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Evaluation of antibacterial, free radical scavenging activities and phytochemical composition of *Boswellia dalzielii* Hutch extracts

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Abstract
Ethanol extract and essential oil of *Boswellia dalzielii* leaves were screened for their antibacterial and antiradical activities by using microdilution and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical methods respectively. Ethanol extract was submitted to a phytochemical screening and the essential oil to Gas Chromatography-Mass Spectrometry (GC/MS) analysis. Antibacterial studies reveal a MIC of 1.25 mg/ml for ethanol extract and 1.25 µl/ml of essential oil on *Methicillin-resistant Staphylococcus aureus*, but no MIC on *Staphylococcus aureus*. The essential oil was more powerful radical scavenging activity with EC_{50} value of 1.25 µg/mL which greater than the standard antioxidants, ascorbic acid (EC_{50} = 5 µg/mL). The phytochemical screening reveals the presence of metabolites mainly 3.56 ±0.06 mg GAE/ g of extract of total polyphenol and 1.24±0.01 mg QE/ g of extract. GC-MS results show the presence of: Zerumbone (33.69%), α-phellandrene (11.80%), alpha α-pinene (9.71%), Germacrene D (9.08%) ß - phellandrene (8.19%) and *Trans* ß – caryophyllene (7.45%) like major compounds. These compounds could be responsible for the antibacterial and scavenging activities.

Keywords: *Boswellia dalzielii*, extracts, MRSA, antioxidant

1. Introduction
The advent of modern medicine opens up much more knowledge about human pathologies and the mode of intervention to fight its discomforts [1]. According to World Bank and WHO: Half the world lacks access to essential health services, 100 million still pushed into extreme poverty because of health expenses [2]. Added to this are other factors including the lack and poor distribution of health infrastructure, the low coverage of regions in doctors. Thus, in order to care for the majority of the African population resorts to plant resources. Statistics of the World Health Organization show that more than 80% of the African population uses medicine and traditional pharmacopoeia to cope with health problems [3]. Firstly, several works shown that oxidative stress (due to an excess of ROS and/or a decrease in antioxidant levels) is sources of different diseases in the world [4, 5], secondly antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today [6]. While on the other hand recent work have shown that the antioxidants from plant uses in traditional medicine origin with free-radical scavenging properties could have great therapeutic importance on several diseases caused due to oxidative stress [7-10] and plant-derived compounds have historically been recognized as effective antimicrobial agents [11-13].
Boswellia dalzielii Hutch (Burseraceae) is a tree of the savanna forest widely used in Africa as medicinal plant: In Nigeria the gum resin is used with other medicines for the treatment of venereal diseases; the bark is boiled to wash for fever, rheumatism; the root decoction boiled along *Hibiscus sabdariffa* is used for the treatment of syphilis [14]. The bark of the stem is used as a poultice following a snakebite, decoction of the aerial parts is taken orally for tuberculosis, decoction of root bark is applied to treat scabies [15]. The bark decoction is used as an antiseptic wash for sores in Ivory Coast and as an ingredient of a complicated prescription for leprosy [16]. In Niger the leaf is used in the treatment of bilharziasis and given as an oxytocic to pregnant women [17]. In Cameroon, people use the leaves of *B. dalzielii* to protect maize, millet and sorghum against weevils' attack [18].
The aim of this study is the analysis of antimicrobial and scavenging activities of two extracts of *B. dalzielii* from Savè (Benin), in order to optimize their medicinal exploitation.

2. Materials and Methods

2.1 Plant material

The leaves of *Boswellia dalzielii* were harvested in April 2018 in Savè in the Colline department of Benin.

2.2 Bacterial strains

Gram negative *Escherichia coli* (CIP 53126) and free Gram positive: *Enterococcus faecalis* (ATCC29212), *Staphylococcus aureus* (ATCC6538), *Methicillin-resistant Staphylococcus aureus* (MRSA); were used for antibacterial test. They were obtained from Laboratory of Biochemistry and Bioactives, Natural Substances, Faculty of Science and Technology, University of Abomey-calavi (Benin).

2.3 Extraction

Two types of extraction were made on the leaves of *Boswellia dalzielii.*

2.3.1 Ethanolic extraction

Ten gram (10g) of *Boswellia dalzielli* leaves powder was extracted by maceration with 100 ml of ethanol for 24 hours under string. After filtration on Buchner, the filtrate was concentrated to dryness using a rotary evaporator then stored at 4 ° C until further use.

