the minimum inhibitory concentrations (MICs) of the Boswellia dalzielii extract against the test organism, 2 millilitres of different concentrations of the extract were poured un-to molten Mueller hinton broth. A loopful of the 0.1ml of the standardised inoculum was dropped unto the mixture. The same procedure was done for the remaining three concentrations and the tubes were incubated for 24hours. The tubes were observed for tubidity and results recorded.

Minimium Bactericidal Concentration (MBC)

The extract's lowest concentration, which showed no signs of growth, and the next concentration following it were inoculated unto Mueller hinton agar. The plates were incubated for 24 hours at 37°C. The MBC was recorded at the plate bearing the least concentration that has no growth

RESULTS AND DISCUSSION

Table 1 reveals the biochemical characteristics of *E. coli* and *Salmonella* spp. After obtaining the gram reaction of the isolates, Indole, Urease, Methylred (MR), Voges proskauer (VP) and Triple Sugar Iron tests were conducted so as to ascertain the biochemical characteristics of the isolates.

Table 1: Biochemical characteristics of *E. coli* and *Salmonella* spp.

Organisms	Indole	Urease	MR/VP	Triple Sugar Iron (TSI)
E. coli	+	-	+/-	AA + gas
Salmonella spp	_	-	+/-	AA + H_2S

Key: - = Negative, + = positive, A/A = Acid/ Acid

Table 2 showed the phytochemical constituents analysis of ethanolic and aqueous extract of the stem bark of *Boswellia dalzielii.* The notable presence of known phyotochemicals like flavonoids, saponins, Tanins, alkaloids and phenols, These phytocontituents were known to bear antimicrobial as well as anti imflammatory properties as concluded in the work of (Mamza e*t al.*, 2022). Similar result was also demonstrated emphasising that flavonoids' capacity to form complexes with bacterial cell walls is what gives them the ability to fight bacteria (Vogt, and Dippold, 2005). Tannin-containing herbs are astringent in nature and are used to treat intestinal conditions like dysentery and diarrhoea (Trease, and Evans, 2002). These results collectively reflect the role of phytochemicals in fighting pathogens

When considered collectively, these results provide credence to the use of this plant in many African communities for the preparation of regional remedies intended to treat ailments.

Table 2: Phytochemical analysis of *Boswella dalzeilii*

Phytochemical compounds	Ethanol	Aqueous
Flavonoids	+	+
Tanins	+	N.D
Saponins	+	+
Glycoside	N.D	+
Alkaloids	+	N.D
Steroids	N.D	+
Phenols	+	+

Key: + = present N.D = not detected

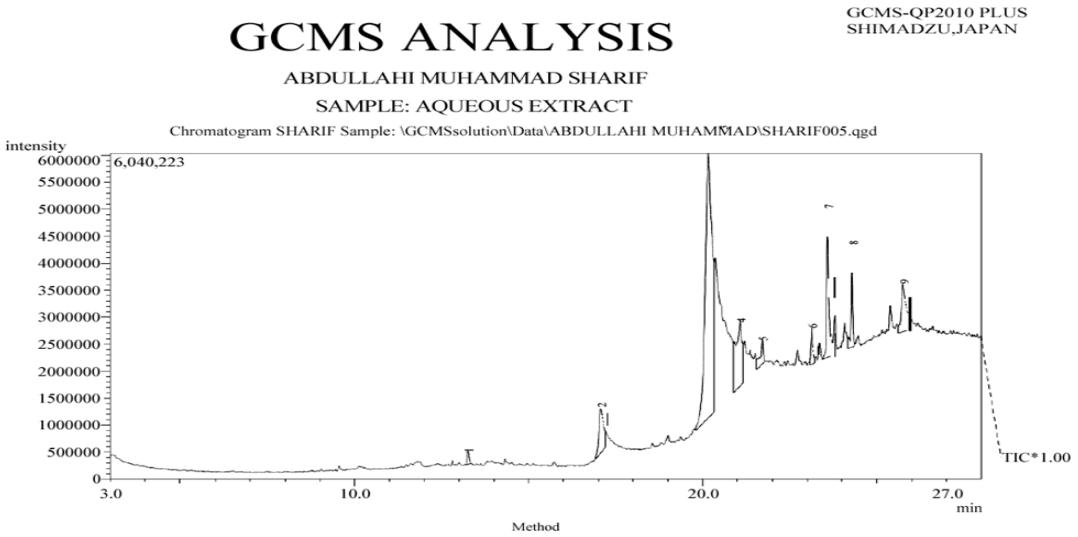


Figure 1: The GC-MS spectral of the *Boswellia dalzielii* Extract

The ethanolic stem bark extract of *Boswellia dalzielii* GC-MS data shows substances that had a 100% match similarity index on the NIST collection. Figures 1, Shows that the ethanolic extract included a number of pharmacogenetic chemicals. The chemical composition of the *Boswellia dalzielii* extract as characterized by GC-MS. Several Compounds are identified which tallied with represented by the prominent peaks on the spectral. The phyto-constituents are made up of aliphatic hydrocarbons, aromatic, carboxylic acids and ester compounds. Carboxylic acids are most prominent with 13-Docosenoic acid, (Z)- ($C_{22}H_{42}O_2$) 45.64% of the constituting compounds. Similar compounds were detected and reported in the work of Magashi *et al.,* (2017).

