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Antibacterial Activity of Oxyanthus Speciosus Dc. (Rubiaceae)

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ABSTRACT: The resistance of microorganisms to antimicrobial necessitated new drugs and lead for potent antimicrobial. The study investigates the antibacterial potential of Oxyanthusspeciosus (Rubiaceae), an evergreen shrub spanning across the Tropical and Southern Africa. The leaf was collected, dried, pulverized and screened for phytochemicals. The powder was extracted with 80% ethanol and further fractionated on vacuum liquid chromatography (VLC). The extract was eluted with a gradient mixture of N-hexane, ethyl acetate and ethanol. Extract and fractions were evaluated on clinical isolates of Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi and Staphylococcus aureus. Phytochemical screening unveiled the presence of alkaloids, flavonoids, saponins, tannins, triterpenoids, and cardiac glycosides and a yield of 0.61%from the 80% ethanol extract. Seven fractions were obtained from VLC. The extract was active against all the organism and only four fractions were selectively active against the test organisms. This result highlights the potential of the plant in treatment of pulmonary, gastrointestinal, wound and soft tissue infections occasioned by these organisms. It also lends credence to the use of Oxyanthusspeciosus leaves by indigenous people in treating infectious diseases.

KEYWORDS: Oxyanthusspeciosus, Vacuum Liquid Chromatography, Bacteria, Microorganisms

I. INTRODUCTION

Antibacterial are substances that either kill bacteria or inhibit their growth. They can be classified as bactericidal (killing bacteria) or bacteriostatic (inhibiting bacterial growth). Antibacterial include antibiotics, which are used in clinical settings to treat infections [1]. The overuse and misuse of antibacterial in healthcare and agriculture have led to bacteria evolving resistance, making infections harder to treat and increasing the risk of disease spread and death [2]. The use of antibiotics in animals contributes to the emergence of antibiotic resistance that can affect human health. Consequently, Improper disposal and excretion of antibacterial pollute water and soil, encouraging the evolution and transfer of resistance genes in the environment [3]. Unfortunately, there is a diminishing pipeline of new antibacterial drugs, largely because of the poor economic returns in this area of pharmaceutical research [4]. Nevertheless, antimicrobial challenges to man will have to be resolved through research of new potent drug candidates. This therefore necessitate the investigation in to Oxyanthusspeciosus.

Oxyanthusspeciosus DC. Belongs to Rubiaceae family. It is a multi-purpose plant species with significant indigenous uses across various applications in medicine and industry. It is a slow-growing evergreen shrub or tree growing up to 16 metres tall [5]. It is a shrub or small tree growing primarily in the wet tropical biome, ranging from Tropical to Southern Africa [6]. The leaves are used for treating pulmonary troubles, stomach troubles and febrifuges. The barks are used for skeletal corrections in humans. The leaves and roots are used as antidote for snake bite and arrow poison. Aro et al. reported the antimycobaterium of extract and fractions of O. speciosus against non-pathogenic (Mycobacterium aurum, M. fortuitum, and M. smegmatis) and the pathogenic strain M. tuberculosis [7]. Cytotoxic activity was also reported for the rotundic acid that was isolated from O. speciosus. The root alkaloid was found to have a potent anti-inflammatory activity in a protein denaturation assay with an IC50 value of 1.930 ± 0.9123 µg/mL.Lutin and rotindic acid were isolated by Aro et al. [7] A pyrrolidinyl-piperazine alkaloid derivative named 2-(7'-methylhexyloxy)furan-5'-yl)-6-(pyrrolidin-7-yl)piperazine (1), along with five known compounds were isolated from methanolic extract of the roots of O. speciosus [8]. Methanolic extract of the leaves characterized with Gas chromatography revealed 2 members of alkaloid, six members of flavonoid, ten members of phenolics and two terpenes [9]. Base on the reported use of the O. speciosus in pulmonary disorder and scarcity of scientific justification and proof of efficacy if

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present, this study investigates the antibacterial activity of the O. speciosus on five bacteria.

II. MATERIALS AND METHODS

2.1 Plant Material

The leaves of Oxyanthusspeciosuswas collected in Ibadan, Oyo State, Nigeria. The plant was authenticated and deposited in the herbarium of the Department of Pharmacognosy and Phytotherapy, University of Port-Harcourt with a voucher specimen number UPHR O652. The leaves were air-dried under room temperature. The dried leaves were ground to fine powder using a blender and stored in air-tight container until further use.

2.1 Phytochemical screening

Phytochemical screening was carried out on the dried powdered sample and extract to detect the presence and absence secondary metabolites according to prescribed methods [10], [11].

