Journal of Advances in Medicine and Medical Research

29(6): 1-8, 2019; Article no.JAMMR.23097
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

In vitro Antimicrobial and Antifungal Efficacy of Ethanol Crude Stem Bark Extract of *Boswella dalzielle*

K. I. Ogbu[1*], I. C. Chukwudi[2], O. J. Ijomanta[3], E. O. Agwu[1], C. N. Chinonye[3] and K. B. Kespo[1]

[1]Federal College of Animal Health and Production Technology, Vom, Plateau State, Nigeria.
[2]Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Authors' contributions

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

Article Information

DOI: 10.9734/JAMMR/2019/v29i630093
Editor(s):
(1) Dr. Emin Umit Bagriacik, Director, Immunology Research Center Gazi University, Faculty of Medicine, Department of Immunology Besevler, Ankara-Turkey.
Reviewers:
(1) Francisco Jose Torres De Aquino, Instituto De Química, Universidade Federal De Uberlândia, Brasil.
(2) Aida Rodriguez-Garcia, Faculty of Biological Sciences, Autonomous University of Nuevo Leon, Mexico.
Complete Peer review History: http://www.sdiarticle3.com/review-history/23097

Original Research Article

Received 14 November 2015
Accepted 11 February 2016
Published 08 April 2019

ABSTRACT

The efficacy of *Boswellia dalzielii* (Frankincense) stem bark extract on some bacterial and fungal organisms was evaluated for its in-vitro antimicrobial activities against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella gallinarium*, *Aspergillus fumigatus* and *Candida albicans*. The research work was carried out in Biochemistry and Microbiology Laboratories of Federal College of Animal Health and Production Technology, National Veterinary Research Institute, Vom. Well diffusion method was carried out on nutrient agar. MIC, MBC and MFC of the test organisms were carried out on nutrient broth. The phytochemistry revealed the presence of saponin, tannin, flavonoids, cardiac glycosides, steroids, terpens and phenol in ethanol extracts while resin, alkaloid and glycosides were absent in hot water extracts. Alkaloid was also absent in ethanolic extract. The aqueous extract of the plant exhibited neither antibacterial nor antifungal effects against all test organisms used in the study while the ethanolic extract of the plant showed both antibacterial and antifungal effects on the study organisms. The

**Corresponding author: E-mail: kenike_mary@yahoo.com;*

results of this study also showed that the ethanolic extract of *Boswellia dalzielii* stem bark has activity against all bacteria species used in the study (broad spectrum activity). For gram-negative and positive bacteria, *Salmonella gallinarium* and *Staphylococcus aureus* were the most sensitive while Escherichia coli and *Streptococcus pyogenes* were the least respectively. *Candida albicans* was more sensitive than *Aspergillus fumigatus*. It was concluded that the test organisms were susceptible to ethanol extracts of the plant and may be good source of antibiotics.

Keywords: Boswellia dalzielii; antibacterial activity; antifungal activit y; plant extract.

1. INTRODUCTION

Herbal medicine is the oldest form of medicine known to mankind [1,2]. It was the mainsta y of many early civilization and still the most widely practiced form of medicine in the world today [3]. Many people in developing countries still rely on traditional healing practices and medicinal plants for their daily healthcare needs, in spite of the advancement in modern medicine [4]. Traditional medicine which is widespread throughout the world has been recognized by World Health Organization (WHO) as an essential building block of primary health care. According to reports of World Health Organization, 80% of the world's population relies mainly on traditional therapies which involve the use of plants extracts or their active substance [5]. There is abundant undocumented traditional knowledge of herbal remedies used to treat diseases in most cultures [6]. Different traditional healing practices worldwide are designed for either therapeutic or prophylactic use in human or animal diseases [7,8]. Several studies carried out in Africa, Asia, Europe, Latin America and North America show that plants are routinely used as remedy for animal diseases [9-14]. Historically, it is documented that humans utilize the same herbal preparations that they use to treat their sick animals [15]. In Nigeria, farmers are known to treat animal diseases with herbs and other traditional medical practices before the advent of orthodox medicine [16]. Traditional medical and veterinary practices remain relevant and vital in many areas in Nigeria due to absence or inadequate provision of modern medical services particularly in rural areas [17]. Ethno- veterinary medical practice is widespread among herdsmen and native livestock producers in northern Nigeria. Traditional remedies in this area include plant extracts from different plant parts [18]. Herdsmen in non-industrialized nations of the world still use medicinal plants for the treatment of livestock diseases, either due to lack of access to trained veterinarians and high cost of orthodox medicines, or the held belief that herbal remedies are more efficacious [19].