2.3.2 Extraction of essential oil

The essential oils of *Boswellia dalzielii* leaves were extracted by hydro-distillation for 3hours using a Clevenger type apparatus. Essential oil was dry using sodium sulfate anhydrous. The oil was weighted and conserved at 4°C until use.

2.4 Phytochemical study

2.4.1 Preliminary phytochemical analysis

Ethanol extract of *B. dalzielli* leaves was subjected to qualitative phytochemical tests to identify various classes of phytochemical constituents using standard procedures [19].

2.4.2 Determination of total phenolic content

Phenolic content was estimated spectrophotometrically using a modified Folin-Ciocalteu method [20]. The total flavonoids content was determined using $AlCl_3$ [21]. Tannin content was determined using the modified Vanillin-HCl method [22].

2.4.3 Gas chromatography-mass spectrometry (GC-MS)

This analysis was carried out using a gas chromatograph coupled to a GCMS-QP 2010 Shimadzu type mass spectrometer detector equipped with an HP 5 MS Agilent capillary column (30m x 0.25mm x 0.25µm). The carrier gas is helium (1 ml / min); it was injected in the Splitless mode in temperature programming: 70 ° C for 2 min Then 3 ° C / min for 240 ° C for 15min Analysis time 73 min. The respective operating temperatures of the injector and detector are 240 ° C, 250 ° C and the Source 200 ° C. The mass spectrometer is used in electronic impact mode at 70ev.

Information retrieved from mass spectrometry is set in FullScan mode. The identification of the compounds was made taking into account retention times (TR) and mass spectra and validated from reference standards (brand Fluka - Sigma - Acros). The libraries used are NIST and WILEY.

2.5 Evaluation of biological activities

2.5.1 Antibacterial assay

The antibacterial test was performed in microplates using the method described by Atindehou *et al.*, 2013 [23]. Briefly, bacteria were cultured aerobically at 37˚C in a Mueller Hinton Broth (MHB, Merck, Darmstadt, Germany) for 18 h. *B. dalzielli* extract (20 mg) was suspended in acetone/water (10:90 v: v, 1 mL) and diluted to a concentration of 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039 mg/mL in MHB in 96 wells microplates in 100 µL. 100 µL of a midlogarithmic phase culture of bacteria with at concentration 10^6 at 620 nm was added. Each assay was performed in triplicate. Negative control which consist of the mixture of MHB without bacteria and acetone/water control were also realized. After 18 h of incubation under agitation at 37˚C, the Minimal Inhibitory Concentration (MIC) was determined by addition of 40 µL of Iodonitrotetrazolium chloride (INT, Sigma Aldrich Chemie GmbH, Steinheim, Germany at 0.2 mg/mL and the plates were incubated for 1 h at 37˚C. Bacterial growth was determined by a reddish-pink color in the well. For essential oil, the same method was used but the concentrations tested are: 25, 12.5, 6.25, 3.12; 1.56; 0.78; 0.39 µL/mL. The essential oil was dissolved in 1% DMSO and the growth control with DMSO was also performed.

2.5.2 Antioxidant assay

Free radical scavenging activity was determined using 2, 2-diphenyl-1-picrylhydrazil (DPPH, Sigma Aldrich, Germany), as described by Povichit *et al.* 2010 [22] with slight modifications. Briefly, 1900 µL of DPPH solution (6.10^{-5} M in Methanol) and 100 mL of ethanol extract or essential oil at different axis and concentration were added. The reaction mixture was shaken and incubated in the dark for 30 min at 37°C. The absorbance was read at 517 nm against blank using the spectrophotometric reader. Ascorbic acid (Sigma Aldrich, Germany) was used as positive control.

DPPH radical scavenging activity was calculated according to the formula:

$$[(A_0 - A_1)/A_0] * 100$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the extract.

Antiradical curves were plotted referring to concentration on the x axis and their relative scavenging capacity on the y axis and the concentration required to obtain a 50% antioxidant effect (EC_{50}) was determined.

