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Invitro Antimalarial and Antibacterial Activities of Methanol Stem Bark Extract of Frankincense Tree (*Boswellia dalzielii)* and Leaves Extract of Kenaf (*Hibiscus cannabinus)*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research investigated the anti-malarial and antibacterial activities of stem bark methanol extract of Frankincense tree and Kenaf leaves extract on *Plasmodium falciparum* parasite and against five clinically significant bacteria.
Study Design: Laboratory-experimental design was used for this study.
Place and Duration of Study: This study was carried out between September 2019 and November 2019 at Biochemistry and Microbiology laboratories, Sokoto State University, Sokoto, Nigeria.
Methodology: The *in vitro* antimalarial activity test was conducted by determining the parasitemia for each sample concentration by manual counting on thin Giemsa smears after a 24-hour incubation with the extracts in order to determine the IC_{50} values. The antibacterial study was done using a modified agar well diffusion technique.
Results: The testing revealed that methanol stem bark extract of Frankincense tree and Kenaf leaves exhibited good and potent (very good) antimalarial activities against *P. falciparum* respectively with IC_{50} values of 1.25 µg/mL and 0.16 µg/mL respectively. They also show good inhibitory activities against *E.coli* but slight inhibitory activities against the other pathogens tested.

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Conclusion: The current study indicates that extracts of these plants exhibit anti-malarial and antibacterial activities and may serve as useful sources of drugs for treatment of malaria caused by *P. falciparum parasite* as well as bacterial infections caused by the tested bacteria.

Keywords: Antimalaria; Kenaf; frankincense; P. falciparum E. coli; IC_{50}.

1. INTRODUCTION

Malaria is known as acute febrile illness in endemic areas and the most infectious diseases in the world [1]. Malaria in humans is caused by five species of parasites belonging to the genus *Plasmodium*, The four of which are *P.* falciparum, *P.* vivax, P. malariaeand *P.* ovale which are human malaria species that are spread from one person to another via the bite of female mosquitoes of the genus *Anopheles*. *P. falciparum* is the most prevalent on the African continent, and is responsible for most deaths from malaria [2]. *Plasmodium falciparum* is a protozoan parasite and the causative agent of the most virulent form of malaria in humans [3]. Nearly one million deaths in malaria endemic areas are due to *P. falciparum* infection, mostly among children under the age of five [3]. According to report by the World Health Organization (WHO), malaria is still one of the major causes of mortality and morbidity, especially in the developing countries. According to report by [4], in 2010, an estimated 3.3 billion people were at risk of malaria and an estimated 1,238,000 deaths were reported that year. Although the disease is present in several regions of the world like Africa, South-East-Asia, Middle and South America, People living in Sub-Saharan Africa have the highest risk of getting infected. It is estimated that 81% of reported cases and 91% of deaths due to malaria in 2010 occurred in the African regions. From all people concerned, children under the age of five years and pregnant women especially in their first pregnancies are the most severely affected [4]. This is a cause for concern.

Bacteria are single celled microorganisms which can contribute to diseases in human. Although some are harmless or often beneficial, others are pathogenic (such as *Pseudomonaeuroginosa, Salmonella typhi*, *Staphylococcus aureus*, *Shigelladysentry* and *Escherichia coli*) in humans and other animals. Pathogenic bacteria contribute to globally significant diseases such as pneumonia, caused by Streptococcus and pseudomonas (*Pseudomonaeuroginosa)*, and food borne illnesses, which can be caused by *Salmonella typhi*, *Staphylococcus aureus*, *Shigelladysentry* and *E. coli*. Microbial pathogenic diseases are the leading causes of illness and deaths in developing countries killing an estimated 1.9 million people annually at global level and one-third of the population are affected by microbial pathogenic food-borne diseases each year [5]. These inform the interest in this proposed study, that is to investigate the antimalarial and antibacterial potencies of locally used medicinal plants such as Frankincense tree (*Boswelliadalzielii)* and Kenaf Leaf (*Hibiscus cannabinus)*.

The search for new remedies from medicinal plant species used as an alternative choice for the treatment of malaria depends on the accurate and specific ethnobotanical and ethnopharmacological information obtained from local healers [6]. In this research two species of plants were evaluated two for thereinvitro antimalarial activities against Plasmodium falciparum parasite and clinically significant bacteria. The species used includes kenaf (Hibiscus cannabinus) and Frankincense stem bark (Boswelliadazielii).