Plants are also potential sources of modern drugs. A recent survey of United Nations Commission for trade and development (UNCTAD) indicated that about 13% of drugs produced within developed countries are derived from plants [20]. Surprisingly, this large quantity of modern drugs comes from less than 15% of the plants, which have been known to have been investigated pharmacologically [21]. Therefore, since there are so many of these naturally occurring substances in plants, it is obvious that the plant kingdom offers better opportunity of providing useful medicinal compounds.

Boswellia dalzielii (family Burseraceae) commonly known as frankincense tree abounds in the Savannah regions of West Africa. *Boswellia dalzielii* is a tree that belongs to the family of Burseraceae, from the genus of Boswellia and species of *B. dalzielii*. It is about 13m high of the wooden savanna with a pale papery bark peeling and ragged characteristic. It is abundantly found in West Africa in countries such as Ghana, Niger, Ivory Coast, Upper Volta and Northern part of Nigeria, where the Hausa speaking people of Nigeria call it "Hano" or "Ararrabi". The plant is popular in the Northern part of Nigeria due to its ethno medicinal importance [22]. The plant has several medicinal uses which include the decoction of the stem bark use to treat rheumatism, septic sores, venereal diseases and gastrointestinal ailments [23,24]. Phytochemical studies of the plant revealed the absence of alkaloids [25], while saponins, tannins, flavonoids, cardiac glycosides, steroids, and terpenes were present [26,27]. Oil from the leaves of *Boswellia dalzielii* was found to exhibit significant activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* [28]. The methanolic and aqueous extracts showed both antibacterial and antifungal activities [29,27]. Recent studies of the aqueous extract of the stem bark of *Boswellia dalzielii* showed no antimicrobial activity against all the microbes used however the extract produced some anti-ulcer activity [26]. Furthermore, recent study also revealed

incensole to be part of the chemical composition of the stem-bark of Boswellia dalzielii [30].

Nowadays, the problem of antimicrobial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [31]. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro [32]. Unfortunately, development of effective antimicrobial agents has been accompanied by the emergence of drug- resistant organisms due to the irrational and over-use of antibiotics, failure to complete a course of treatment, genetic versatility of microbes and horizontal transfer of resistant genes among bacterial species. All the mentioned factors diminish the clinical effectiveness of antibiotics [33,34].

In recent time, there has been renewed interest on plants as sources of antimicrobial agents due to their use historically and the fact that a good portion of the world's population, particularly in developing countries rely on plants for the treatment of infectious and non-infectious diseases [35]. There is paucity of informations on the antifungal activity of the plantextract and also comparative study on the antifungal and antibacterial activities of the plant extracts. Therefore, the aim of this research is to determine the susceptibility of some bacterial and fungal organisms to the ethanolic and aqueous plant extracts and also to determine the minimum antibacterial and antifungal concentrations of the plant extracts.

Fig. 1.

2. MATERIALS AND METHODS

2.1 Methodology

The Standard qualitative method as described by Sofowora, [36] was used for phytochemical screening of the plant using ethanol and hot water as the solvents in the biochemistry laboratory. Well diffusion and tube dilution methods were used to determine the antimicrobial properties, minimum inhibitory concentration and minimum bactericidal concentrations of the plant extract as described by Cheesbrough, [37] while minimum fungicidal concentrations of the plant extract was determined as described by Picman et al. [38].

