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Research Article

Inhibition of *Methicillin* Resistance *Staph. aureus* (MRSA) and Fungi by *Canarium schweinfurthii* (Engl) Extracts

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Abstract: The leaf and stem bark of *Canarium schweinfurthii* were investigated. The result reveals the presence alkaloids, tannins, phenolic compounds, flavonoids, cardiac glycoside, saponins and steroids. The leaf and stem bark crude extracts showed Zone of inhibition ranging between 20 to 30 mm on Methicillin-resistant *Staphylococcus aureus*, other zone of inhibition in the range, 25 to 26 mm (Hexane), 25 to 30 mm (chloroform), 26 to 32 mm (ethylacetate) and 27 to 42 mm (methanol) were also observed. Against the test organisms*; MRSA, Vancomycin Rest Enterococci*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillcus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Shigella dysenteria*, *Candida albicans*, *Candida krusei*, *Candida tropicalis* and *Candida Stellatoidea.* The ethyl acetate fraction (leaves) recorded a lowest Minimum Inhibitory Concentration of 6.25 mg/mL against *MRSA* and most of the bacteria except *Salmonella typhi.* The stem bark, hexane, ethylacetate and chloroform fractions recorded a MIC of 6.25 mg/mL against *Staphylococcus aureus, Bacillcus subtilis, Salmonella typhiand Klebsiella pneumonia.* Both recorded a MIC of 12.5 mg/mL against *Candida.* The minimum bactericidal/fungicidal concentration (MBC/MFC) determination showed that a concentration of 12.5-50 mg/mL of the hexane, chlororform, ethyl acetate and methanol fraction could completely kill the entire test organism except *Streptococcus pyogenes and Proteus mirabilis*.
Keywords: MRSA, antifungal, antibacterial, *Canarium schweinfurthii*.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans [1]. MRSA is any strain of *Staphylococcus aureus* that has developed, through the process of natural selection, resistance to beta-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins. Strains unable to resist these antibiotics are classified as methicillin-sensitive *Staphylococcus aureus*, or MSSA. The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of *S. aureus* that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous.

The spread of multiple resistant pathogenic bacteria has been recognised by the World Organisation for Animal Health [2], Food and Agriculture Organisation (FAO) and the World Health Organization [3] as a serious global human and animal health threat. The development of bacterial resistance is neither an unexpected nor a new phenomenon. It is, however, an increasingly troublesome situation due to the frequency with which new emerging resistance phenotypes are occurring among many bacterial pathogens and even commensal organisms.

In more than 80% of developed countries, plants have been used as traditional medicine as they are the good source of compound derivation. Therefore, plants are investigated for better understanding of their properties, safety and efficacy. Many plants have been used for their antimicrobial traits, which are chiefly due to the synthesis of secondary metabolites [4] and their inhibitory effect against the growth of human pathogens. Keeping this in view, efforts are underway to search for economic and safe phytochemicals for disease control. Despite the existence of potent antibiotic and antifungal agents, resistant microbial strains are continuously appearing, suggesting the need for a permanent search and the development of new drugs [5].The investigation of anti-microbial agents of plant origin which are used in traditional medicine is thus of great importance.

Canariumschweinfurthii Engl. (Burceraceae) is a forest region plant, wide spread over all Africa, from Senegal to Democratic Republic of Congo, Sudan and Ethiopia. *C. schweinfurthii* locally known as "Bete" is a big tree up to 40 m high and 1.5 m diameter with cylindrical bole scaly bark exuding resin, with buttresses not much developed [6]. The stem bark decoction of *C. schweinfurthii* is used as a remedy for roundworms, colic, stomach pains, pains after child birth, gale, dysentery and gonorrhoea [7]. Traditionally, wild plant parts are used as a source of herbal preparation for treatment of various ailments [8].They are novel source of medicines as they have a reservoir of chemical agents with therapeutic properties [9] and plants are the cheapest and safer alternative sources of antimicrobials [10].Plant extracts have both antibacterial and antifungal properties and can be of great significance in therapeutic treatments [11].Therefore, screening and testing the efficacy of plants are undertaken to explore their antimicrobial activity [12].