Kenaf is a valuable medicinal plant [7] that produces a diverse array of interesting potential bioactive molecules for instance phenolic compounds, anti-tumor compounds, and phytosterols, with antioxidant, cardio-protective, anti-inflammatory, anti-hypertensive and anti-proliferative activities, which have been pharmacologically investigated [8]. Plants rich in natural antioxidants such as polyphenols, flavonoids are related to reduce the risk of certain types of cancer that has led to a revival of interest in plant-based foods and drugs [9]. Moreover, kenaf has been prescribed quite a long time in traditional folk medicine in Africa and India, indeed, it is composed of various active components including tannins, saponins, polyphenolics, alkaloids, essential oils and steroids [10] and [11]. It has been recognized that Africans have also used peelings from the stems to treat fatigue and anemia as a hematinic agent [10]. It is used as vegetable, blood tonic and a remedy for liver diseases. An earlier study revealed its antioxidant activity in the protection of the cell membrane integrity from the effect of

oxidant [10]. Kenaf leaves were also applicable in treating dysentery, and blood and throat disorders [12]. In addition, the leaf extract of H. cannabinus may therefore have implications in the management of artherosclerosis [13].

Due to its therapeutic properties, the *Boswellia*plant is of interest both to doctors and to nutritionists. The extracts and essential oils of frankincense have been used as antiseptic agents in a mouthwash as well as in the treatment of coughs and asthma [14]. Many studies have been reported on the anticancer, anti-inflammatory, immunomodulatory, antimicrobial, antiviral and even antidiabetic activities of several *Boswellia* species [15].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The stem bark of *Boswellia dalzielii* was collected from Zuru local government area of Kebbi State, Nigeria, while kenaf leaf was collected in Modurawa, one of the villages in Wamako local government area of Sokoto state, Nigeria. The stem bark and the leaves were washed with clean water to remove dust particles, and were air dried under shade.

2.2 Preparation of Extracts

Pestle and mortar was used for grinding the plant to powder form. 70g of powdered *Boswelliadalzielii* stem bark and 30g powdered kenaf leaves were weighed using a weighing balance and transferred into two different 1000ml beakers. 800ml of 70% methanol was added to the beaker containing stem bark and 400ml of the same methanol was added into the beaker containing kenaf powder and left for 72 hours after which the extracts were filtered using what-man filter paper. The filtrates were dried using a hot air oven at reduced temperature (45^{o}c) to produce the extracts which were then used for the analysis.

2.3 Anti-malaria Bioassay

The *Plasmodium falciparum*malaria parasite infected human blood was obtained from UsmanuDanfodio Teaching Hospital, Sokoto State, Nigeria. The sample was transported to the laboratory at Sokoto State University Sokoto State, Nigeria by mixing with anti-coagulant in bottle. It was centrifuged to obtain infected red blood cells and were washed 3 times in CPD to remove serum, and leukocytes if present. *Plasmodium falciparum* infected erythrocytes were cultured in RPMI 1640 media supplemented with 37.5 mM HEPES, 7 mMD-glucose, 6 mMNaOH, 25 µg/mL gentamicin sulfate,2 mM L-glutamine and maintained under a gas mixture of 5% O2, 5% CO2, and 90% N2 [16]. Culture was synchronized by using 10% sorbitol. The erythrocytes with a parasitaemia of 1–2% and a haematocrit of 2% were incubated with compounds over a concentration range of 0.001to 100 µM for 48 hours. Diluted to 5% hematocrit with cMCM in small flasks of 25 cm2 (0.2 mL of packed cells to 4 mL of cMCM) or in 75-cm2 flasks (1.0 mL to 20 mL). The flask was put into a candle jar and loosen the screw cap in other to produce low oxygen by burnt out candle and place the jar at 37 °C. The culture media was replaced every day. 100µl of distilled water was first distributed into well plates after which 100µl of culture medium containing extracts at various concentrations was added into well plates. 100µl of parasiteculture were finally added. Titre plates were incubated in CO_2 condition at 37 ºC in candle jar for 24 hours [16]. After incubation, contents of the wells were harvested and the red cells transferred to a clean microscopic slide to form a series of tin films. The films were stained for 10 minutes in 10 % Giemsa solution of pH 7.3. Schizont growth inhibition per 200 asexual parasites was counted in 10 microscopic fields. The control parasite culture freed from extracts was considered as 100 % growth. The percentage inhibition per concentration was calculated using the formula:

$$\% = \frac{\%\ of\ parasite\ in\ control\ well - \%\ parasitemea\ in\ test\ well}{\%\ parasitemea\ in\ control\ well} \times 100$$

The IC_{50} values, the concentration required to inhibit schizont growth by 50% were determined by linear interpolation from the schizont growth inhibition curves (Log of concentration versus percent inhibition) generated from each parasite-extract interaction. The definition of anti-malarial activity used is: IC_{50}≤1µM, potent (very good) activity; IC_{50} 1-10 µM, good activity; IC_{50} 10-30 µM, moderate activity; IC_{50}≥30 µM, inactive [17].