Molten nutrient agar was prepared and 0.2ml of the organism from the broth culture was inoculated into molten nutrient agar and was poured into plates and was left on the bench to solidify [39]

Six wells were bored using sterile borer. The extract were dispensed into each well using a sterile micropipette at different concentrations of 500mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml. Gentamycin and amphoteracin B injections were used as positive controls for both bacterial and fungal organisms respectively. The plates were incubated at 37°C for 24 hours.

2.2 Phytochemical Screening

The plant Boswellia dalzielii was obtained from National Veterinary Research Institute, Vom, Plateau State, Nigeria. It was identified as Boswellia dalzielii by Mr. Okonkwo, a plant taxonomist attached to the Federal College of Forestry, Jos. The powdered stem bark (100g) was filtered and extracted exhaustively with petroleum ether 60-80°C in a Soxhlet apparatus for 24 hrs. The marsh was air dried and re-extracted with ethanol. The aqueous and ethanolic extracts were separately evaporated under reduced pressure to give solid residues weighing 10.76 g and 21.82 g, respectively. The residues were then subjected to phytochemical screening using standard tests to show the different types of phytochemical constituents present in the stem [36,40-42].

Test for Tannins: 10 mls of distilled water was added to 0.5 g of the plant extract and was stirred and filtered. To the filtrate, a few drop of ferric chloride solution was added. Deep green

coloration was seen which indicates the presence of tannin.

Test for Alkaloids: 3 ml of 1% aqueous solution of HCL was added to 0.5g of the plant extract on steam bath. It was filtered and divided into 2 test tubes. Few drops of Meyers reagent was added to one of the test tubes and picric solution to the other. Formation of precipitate indicates the presence of alkaloids.

Test for Flavonoids: 0.5 g of the extract was dissolved in 2 mls of dilute sodium hydroxide.

A few drops of concentrated sulphuric acid were then added. A yellow solution indicates the presence of flavonoids.

Test for Glycoside: 10 mls of boiling distilled water was added to 0.5 g of the plant extract, stirred thoroughly and filtered. 2 ml of the filtrate was dispensed with a few drops of concentrated HCL. Few drops of ammonia solution were added to render it alkaline. 2ml of Benedict's reagent was added to 5 drops of filtrate solution and was boiled. A reddish brown precipitate shows the presence of glycoside.

Test for Saponins: Distilled water was added to 0.5 g of the extract inside test tube. Persistent frothing which warmed the tube was an evident for the presence of saponin.

Test for steroids and terpens: 0.1 g of the extract was dissolved in 1 ml of the chloroform. 1ml of acetic anhydride and 2 drops of concentrated H2S04 were added. A pink colour was noticed which changes to bluish green on standing, indicates the presence of steroids and terpens.

Test for cardiac glycoside: 0.1 g of the extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. 1 ml of concentrated sulphuric acid was also added. A pink colour which changes to bluish green on standing was an indication for the presence of cardiac glycoside.

2.3 Source of the Organisms

The organisms were collected from the Central Diagnostic Laboratory of the National Veterinary Research Institute Vom and the work was carried out in college Microbiology Laboratory, Federal College Animal Health and Production Technology Vom, Plateau State. These organisms were *Escherichia coli, Salmonella gallinarum, Staphylococcus aureus, Aspergillus fumigatus, Candida albicans* and *Streptococcus pyogenes*. These are the most common pathogenic organisms in the area of study.

2.4 Sensitivity Test Using Well Difussion Method

Molten nutrient agar was prepared and 0.2 ml of the organism from the broth culture was inoculated into molten nutrient agar and was pour plated and was left on the bench to solidify [43]. Six wells were created in the agar. The extract was dispensed into each well using a sterile micropipette at different concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL. Gentamycin and Miconazole were used as positive controls for both bacterial and fungal organisms respectively. The plates were incubated at 37°C for 24 hours.