The aim of this study was to evaluate phytochemical constituent of C. *schweinfurthii and* investigate their antimicrobial activities as claimed by ethno-medicinal practitioners [13].

MATERIALS AND METHODS

Collection and identification of plant materials

The *Canarium schweinfurthii Engl* leaf and bark were collected from Pankshin Jos, Plateau (9°10'N9°45'E) state in September 2012 and were identified and authenticated by the curator; Mallam Musa Mohammed, Department of Biological Sciences, Ahmadu Bello University. A voucher specimen #7232 was deposited at the herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

Extraction of plant materials

The plant parts were pulverized and the powdered. plant materials were extracted with methanol using soxhlet method. After filtration and concentration, the residues were partitioned successively in a 4.5 mm by 101 mm glass column with solvent of different polarity starting with (5x250 mL) n-hexane, chloroform, ethyl acetate and methanol, in order of increasing polarity.The different extract partitions obtained were evaporated to dryness and subjected to antibacterial and antifungal screening.

Preliminary phytochemical tests

Phytochemical properties of the sub-extracs of *C. schweinfurthii* were tested by the method of Odebiyi, and Sofowora [14], using the following chemicals and reagents: alkaloids with Mayerand Dragendoff's reagents, tannin ($FeCl_3$), saponin (frothing test), flavonoids (chip of magnesium and HCl), glycosides (NaCl, and Fehling's solutions A and B), steroids (ether ethylic, sulfuric acid and anhydride acetic), and phenols ($FeCl_3$ and $K_3Fe(CN)_6$).

Preparation of inoculum

The test organism *Methicillin Rest staph aureus,Vancomycin Rest Enterococci*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillcus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Shigella dysenteria*, *Candida albicans*, *Candida krusei*, *Candida tropicalis* and *Candida stellatoidea* were obtained from the Department of Medical Microbiology A.B.U Teaching Hospital Zaria,Kaduna State, Nigeria. Stock culture was maintained at 5 ^oC on slants of nutrient agar. Active stock culture was inoculated in fresh tubes of Muller–Hinton broth medium (MHB) and the bacteria and fungi were incubated for 24 h at 37 ^oC and 2-7 days respectively. Fifteen fresh subculture Muller–Hinton agar slants were prepared and stored in refrigerator at 5 ^oC for future requirements.

Antibacterial/fungal susceptibility test

Spectrum of antibacterial activity was studied by using the technique described by *Bauer et al*.[15].The leaf and stem bark extracts of the *C. schweinfurthii* were investigated for their antibacterial and antifungal activities against ten bacteria and four candida strains. Ciprofloxacin, Erythromycin and Fluconazole sensitivity disc were used as a positive antibacterial and antifungal controls and dimethylsulphoxide was taken as negative control. At the end of incubation, inhibition zones formed around the well were measured with transparent ruler in millimeter. These tests were performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum Inhibition Concentration of the extract was carried using broth dilution method. Mueller Hinton broth was prepared; 10mls was dispersed into test tube and was sterilized at 37 ^oC for 15 minutes, the broth was allowed to cool. Mc–farland's standard turbidity scale number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10mls was dispensed into sterile test tube and the test microbe was inoculated and incubation was made at 37 ^oC for 6 hours. Dilution of the test microbe in the normal saline was made until the turbidity marched that of the Mc-farland's scale by visual comparison at this point the test microbe has a concentration of about 1.5 x 10^8 cfu/L. Two fold serial dilution of the extract in the sterilized broth was made to obtain the concentration of 50, 25, 12.5 and 6.25mg/mL. The initial concentration was obtained by dissolving 0.5 g of the extract in 10 mls of the sterile broth. Having obtained the different concentrations of the extract in the sterile broth, 0.1mL of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at

37^{o}C for 24 hours, after which each test tube was observed for turbidity (growth) the lowest the lowest concentration of the extract in the broth which shows no turbidity was recorded as the minimum inhibition concentration

Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Minimum bactericidal concentration/fungicidal concentration were carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121 ^{o}C for 15 minutes, poured into sterile Petri dishes and was allowed to cool and solidify.