2.4 Anti-bacteria Bioassay

This was done using a modified agar well diffusion technique described by [18]. 0.2ml of standardized microbial suspension of test organism was seeded into 600ml molten Mueller Hinton agar at 40□C. The seeded agar was poured epically into sterile Petri dishes and allowed to set at room temperature.

The solidified agar was bored with sterile 8mm cork borer to create 3 wells on the agar plate about 10mm deep. The wells were filled with 0.1mg/2ml and 0.05mg/2ml of crude extract and the third well filled with distilled water as control. Similarly, a plate was prepared for the standard antibiotic (amoxicillin). The plates were incubated for 24 hrs at 37□C. Areas showing clear zone around bored holes indicates inhibition of the organisms by the extracts. The resulting inhibition zones (mm) were measured using a transparent ruler.

3. RESULTS AND DISCUSSION

Table 1.*In Vitro* antimalarial activities of methanolic stem bark extract of frankincence tree (*boswellia dalzielii*) and leaves extract of kenaf (*Hibiscus cannabinus)* against *P. falciparum*

Botanical names	Plant part	IC_{50} (µg/mL)	Antimalarial activity
Frankincense tree(B. dazielii)	stem bark	1.25 ± 0.15	Good
Kenaf (Hibiscus cannabinus)	leaf	0.16 ± 0.01	potent (very good)

IC_{50} values are presented as mean ± SEM

Table 2. Zones of Inhibition of some bacterial strains treated with methanolic stem bark extract of frankincence tree (*Boswelliadalzielii*) and leaves extract of kenaf (*Hibiscus cannabinus)*

		Zone of Inhibition (mm)				
		E. coli	***S. aureus***	***P. aeuroginosa***	***S typhi***	***S. dysentry***
Frankincense tree *(B. dazielii*)	0.1mg/2ml	+	±	+	±	±
Frankincense tree (*B. dazielii*)	0.05mg/2ml	±	±	±	±	±
kenaf (*Hibiscus cannabinus)*	0.1mg/2ml	+	±	NT	±	-
kenaf (*Hibiscus cannabinus)*	0.05mg/2ml	±	±	NT	±	-
Amoxicilin	0.05mg/ml	±	±	+	++	±

Grading of results: (−) no zone of inhibition; (±) slight inhibition (1–5 mm in diameter); (+) zone of inhibition (6–10 mm in diameter); (++), zone of inhibition (≥11 mm in diameter); NT, not tested

The results from the in vitro antimalarial testing of methanol extracts of Frankincense stem bark (*Boswellia dalzielii*) and Kenaf leaves (*Hibiscus cannabinus*) against *P. falciparum* are presented in Table 1. It revealed that Frankincense stem bark (*Boswellia dalzielii*) extract exhibited good antimalarial activity against *P. falciparum* with an IC_{50} values of 1.25 µg/ml, while Kenaf leaves (*Hibiscus cannabinus*) extract exhibited very good (potent) antimalarial activity against *P.* falciparumwith an IC_{50} values of 0.16 µg/ml. This result is in line with the work of [19] who used two leaves extract and test the anti-malarial activities on *Plasmodium falciparum* parasite.

Results for the antibacterial study reveals that at 0.1mg/2ml, methanol extracts of Frankincense stem bark inhibited *E.coli* and *P. aeuroginosa* but slightly inhibited all the tested bacteria at 0.05mg/2ml (Table 2), while kenaf leaves extract inhibited *E.coli* at 0.1mg/2ml, but slightly inhibited *S. aureus, S. typhi and E.coli* at 0.05mg/2ml (Table 2). These findings are in agreement with that of [20]. The antibacterial activities of these plants may be attributed to the presence of tannins. It was reported that several plants rich in tannins have been shown to possess antimicrobial activities against a number of microorganisms [21].