3. Results and Discussion

3.1 Chemical composition

3.1.1 Chemical composition

The phytochemical screening of ethanol leaf extract of *Boswellia dalzielii* revealed the presence of alkaloid, coumarin, flavonoid, tannin, anthocyanin, carbohydrates, anthraquinone and terpenoid (Table 1). Regarding the total phenol, the results show low tannin content: 0.02 mg CE/g Extract (Table 2)

Table 1: Phytochemical constituents of ethanol extract of leave of *Boswellia dalzielii*

Phytochemicals	Result
Alkaloids	+
Flavonoids	+
Anthocyans	+
Tannins	+
Mucilage	+
Anthraquinone	+
Coumarins	+
Saponins	+

+ = present

Table 2: Phenolic content in the ethanol extract of *Boswellia dalzielii* leaves

Constituents	Quantity
Total polyphenols (mg GAE/g Extract)	3.56 ± 0.059
Flavonoids (mg QE/g Extract)	1.24 ± 0.012
Tannins (mg CE/g Extract)	0.02 ± 0.004

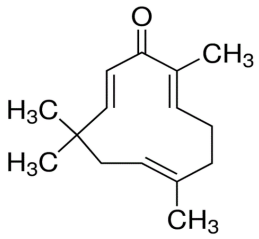
3.1.2 Chemical composition of essential oil

Hydrodistillation extraction yield of the essential oil is 2.1 %. This essential oil was characterized by the higher content of sesquiterpernes (61.09%) with 33.69 % of Zerumbone 9.08% of Germacrene D and 7.45 % of Trans -b- caryophyllene, a-Phellandrene (11.8 %), a-Pinene (9.71 %) and b- Phellandrene (8.19 %) were the major compounds of the 34.29 % of monoterpenes of this essential oil (Table 3).

In literature, 3-carene (27.72%), a-pinene (15.18%), p-cymene (9.54%), b-phellandrene (8.48%), isolongifolene (6.15%) myrcene (5.72%) were the predominant compounds of the essential oil from Segbana, in the north of Benin [24]. In Nigeria essential oil, the most abundant constituents were a - pinene (45.7%) and a-terpinene (11.5%) when α-phellandrene, β- phellandrene, Germacrene D, β - caryophyllene were minority compounds [25]. Among the new compounds identified in the essential oil of the present study and which are not in Kohounde [24] and Kubmarawa [25] works results, there are mainly the Zerumbone (33.69%). Zerumbone is a sesquiterpene found in subtropical ginger and known for its various pharmacological properties: anti-inflammatory, anti-cancer, anti-oxidant [26, 27].

Table 3: Main components of essential oil from *Boswellia dalzielii* leaves

Retention Time (min)	Components	Yield (%)
4.6	alpha - pinene	9.71
6.10	alpha - phellandrene	11.8
6.92	delta - 3- carene	0.45
6.99	gamma - terpinene	1.35
7.1	beta - phellandrene	8.19
7.7	beta - ocimene	1.09
12.5	Terpinene – 4 – ol	0.68
13	alpha - terpineol	1.02
19.5	alpha - cubebene	2.68
20.5	Copaene	2.85
20.9	alpha – bourbonene	0.63
22.3	Trans beta - caryophyllene	7.45
23.7	Alpha – Humulene	0.97
24.8	Germacrene D	9.08
26.5	delta - cadinène	1.64
31	tau-Muurolol	1.32
31.5	Alpha - cadinol	0.78
34.3	Zerumbone	33.69
Monoterpene hydrocarbon		32.59
Oxygened monoterpene		1.7
Sesquiterpene hydrocarbon		61.09
Oxygened sesquiterpene		35.79
Unidentified		4.62



Zerumbone

Studies on the oleogum resin of most other species of the genus Boswellia have shown they are rich in monoterpenes. It is the case of *Boswellia sacra Boswellia dioscorides, Boswellia elongate, Boswellia socotrana, Boswellia carteri* and *Boswellia frereana* [28-31].

3.2 Antibacterial activity

The minimal inhibitory concentration (MIC) value (Table 4) revealed that among the four strains on which the extracts were tested, only *S. aureus* was not sensitive to different concentrations of extracts tested, while MRSA has been the most sensitive with a MIC of 1.25mg/ml for the ethanol extract and 12.5 mg/ml for the essential oil.