The content of the MIC in the serial dilution were then sub cultured onto the prepared medium, incubation was made at 37oC for 24 hours, after which the plates of the medium was observed for colony growth, the MBC/MFC were the plates with lowest concentration of the extract without colony growth.

RESULTS AND DISCUSSION

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [16]. Analysis of the plant extracts revealed the presence of phytochemicals (Table I) such as tannins, saponins, cardiac glycosides, steroid , phenolic compounds, flavonoids and alkaloids this observation is in line with the report of *Ngbede et al.* [17]. The presence of these could account for high antimicrobial activity demonstrated by the plant. Tanins posed physiological astringent and haemostatics properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibited the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them, they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent antioxidants [18-21]. They act as binders for treatment of diarrhoea and dysentery [22]. Tannins encompass a heterogeneous group of compounds and polymers (polyphenols). In general their non-specific activity has been ascribe to their ability to complex metal ions, scavange radicals and reduce active oxygen species and form tight complexes with a wide array of proteins and polysaccharides [23]. Hence, they have antioxidative properties.

Saponins, a special class of glycosides, have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotonic in nature and are reported to have anti-diabetic and anti-fungal properties [24-26]. Saponins are known to produce inhibitory effect on inflammation [27] and have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [28-29].

Steroid have been reported to have antibacterial properties [30], and they are very important compounds especially due to their relationship with compounds such as sex hormones [31]. Alkaloids are reported to have produce some highly efficacious antimicrobial which binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation [32]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [33]. Several workers have reported the analgesic, antispasmodic and antibacterial [28] properties of alkaloids.

Glycosides are known to lower the blood pressure according to many reports [34]. Flavonoids have shown to have a wide range of antibacterial and antifungail activities in in-vitro studies [35-36].

The presence of these phytochemicals in *Canarium schweinfurthii Engl* extracts suggests that the plant is pharmacologically active; supporting the claim by the traditional healers proving the plant to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.The present study further strengthens earlier reports by Moshi et al.,[13] that *Canarium schweinfurthii Engl* has antibacterial activities. This supports the claim of herbal healers for the use of the plant in the treatment of diarrhoea, dysentery and wounds [37-38].

Two Gram-positive, two Gram-negative bacteria and a candida were resistant against the leaf extract while a Gram-positive bacterium and two Gram-negative bacteria and two candidas showed resistant against the stem bark extracts. The leaf extracts are potentagainst Gram-positive bacteriaas aresult of the presence of the phytoalexinsuch as alkaloids (Table-1) which punctures the proteoglycan cell wall [39] which is clearly absence in all the stem bark extract. Further to support, the activity is due to the presence of alkaloid in the ethylacetate and methanol extracts is responsible for observed high zone of inhibition against Gram-positive bacteria (25 ± 0.2 mm - 32 ± 0.5 mm)against Gram-positive bacteria (25 ± 0.2 mm -32 ± 0.5 mm) as compared with other leaf extractsand stem bark extracts(Fig-1 & 3).The stem bark showed a comparable antibacterial activity against Gram-negative bacteria as observed in the leaf extracts (Table I)but lower anti-candidaactivity (Fig -2 & 4)which is in agreement with Cowan [31] report on the mechanism of action of alkaloids.