Table 3: Gas chromatography-Mass spectrometry (GC-MS) of Phytochemicals from *Boswellia dalzeilii* stem bark

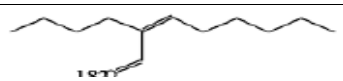
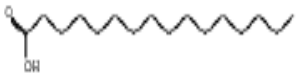
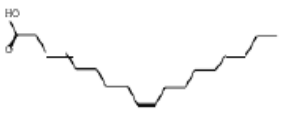
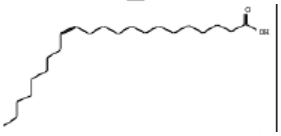
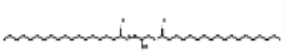
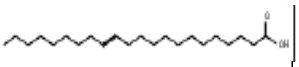
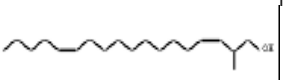
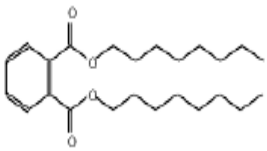
	Retention time	Base peak value	Pyto-constituent	Structure of phyto- constituent	Molecular weight	Molecular formular	SI %	Area %
1	13.267	111.25	2-Octenal, 2-butyl-		182	$C_{12}H_{22}O$	76	0.74
2	17.067	43.10	n-Hexadecanoic acid		256	$C_{16}H_{32}O_2$	86	6.28
3	20.158	55.10	9-Octadecenoic acid (Z)-		282	$C_{18}H_{34}O_2$	89	57.83
4	21.067	55.10	13-Docosenoic acid, (Z)-		338	$C_{22}H_{42}O_2$	82	12.46
5	21.708	57.10	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester		624	$C_{39}H_{76}O_5$	72	2.33
6	23.133	55.10	13-Docosenoic acid, (E)-		338	$C_{22}H_{42}O_2$	86	2.22
7	23.583	55.10	2-Methyl-Z,Z-3,13-octadecadienol		280	$C_{19}H_{36}O$	84	7.85
8	24.283	57.10	1,2-Benzenedicarboxylic acid, dioctyl ester		390	$C_{24}H_{38}O_4$	84	4.09
9	25.742	55.10	9,12-Octadecadienoyl chloride, (Z,Z)-		298	$C_{18}H_{31}C_{lO}$	85	6.18

Table 4 reveal the Antibacterial sensitivity pattern of the ethanolic extract of the stem bark of *Boswellia dalzielii* against *Salmonella* spp and *Escherichia coli*. The extracts of the solvent exhibited varying degrees of antibacterial activity against on the test isolates. The ethanolic extract of *Boswellia dalzielii's* stem bark exhibited the largest zone of inhibition against the test organisms among the two extracts. However, aqueous extracts exhibited modest action against test organisms in consistency with the work of (Balarabe *et al*., 2023). This indicates that ethanol could work well as a solvent for isolating and purifying the active ingredients found in *Boswellia dalzielii* stem bark.

Table 4: Antibacterial sensitivity test of the ethanolic extract of the stem bark of *Boswellia dalzielii* against *Salmonella* spp and *Escherichia coli*

Organisms	ZONE OF INHIBITIONS (mm)				
	Concentrations(mg/ml)				
	200	**400**	**600**	**800**	**Control (Tetracycline)**
E. coli	9	10	14	16	30.67
Salmonella	8	9	12	14	28

Tetracycline = as positive control

Table 5: The Antibacterial sensitivity of the aqueous extract of the stem bark of *Boswellia dalzielii* against *Salmonella* spp and *Escherichia coli*

Organisms	ZONE OF INHIBITIONS (mm)				
	Concentrations(mg/ml)				
	100	**50**	**25**	**12.5**	**Control (Tetracycline)**
E. coli	9.5	11	12	14	31
Salmonella Spp.	6	9	12	13	34

Tetracycline = as positive control

Table 6 displays the minimum inhibitory concentration (MIC) of *Boswellia dalzielii* against the test organisms (mg/ml) in both the aqueous and ethanolic extracts. Additionally, MIC was investigated for both the crude ethanolic and aqueous stem bark extracts. From the values of MIC, the crude aqueous extract against test bacteria was higher compared to the ethanolic extracts. Thus, this implies that the test bacteria have greater sensitivity to crude ethanolic extract compared with aqueous extracts and the finding agrees with prior work (Tegasne, 2020).

Table 6: Minimum inhibitory concentration (MIC) of *Boswellia dalzielii*

Extract	***E. coli***	***Salmonella* spp**
Ethanolic	50mg/ml	25mg/ml
Aqueous	50mg/ml	12.5mg/ml

The Minimum bactericidal concentration (MBC) results is depicted in the above table. Both aqueous and ethanolic extract of the stem bark of *Boswellia dalzielii in* (mg/ml) Against *E. coli* and *Salmonella* spp. This is an indication that the ethanolic and aqueous extracts of *Boswellia dalzielii* have both bactericidal effect even at low concentrations. The result is in contrast with the work reported by (Mukerjee *et al*., 2003) which stated the bacteriocidal activity of Bosweilii dalzeilii at very high concentrations of 200mg/ml.

Table 7: Minimum bactericidal concentration (MBC)

Extract	***E. coli***	***Salmonella* spp**
Ethanolic	25mg/ml	12.5mg/ml
Aqueous	25mg/ml	12.5mg/ml

CONCLUSION

This study revealed the characteristic structures and chemical composition of the stem bark *Boswellia dalzielii* as well as its antibacterial efficacy against *S.*Typhi *and E.coli.* This finding support its potential as a source of novel antimicrobial aagents. may provide a greater insight to the possible use and harnessing of this plant as an alternative to some commonly used antibiotics. Also, the plant's ethnomedical uses may have scientific backing because of the extracts' demonstrated activity against tested bacterial species linked to a range of infectious diseases. However, additional investigations on in vivo tests, clinical trials, and the extract's mechanism of action are needed to provide more conclusive evidence of the antibacterial activity of the extracts of *Boswellia dalzielii* used.

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