2.5 Determination of Minimum Inhibitory and Minimum Bacteriocidal Concentrations

Tube dilution method was used in varying concentration of the liquid media and the extracts in test tubes at 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL were dispensed in tubes, and 0.2 ml of the standardized organism was also dispensed in the same tubes. The tubes were incubated at 37°C for 24 hours, positive control were also setup. The least concentration without growth gives the MIC. The MIC is then subcultured into a broth culture tubes that contain no extracts, the lowest concentration that result in no growth of the subcultured is noted which indicated MBC, [37].

2.6 Determination of Minimum Fungicidal Concentration

The hyphal growth inhibition test was used to determine the antifungal activity of the plant extract against fungal strains as previously described Picman et al. [38]. Briefly, dilutions of the test solutions dissolved in vehicle were added to sterile melted PDA at 45°C to give final concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml of plants extracts. The resultant solution was thoroughly mixed and approximately 15 mL was poured onto the petri plate. Plugs of 1 mm of fungal mycelium cut from the edge of actively growing colonies were inoculated in the center of

the agar plate and then incubated in a humid chamber at 25°C. Control cultures also received an equivalent amount of vehicle. Three replicates were used for each concentration. Radial growth was measured when the control colonies almost reached 1.5 cm.

3. RESULTS AND DISCUSSION

The phytochemical screening of Boswellia dalzielii plants showed that it contains saponnin, tannins, flavonoids, cardiac glycosides, steroids, terpens and phenol in both ethanol and hot water extraction while resins and glycosides were present only in ethanolic extract but absent in aqueous extract. Alkaloids was found to be absent in both hot water and ethanol extraction (Table 1). This is in accordance with Nwinyi et al. [28] and Anago et al. [44] who reported the presence of tannin among the phytochemical properties of the plant and absence of alkaloid in their aqueous and ethanolic extracts respectively.

Table 1. The phytochemical components of *B. dalzielii* stem bark extracts

S/N	Phytochemicals	Ethanol extract	Hot water
1	Saponin	++	+
2	Tannins	++	+
3	Resins	+	-
4	Alkaloids	-	-
5	Flavonoids	++	+
6	Glycosides	+	-
7	Cardiac glycosides	++	-
8	Steroids	++	+
9	Terpens	++	+
10	Phenol	+++	+

Key - = absent+= slightly present++= moderately present +++= heavily present

Hassan et al. [42] also reported the presence of tannins, saponins, flavonoids, cardiac glycosides, steroids and terpenes in methanolic etract of the plant.

The aqueous extract of the plant exhibited neither antibacterial nor antifungal effects against all test organisms used in the study (Table 2). This agreed with the report of Nwinyi et al. [28] and Taiwo et al. [43] who stated that aqueous extract of the plant has no antibacterial effect.

The ethanolic extract of the plant showed both antibacterial and antifungal effects on the study organisms (Table 3). This also agreed with Olukemi et al. [45], Nwinyi et al. [28], Noumbo et al. [46] who reported that ethanolic extract from Boswellia dalzielii have antimicrobial property. According to Campbell [47], the presence of substantial level saponnin, phenols and tannins in an extract encourage antimicrobial properties.

The results of this study showed that the ethanolic extract of the stem bark of Boswellia dalzielii has activity against some gram-positive and gram-negative bacteria (broad spectrum of activity) [29]. For gram-negative bacteria (Table 4), Salmonella gallinarium was the most sensitive while Escherichia coli was the least. For gram-positive (Table 4), Staphylococcus aureus was the most sensitive while Streptococcus pyogenes was the least. Candida albicans was more sensitive than Aspergillus fumigatus (Table 4). In general, this herb was more active with bacteria than fungi (Tables 4). This is due to the complex nature of fungal cell wall which makes entry of drugs and other chemotherapeutic agents extremely [48]. Nwinyi et al. [28] stated that presence tannin is responsible the antibacterial activity of Boswellia dalzielii ethanolic extract. According to Olukemi et al. [45], Staphylococcus aureus is very sensitive to Boswellia dalzielii ethanolic extract and also reported that gram-negative bacteria are less susceptible to the extract than gram-positive. The result of the study also correlated with the use of the stem bark of Boswellia dalzielii by herbal practitioners in Jos to treat gastroenteritis [28].