The chloroform and ethyl acetate extracts of the stem bark gave a high zone of inhibition of 30 ± 0.5

mm and 26±0.2 mm (Fig-3) against *Bacillcus subtilis* and *Candida albicans* respectively, since a hydrolase activity of the alkaloid was not required because of the cell walls are made up of an inner layer chitin and an outer layer of mannoproteins which differ in hydrophobicity [40]. The positive controls ciprofloxacin (40±0.2 mm), erythromycin (37±0.5 mm) and fluconazole (35±0.2 mm) (Fig-2) showed higher antibacterial and antifungal activities on *Bacillcu ssubtilis*, *Candida albicans* and *Candida stellatoidea*. Mogana , et. al., [40], also reported that the highest sensitivity obtained was with the ethanol extract of leafs which inhibited the growth of Gram-positive *Staphylococcus aureus*, Bacillus cereus, methicillin-resistant *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* with an inhibition zone of 14mm, 13mm, 13mm and 15mm respectively.

The minimum inhibitory concentration of 6.25 mg/mL was recorded for the ethyl acetate leaf extract against Methicillin Rest staph aureus, *Staphylococcus aureus* ,Bacillcus subtilis, Escherichia coli, *Klebsiella pneumonia* and Shigella dysenteria. The chloroform, ethyl acetate and methanol bark extract indicated a minimum inhibitory concentration of 6.25 mg/mL in against bacteria only (Table-3).This confirms the earlier assertion of the potency of the leaf against Gram-positive, negative bacteria and candida. The work of Mogana, et. al., [40] on the ethanol extract of leafs and barks and hexane extract of barks of *Canarium patentinervium* Miq, exhibited similar significant antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus*, Bacillus cereus, methicillin-resistant *Staphylococcus aureus* and Gram-negative-*Pseudomonas aeruginosa*.

Minimum bactericidal and fungicidal concentration of the chloroform extract of the leaf was 12.5 mg/mL against the entire test organism while the stem bark ethyl acetate extract showed bactericidal activity against *Staphylococcus aureus* and *Bacillcus subtilis* at the same concentration and fungicidal activity at 25 mg/mL (Table-4).Most importantly is the potency of all the leaf extract against Methicillin Resistance *Staphylococcus aureus* and *Salmonella typhi* which showed resistance against narrow spectrum erythromycin control and the stem bark extracts sensitive against the ciprofloxacin resistance Methicillin Resistance *Staphylococcus aureus*, *Staphylococcus aureus* and *Klebsiella pneumonia* (Fig-4). The methanol extract was more potent than ciprofloxacin and erythromycin antibiotic against most the bacteria.

Table-1: Phytochemical screening

	leaf extracts				bark extracts			
Phytochemical	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
Alkaloid	-	-	+	+	-	-	-	-
Cardiac glycoside	-	-	+	+		-	+	+
Saponins	-	-	-	+	-	-	+	+
steroids	+	+	+	+	+	+	-	-
Phenolic compounds	-	-	-	+	-	-	+	+
Flavonoids	-	+	+	+	+	+	+	+
Tannins	-	-	+	-	-	-	+	+

Key: [+]-Presence, [-]- Absence, I= Hexane fraction, I= Chloroform fraction, III= Ethyl acetate fraction and IV-=Methanol fraction