4. CONCLUSION

Methanol stem bark extract of Frankincence tree and leaves extract of kenaf have shown appreciable inhibitory activities against *P. falciparum* parasite, and have shown good inhibitory activities against *E.coli* but slight inhibitory activities against the other pathogens tested. Therefore, these plants may serve as useful sources of drugs for treatment of malaria caused by *P. falciparum parasite* and bacterial infections caused by the tested microorganisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Berman H, Ford-Gilboe M, Moutrey B, CekicS.Can JNurs Res. 2001;32(4):21-41. PMID: 11928299
2. World Health Organization, Guidelines for the treatment of Malaria, WHO Press, Geneva, Switzerland, 3rd edition; 2015.
3. Baumeister S, Winterberg M, Przyborski JM, Lingelbach K. The malaria parasite

Plasmodium falciparum: Cell biological peculiarities and nutritional consequences. *Protoplasma*. 2010;240:3–12.

4. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD. Global malaria mortality between 1980 and 2010: A systematic analysis. *Lancet. 2012;*379:413-431.
5. Ifeadike CO, Ironkwe OC, Adogu POU, Nnebu CC, Emelumadu OF, Nwabueze SA. Prevalence and pattern of bacteria and intestinal parasites among food handlers in the FCF Nigeria. Nigerian Medical Journal. 2012;53(3):166.
6. Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernans N, Van Miert S,Pieters L, Totté J, Vlietinck AJ.J Ethnopharmacol. 2004; 93(1):27-32.
7. Wong YH, WY Tan, CP Tan, K Long, KL Nyam. Cytotoxic activity of kenaf (*Hibiscus cannabinus* L.) seed extract and oil against human cancer cell lines; 2014.
8. Maganha EG, RC Halmenschlager, RM Rosa, JA Henriques, AL Ramos, J. Saffi. Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. Food Chemistry. 2010;118:1–10.
9. Durgo K, Belščak-Cvitanović A, Stančić A, Franekić J, Komes D. The bioactive potential of red raspberry (*Rubusidaeus* L.) leaves in exhibiting cytotoxic and cytoprotective activity on human laryngeal carcinoma and colon adenocarcinoma. J Med Food. 2012;15:258–268.
10. Agbor GA, JE Oben, JY Ngogang. Haematinic activity of Hibiscus cannabinus.African Journal of Biotechnology. 2005;4(8):833–837.
11. Kobaisy M, Tellez MR, Webber CL, Dayan FE, Schrader KK, Wedge DE. J Agric Food Chem. 2001;49(8):3768-71. PMID:11513663
12. Lawton BP. Hibiscus: Hardy and tropical plants for the garden, 41. Cambridge: Timber Press, Inc. Le Roux, M. 2007. South Africa: Seardel Hopes New Plant will Oust Plastics; 2004. Available:http://allafrica.com/stories/20070 2060312
13. Agbor AG, JE Oben, OB Brahim, YJ Ngogang. Toxicity Study of Hibiscus cannabinus. Journal of the Cameroon Academy of Science. 2004;4(1):27–32.
14. Banno K, Manfreda J, Walld R, Delaive K, Kryger MH. Sleep. 2006;29(10):1307-11. PMID: 17068984
15. Assefa D, Shibre T, Asher L, Fekadu A. BMC Psychiatry. 2012;29;12:239. DOI: 10.1186/1471-244X-12-239. PMID: 23272796
16. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976;193:673–675.
17. Ramalhete C, Da Cruz FP, Lopes, D., Mulhovo S, Rosario VE, Prudencio M, Ferreira M. Triterpenoids as inhibitors of erythrocytic and liver stages of Plasmodium infections. Bioorg Med Chem. 2011;19,7474–7481
18. Perez, C., Pauli, M. and Bazerque, P (1990). An antibiotic assay by the agar well diffusion method. Act. Bio. Med. Exp, 15:113-115.
19. Ferry FS, Tjtraresmi A, Runadi D, Tanti GA, Hamida A, Halimah E, Subarnas A, Budi P, Asih S. *In Vitro* Antimalarial Activities of D. caulostachyum and G. celebica Leaf Extract against *P. falciparum.* Journal of Pharmaceutical Science and Research. 2018;10(2):391-393.
20. Olukemi MA, Kandakai YT, Mawak JD. Antibacterial activity of the stem bark of *Boswellidalzielli*. Journal of Pharmacology, 2005;3(2):153-157.
21. Adebajo AO, Adewumi CO, Essein EE. Anti infective agents of higher plants. J of Inter Sym of Med Plants. 1983;5:152-158.

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