Table 2. Antimicrobial activity of the Hot water extracts

Isolates	Concentration in mg/ml						
	500	250	125	62.5	31.5	-ve	+ve
Aspergillus fumigatus	-	-	-	-	-	-	17
Candida albicans	-	-	-	-	-	-	18
Staphylococcus aureus	-	-	-	-	-	-	10
Streptococcus pyogenes	-	-	-	-	-	-	15
Escherichia coli	-	-	-	-	-	-	9
Salmonella gallinarum	-	-	-	-	-	-	12

Key: -ve = control negative (sterile water), +ve = control positive (Gentamycin and Miconazole for antibacterial and antifungal respectively)

Table 3. Antimicrobial activity of the ethanol extracts

Isolates	Concentration in mg/ml						
	500	250	125	62.5	31.5	-ve	+ve
Aspergillus fumigatus	4	4	3	3	2	-	17
Candida albicans	5	4	3	3	2	-	18
S. aureus	10	6	4	4	3	-	10
S. pyogenes	4	3	2	2	1	-	15
E. coli	9	7	5	2	2	-	9
S. gallinarium	7	6	5	3	3	-	12

Key: -ve = control negative (sterile water), +ve = control positive (Gentamycin and Miconazole for antibacterial and antifungal respectively)

Table 4. MIC, MBC AND MFC of the extracts

Isolates	MIC (mg/ml)	MBC (mg/ml)	MFC (mg/ml)
Aspergillus fumigatus	125	NA	125
Candida albicans	125	NA	-
S. gallinarium	62.5	62.5	NA
S. aureus	62.5	62.5	NA
E. coli	125	125	NA
S. pyogenes	250	250	NA

4. CONCLUSION AND RECOMMENDATIONS

From the results of the of phytochemical, and antibacterial and antifungal screening of the bark of *Boswellia dalzielii*, the study justifies the use of the bark of the plant in traditional medicine for the treatment of various diseases caused by microbes.

Root, stem and leaves extracts of Boswellia dalzielii were recommended to be tried on other microorganisms to ascertain its efficacy. More so, phytotoxicity of *Boswellia dalzielii* should be carried out to determine the possible toxicity of the pharmaco-active ingredients of the plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Eisenberg D, Davis R, Ettner S. Trends in alternative medicine use in the United States 1990-1997; Results of a follow up survey. Journal of American Medical Association. 1998;280:1569-1575.
2. Kubmarawa D, Akiniyi JA, Okorie DA. Ethnomedicinal survey of the traditional medicine of Lala people of Nigeria. International Journal of Medicinal Plant and Alternative Medicine. 2013;1(3):39-57.
3. Srivastava J, Lambert J, Victmeyer N. Medicinal Plants: An expanding role in development. World Bank Technical Paper. 1996;320.
4. Ojewole JAO. Evaluation of the analgesic, anti-inflammatory and antidiabetic properties of *Sclerocarya birrea* (A. Rich.) Hochst. Stem bark aqueous extract in mice and rats. Phytotherapy Research. 2004; 18:601-608.
5. World Health Organization. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram. 2010;28: 13-14.
6. Raul P, Pedrazo M, Manuela P. Animal health care in India. Information Centre for Low External Input in sustainable Agriculture (ILEIA) Newsletter. 1990;8(3): 22-23.
7. McCorkle CM. An introduction to ethnoveterinary research and development. Journal of Ethnobiology. 1986;129-140.
8. Mathias ME. Magic, myth and medicine. Economic Botany. 1994;48(1):3-7.
9. Ali ZA: Folk veterinary medicine in Moradabad District (Uttar Pradesh) India. Fitoterapia. 1999;70:340-347.