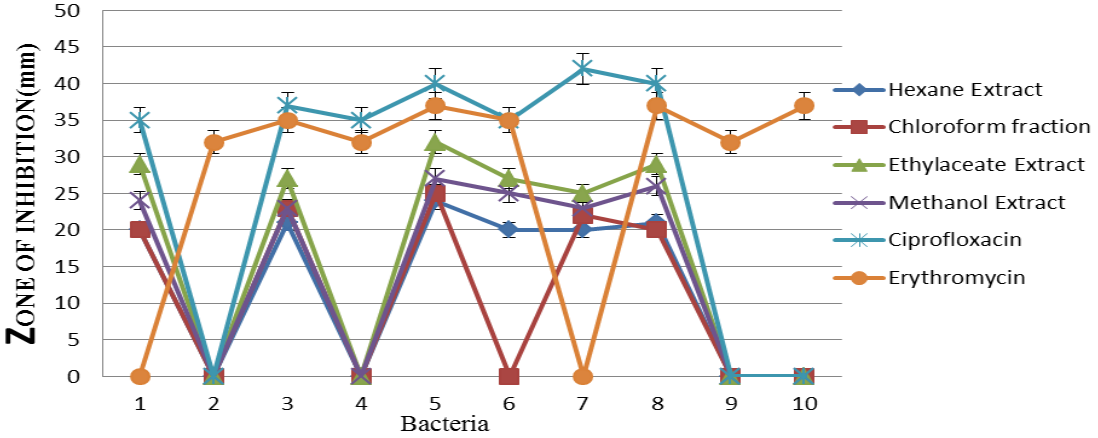

Fig-1: Zone of inhibition (mm) of the *C.schweinfurthii leaf* extracts against the test bacteria

Key:*1-Methicillin rest staph aureus, 2-Vancomycin rest Enterococci, 3-Staphylococcus aureus,4-Streptococcus pyogenes,5-Bacillcus subtilis, 6-Escherichia coli, 7-Salmonella typhi,8-Klebsiella pneumonia, 9-Pseudomonas aeruginosa, 10-Proteus mirabilis and 11-Shigella dysenteria*

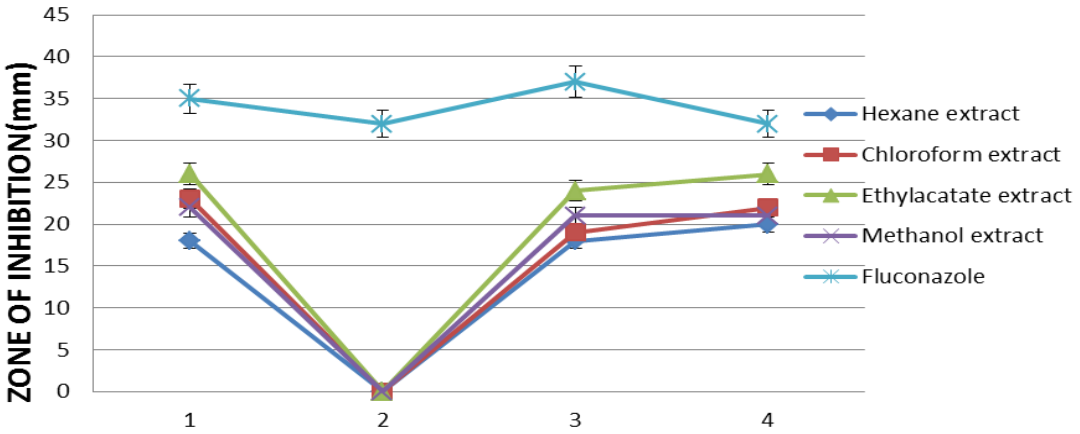

Fig-2: Zone of inhibition (mm) of the *Canariumschweinfurthiileaf*extracts against the test fungi Fungi
Key: *1-Candidaalbicans, 2-Candida krusei, 3-Candida tropicalis and 4-Candida Stellatoidea*