Table 4: MICs of *Boswellia dalzielli* extracts

Strains / Extracts	*E. coli*	*E. faecalis*	MRSA	*S. aureus*
Ethanol extract (mg/ml)	2.5	1.25	1.25	ND
Essential oil (µl/ml)	25	25	12.5	ND

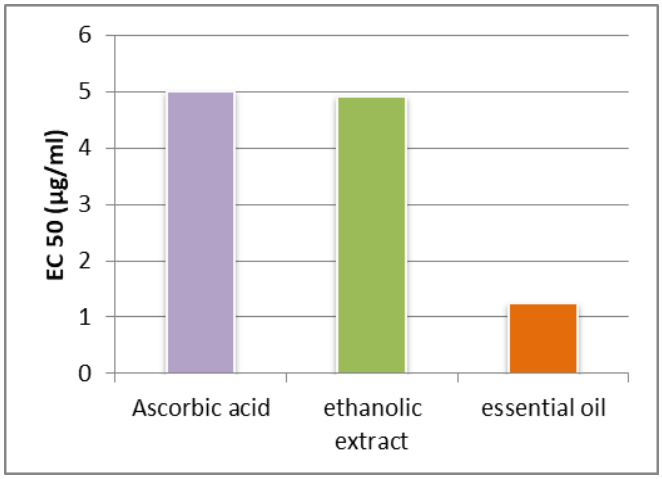
ND: not determined

Medicinal plants are the richest source of metabolites with various biological activities. For this purpose, they are used in traditional medicine in the form of volatile or non-volatile extracts. In non-volatile extracts, alkaloids and saponins are known to have antimicrobial properties [11]; by precipitating microbial protein, tannins inhibit growth of microorganisms [32]. Antibacterial activity of flavonoids is being increasingly demonstrated [33, 34]. Most of secondary metabolites revealed in ethanol extract of *B. dazielli* (alkaloids, terpenoids, flavonoids) have been reported to have anti-MRSA activity [35, 36]. MRSA is one of the leading causes of morbidity and mortality worldwide, it is a major public health concern and is responsible for both hospital and community-associated infections worldwide [37, 38]. Previous work showed significant antimicrobial activity of ethyl acetate and ethanol extracts against *Escherichia coli* and *Klebsiella pneumonia* [16].

The volatile compounds contained in certain essential oils are known for their antibacterial effect. It is the example of the major compounds of the essential oil subject of the present study: Zerumbone, a-pinene [39, 40]. Kubmarawa [41] results show that essential oil inhibited the growth of *Bacillus subtilis, Staphylococcus aureus* and *Candida albicans* at minimum inhibitory concentrations (MIC) of 250µg/ml, 1000µg/ml and 2000µg/ml respectively.

3.3 Free radical scavenging activity

Graph 1 show the free radical scavenging of the two extracts of *Boswellia dalzielii* compare to ascorbic acid used as positive control. Essential oil presented more radical scavenging activity (EC_{50} = 1.1mg/ml) than that of ethanol extract and ascorbic acid (EC_{50} ≈5.0mg/ml).



Graph 1: Free radical scavenging of *Boswellia dalzielii* leaves extract

Scavenging stable DPPH free radicals can be used to evaluate antioxidant activities in a relatively short time. Essential oil of *Boswellia dalzielii* leaves was a strong antioxidant comparable to vitamin C antioxidant power and was in good agreement with the number of terpenes, a major category of plant-derived compounds.

A previous work assessed the antioxidant effectiveness of most common terpenes from essential oils [42]. The antiradical scavenging activity is more important in the presence of phenols and terpene alcohols than ketones or sesquiterpenes [43, 44]. Despite that in the present work only 3.8% of the terpenes are hydroxylated (T-Muurolol, a- cadinol, Terpinene-4-ol and a- terpineol), the essential oil has been more active on DPPH radical. This Can be justified by the presence of other compounds like a-pinene, Zerumbone which have been reported to exhibit radical scavenging activity [45, 46]. Plant phenolic have multifunctional properties and can act as scavenge free radicals by virtue of the hydrogen-donating properties of their phenolic hydroxyl groups, as well as by donating electrons to stop free radical chain reactions [47]. In the present study, ethanolic extract contains 3.56 mg GAE/g of extract including: anthocyanins, flavonoids, tannins. Its free radical scavenging potential equal to that ascorbic acid (reference product) is due to the presence of these compounds with hydroxyl group in its chemical composition.

4. Conclusion

In present study, the phytochemical analysis has shown that essential oil from *Boswellia dalzielii* leaves contains as main compound the Zerumbone. A compound known as well for its antibacterial and antiradical potential. The ethanolic extract has revealed the presence phenolic compounds. From DPPH assay, the essential oil is the best a source of natural antioxidants compared to the ethanolic extract. In regards to *in vitro* antibacterial test, interesting results were obtained for the both extracts. Further work on the activity of the two extracts on methicillin-resistant *Staphylococcus aureus* and on *Staphylococcus aureus* is important to complete this study.

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