10. Passalacqua NG, De Fine G, Guarrera PM: Contribution to the knowledge of the veterinary science and of the ethnobotany in Calabria region (Southern Italy). Journal of Ethnobiology and Ethnomedicine. 2006; 2:52.
11. Kelbessa HE, Bekele T, Lulekal E. Ethnoveterinary medicinal plants in Bale Mountains National Park, Ethiopia. Journal of Ethnopharmacology. 2007;112:55-70.
12. Lans C, Turner N, Khan T, Brauer G, Boepple W. Ethnoveterinary medicines used for ruminants in British Columbia Canada. Journal of Ethnobiology and Ethnomedicine. 2007;3:11.
13. Alves RRN, Lima HN, Tavares Souto WMS. Plants used in animal healthcare in South and Latin America an overview. In Ethnoveterinary Botanical Medicine: Herbal medicines for animal Health. 1st edition. Edited by: Katerere RD, Luseba D. CRC Press New York USA. 2010;231-256.
14. Mathias E, McCorkle CM. Traditional livestock healers. Rev Sci Tech. 2004; 23(1):277-284.
15. Offiah NV, Makama S, Elisha IL, Makoshi MS, Gotep JG, Dawurung CJ, Oladipo OO, Lohlum AS, Shamaki D. Ethnobotanical survey of medicinal plants used in the treatment of animal diarrhoea in Plateau State, Nigeria. BMC Veterinary Research. 2011;7:36,1-9.
16. Nwude N, Ibrahim MA. Plants used in traditional veterinary medical practice in Nigeria. Journal of Veterinary Pharmacology and therapeutics. 1986;3:261-273.
17. Kudi AC, Myint SJ: Antiviral activity of some Nigerian medicinal plant extracts. Journal of Ethnopharmacology. 1999;68: 289-294.
18. Alawa JP, Jokthan GE, Akut K. Ethnoveterinary medical practice for ruminants in the subhumid zone of northern Nigeria. Preventive Veterinary Medicine. 2002;54(1):79-90.
19. Rashid MH, Tanzin R, Ghosh KC, Jahan R, Khatun MA, Rahmatullah M An ethnoveterinary survey of medicinal plants used to treat cattle diseases in Birishiri area, Netrakona district, Bangladesh. Advances in Natural and Applied Sciences. 2010;4(1):10-13.
20. UNCTAD/GATT. Markets for selected medicinal plants and their derivatives. United Nations Conference on Trade and Development, Headquarters, Geneva. 1974;1-2.
21. Farnswath RN, Bingel AS. Problems and prospects of discovering new drugs from higher plants by pharmacological screening. In Sofowora A. (ed)., Medicinal Plants and Traditional Medicine in Africa. 1993;128-161.
22. Uzama D, Gbubele JD, BMD, Kabir MG. Phytochemical, Nutritional and Antimicrobial Screening of Hexane, Ethyl Acetate and Ethanolic Extracts of Boswellia Dalzielii Leaves and Bar. American Journal of Bioscience and Bioengineering. 2015;3(5):76-79.
23. Burkill HM. Useful plants of west Tropical Africa. Second ed., Royal Botanic Gardens Kew. 1995;1:300-301.
24. Evans WC. Trease and Evans Pharmacognosy, 13th ed. ECBS/Baillere Tindal, United Kingdom. 1989;474-475.
25. Baoua M, Fayn J, Bassiere J. Preliminary phytochemical testing of some medicinal plants of Niger; Plant Med. Phytother. 1976;10:251-266.
26. Alemika TOE, Oluwole FS. An investigation of the potentials of Boswellia dalzielii and Commiphora kerstingii in the treatment of peptic ulcer. West African Journal of Pharmacology and Drug Research. 1991;9(10):91-94.
27. Adelakun EA, Finbar EAV, Agina SE, Makinde AA. Antimicrobial activity of *Boswellia dalzielii* stem bark; Fitoterapia. 1991;72(7):822-824.
28. Nwinyi FC, Binda L, Ajoku GA, Aniagu SO, Enwerem NM, Orisadipe A, Kubmarawa D, Gamaniel KS. Evaluation of the aqueous extract of *Boswellia dalzielii* stem bark for antimicrobial activities and gastrointestinal effects. African Journal of Biotechnology. 2004;3(5):284-288.
29. Ntiejumokwu S, Alemika TOE. Antimicrobial and phytochemical investigation of the stem bark of *Boswellia dalzielii*. West African Journal of Pharmacology and Drug Research. 1991; 9110:100 - 104.
30. Alemika TOE, Onawunmi GO and Olugbade TA. Isolation and characterization of incensole from *Boswellia dalzielii*. Journal of Pharmacy and Bioresources. 2004;I(I):7 -11.
31. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic