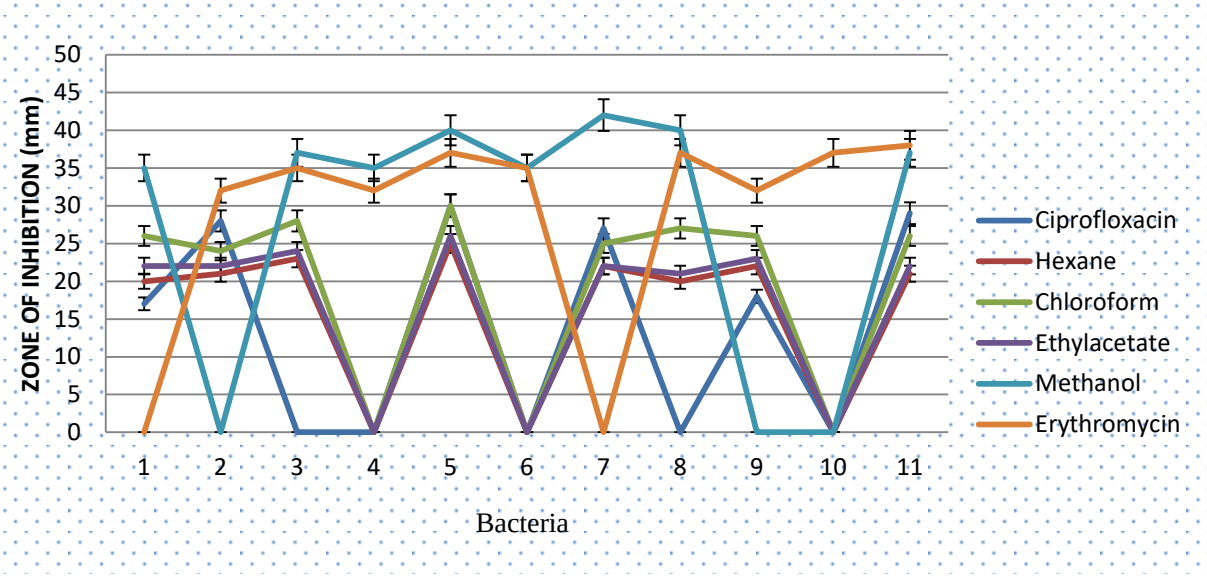

Fig-3: Zone of inhibition (mm) of the *Canariumschweinfurthii* bark extracts against the test bacteria
Key: *1-Methicillin rest staph aureus, 2-Vancomycin rest Enterococci, 3-Staphylococcus aureus, 4-Streptococcus pyogenes,5-Bacillcus subtilis, 6-Escherichia coli, 7-Salmonella typhi,8-Klebsiella pneumonia, 9-Pseudomonas aeruginosa, 10-Proteus mirabilis and 11-Shigella dysenteria*

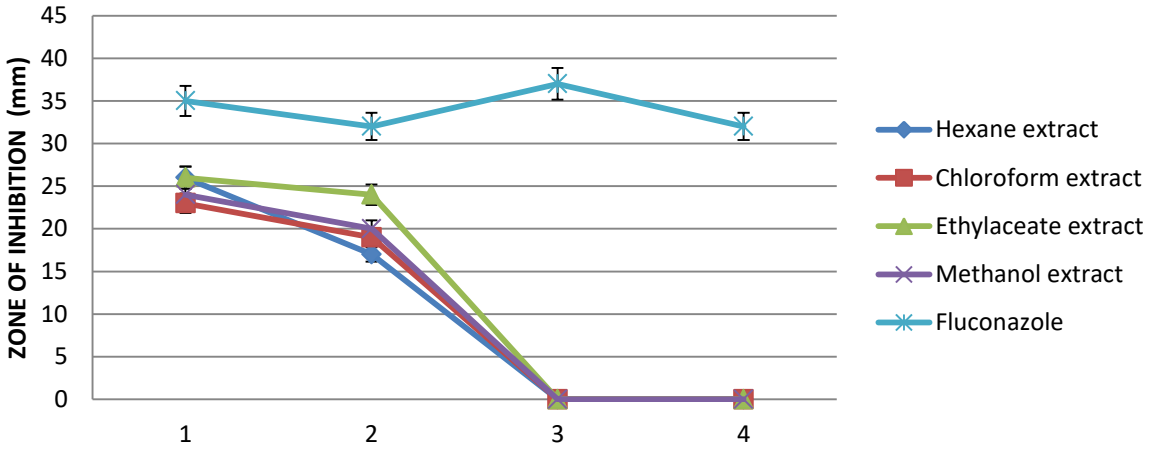

Fig-4: Zone of inhibition (mm) of the *canariumschweinfurthii bark* extracts against the test fungi
Key: 1-*Candida albicans,2-Candida krusei, 3-Candida tropicalis and 4-Candida Stellatoidea*

Table-3: Minimum Inhibitory Concentration (mg/ml) of Extracts against the Test Microorganism

S/N	TEST ORGANISMS	Leaf extracts				Stem bark extracts			
		I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
1	*Methicillin Rest staph aureus*	25	12.5	**6.25**	12.5	12.5	12.5	12.5	12.5
2	*Vancomycin Rest Enterococci*	-	-	-	-	12.5	12.5	12.5	12.5
3	*Staphylococcus aureus*	25	12.5	**6.25**	12.5	**6.25**	**6.25**	**6.25**	12.5
4	*Streptococcus pyogenes*	-	-	-	-	-	-	-	-
5	*Bacillcus subtilis*	25	12.5	**6.25**	–	**6.25**	**6.25**	**6.25**	12.5
6	*Escherichia coli*	25	12.5	**6.25**	12.5				
7	*Salmonella typhi*	25	12.5	12.5	12.5	**6.25**	12.5	**12.5**	12.5
8	*Klebsiella pneumoniae*	25	12.5	**6.25**	12.5	**6.25**	**6.25**	**6.25**	12.5
9	*Pseudomonas aeruginosa*	-	-	-	-	12.5	12.5	12.5	12.5
10	*Proteus mirabilis*	-	-	-	-	-	-	-	-
11	*Shigella dysenteria*	25	12.5	**6.25**	12.5	12.5	12.5	12.5	12.5
12	*Candida albicans*	50	12.5	12.5	12.5	12.5	12.5	12.5	12.5
13	*Candida krusei*	-	-	-	-	12.5	12.5	12.5	12.5
14	*Candida tropicalis*	50	12.5	12.5	12.5	-	-	-	-
15	*Candida Stellatoidea*	25	12.5	12.5	12.5	-	-	-	-

Key: I-Hexane extract, II- Chloroform extract, III- Ethyl acetate extract and IV- Methanol extract

Table-4: Minimum bactericidal (MBC)/fungicidal concentration (MFC) of the *C. schweinfurthii* extracts against the test microbes (mg/mL)

S/N	TEST ORGANISMS	Leaf extracts				Stem bark extracts			
		I	II	III	IV	I	II	III	IV
1	*Methicillin Rest staph aureus*	50	**12.5**	**12.5**	25	50	50	25	50
2	*Vancomycin Rest Enterococci*					50	50	25	50
3	*Staphylococcus aureus*	50	**12.5**	25	25	25	25	**12.5**	25
4	*Streptococcus pyogenes*								
5	*Bacillcussubtilis*	25	**12.5**	**12.5**	25	50	25	**12.5**	25
6	*Escherichia coli*	50	**12.5**	25	25				
7	*Salmonella typhi*	50	**12.5**	25	25	50	50	25	50
8	*Klebsiellapneumoniae*	50	**12.5**	**12.5**	25	25	50	25	50
9	*Pseudomonas aeruginosa*					50	25	25	25
10	*Proteus mirabilis*								
11	*Shigelladysenteria*	50	**12.5**	**12.5**	25	50	50	25	50
12	*Candida albicans*	50	**12.5**	25	50	25	25	25	25
13	*Candida krusei*					50	50	25	50
14	*Candida tropicalis*	50	**12.5**	25	50				
15	*Candida Stellatoidea*	50	**12.5**	25	50				

Key: I-Hexane extract, II- Chloroform extract, III- Ethyl acetate extract and IV- Methanol extract

CONCLUSION

The results showed that the leaf extract of *Canarium schweinfurthii* Engl is bioactive on both bacterial and fungal which can be the source of a novel antibacterial and antifungal drug, while the stem bark can be explored for its potentials against Methicillin-resistant *Staphylococcus aureus* and other gram-negative bacteria. It is suggested that the leaves of the plant is evaluated for toxicity and for possible herbal drug formulation, since the leaves is renewable.

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