resistant bacteria. Brazil Journal of Microbiology. 2000;31:247-256.
32. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Review. 1999;12:564-582.
33. Amit R, Shailendra S. Ethnomedicinal approach in biological and chemical investigationof phytochemicals as antimicrobials, (2006). In: Anago E, Lagnika L, Gbenou J, Loko F, Moudachirou M, Sanni A. Antibacterial activity and phytochemical study of medicinal plants. Pakistan Journal of Biological sciences. 2011;14:449-455.
34. Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T, Dugbemi T. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. Afr. J. Trad. CAM. 2007;4: 185-195.
35. Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HAB, Odugbemi TO. Chemial analysis and antimicrobial activity of the essential oil of *Syzigium aromaticum* (clove). African Journal of Microbiology Research. 2008;2: 162-166.
36. Sofowora A. Medicinal Plants and Traditional Medicine in Africa, 2nd edition, Spectrum Book Limited, Ibadan, Nigeria. 1993;134-156.
37. Cheesbrough M. District laboratory practice in tropical countries part 2. Antimicrobial sensitivity testing. Published by the press syndicate of the University of Cambridge. 2000;132-140. ISBN 0521665469.
38. Picman AK, Schneider EF and Gershenzon J. Antifungal activities of sunflower terpenoids. Biochemical Systematics and Ecology. 1990;18:325–328.
39. Priya KD. Antibacteria activities and phytochemical analysis of different plant part of Nyctanthes arboratritis (Linn). Research Journal of Phyto-Chemistry. 2007;1:61-67.
40. Evans WC. Trease and Evans' Pharmacognosy, 14th Edition, W.B Saunders Company Ltd., U.K. 1996;338-542.
41. Silva GL, Lee I, Kington AD. Special problems with the extraction of plants. In: Cannell, R.J.P. (Ed.) Methods of Biotechnology, Natural Products Isolation, Humana Press Inc., Totowa, New Jersey, U.S.A. 1998;4:343-363.
42. Hassan HS, Musa AM, Usman MA, Abdulaziz M. Preliminary Phytochemical and Antispasmodic Studies of the Stem Bark of *Boswellia dalzielii*. Nigerian Journal of Pharmaceutical Sciences. 2009;8(1): 1-6.
43. Priya K, Ganjewala D. Antibacterial Activities and Phytochemical Analysis of Different Plant Parts of Nyctanthes arbor-tristis L. Research Journal of Phytochemistry. 2007;1:61-67.
44. Anago E, Lagnika L, Gbenou J, Loko F, Moudachirou M, Sanni A. Antibacterial activity and phytochemical study of medicinal plants. Pakistan Journal of Biological Sciences. 2011;14:449-455.
45. Taiwo IH, Binda L, Kim YC. Antibacterial activity of the stembark of *Boswellia dalzielii*. Journal of Ethnopharmacology. 2006;95:421-424.
46. Olukemi MA, Kandakai-Olukemi YT and Mawak JD. Antibacterial activity of the stem bark of *Boswellia dalzielii*. Journal of Pharmacy and Bioresources. 2005;2(2): 131-136.
47. Noumbo T, Serferbe SG, Yaouba A, Fovo JD, Keuete EK. Efficacy of three Local Plant Extracts as Seed Treatment on the Germination, Infection And Vigour Index of two Cotton Seed Varieties from Chad. International Journal of Applied Biology and Pharmaceutical Technology. 2015; 6(2):39-45.
48. Campbell WE. Antimicrobial Chemo-therapy in Medicinal Microbiology, 6th Edition, Bailiere-London. 1999;205-229.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/23097