Table 1. Phytochemical screening result of leaf of Oxyanthusspeciosus

S/No	Phytochemical Test	Crude sample(powder)
1	**Alkaloids:**	+
2	**Flavonoids**	+
3	**Anthraquinone**	-
4	**Saponin**	+
5	**Tannins**	+
6	**Carbohydrate**	+
7	**Triterpenoids**	+
8	**Steroids**	+
9	**Cardiac Glycosides:**	+
10	**Cyanogenic Glycosides**	-

Key: + means Present; - means Absent

2.3 Extraction and Fractionation

About 1000g of the powdered leaves of Oxyanthusspeciosuswas macerated with 80% Ethanol for 72 hours with intermittent shaking. Extract was filtered and replaced with solvent every 24 hours. The combined extract was concentrated in vacuo in a rotary evaporator at 40ºC the dried extracts were weighed and subjected to vacuum liquid chromatography (VLC) separation. The glass column of the VLC was wet packed with a slurry of silica gel in n-hexane. The crude extract was loaded onto the silica gel column, The extract was eluted with a gradient mixture of N-hexane, ethylacetae and ethanol(N-Hexane, N-Hexane: Ethyl acetate (70:30), N-Hexane: Ethyl acetate (30:70,), Ethyl acetate (100%), Ethyl acetate: Ethanol (70:30), Ethyl acetate: Ethanol (30:70) and Ethanol (100%)) ware used as mobile phase. The eluates were stored inside the laboratory fume cupboard to concentrate in to a dried extract for further antibacterial tests.

2.4 Collection of Bacterial Isolates

Stock cultures of clinical isolates of bacteria which served as test organisms were obtained from the Emadavistic medical and research laboratory, Port Harcourt. These organisms were Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi and Staphylococcus aureus.

2.5 Antibacterial Susceptibility Test of Oxyanthusspeciosuson clinical isolates Using Agar Diffusion Method

Bauer-Kirby Agar Diffusion method with slight modification was used to carry out this experiment. Sterile Petri dishes were labelled in duplicates for the various test organisms. A 0.1ml of each of the microorganisms was added aseptically to the prepared Mueller Hinton Agar pour in the universal bottle and properly mixed. The mixture was then

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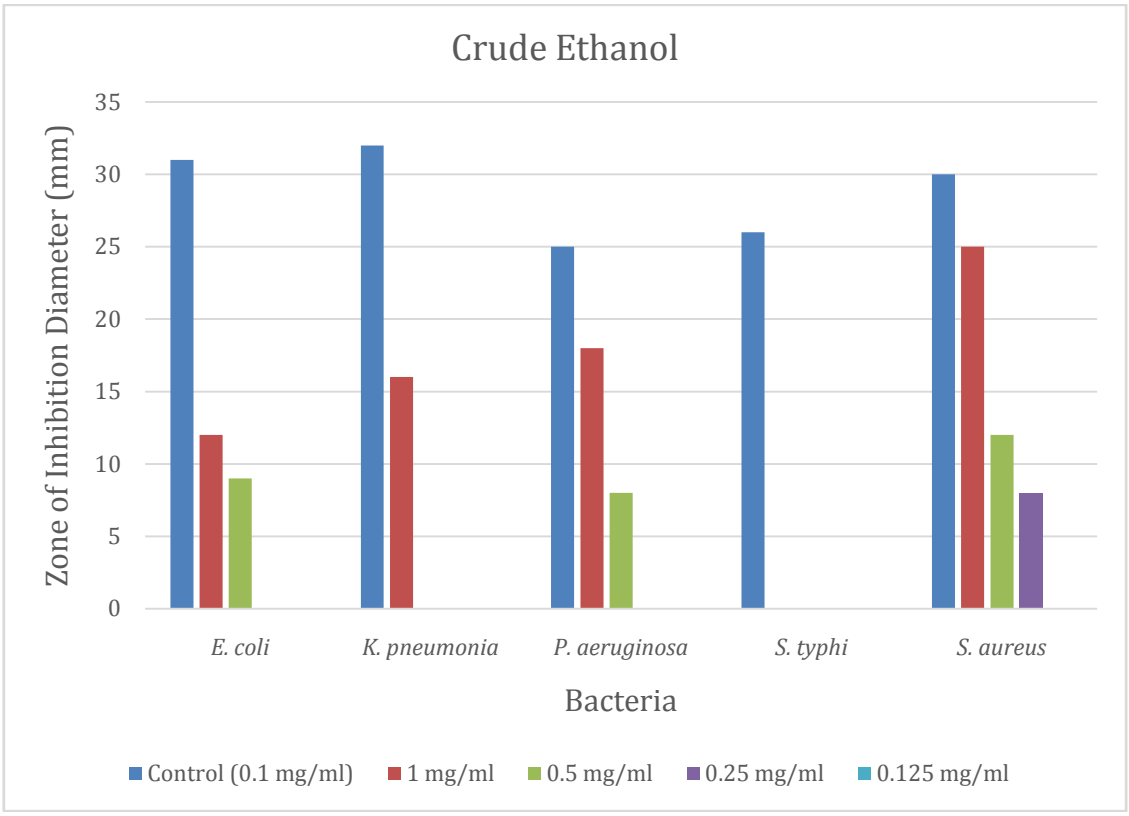

Figure 1. Effect of crude Ethanol extract of O. speciosus on Bacteria

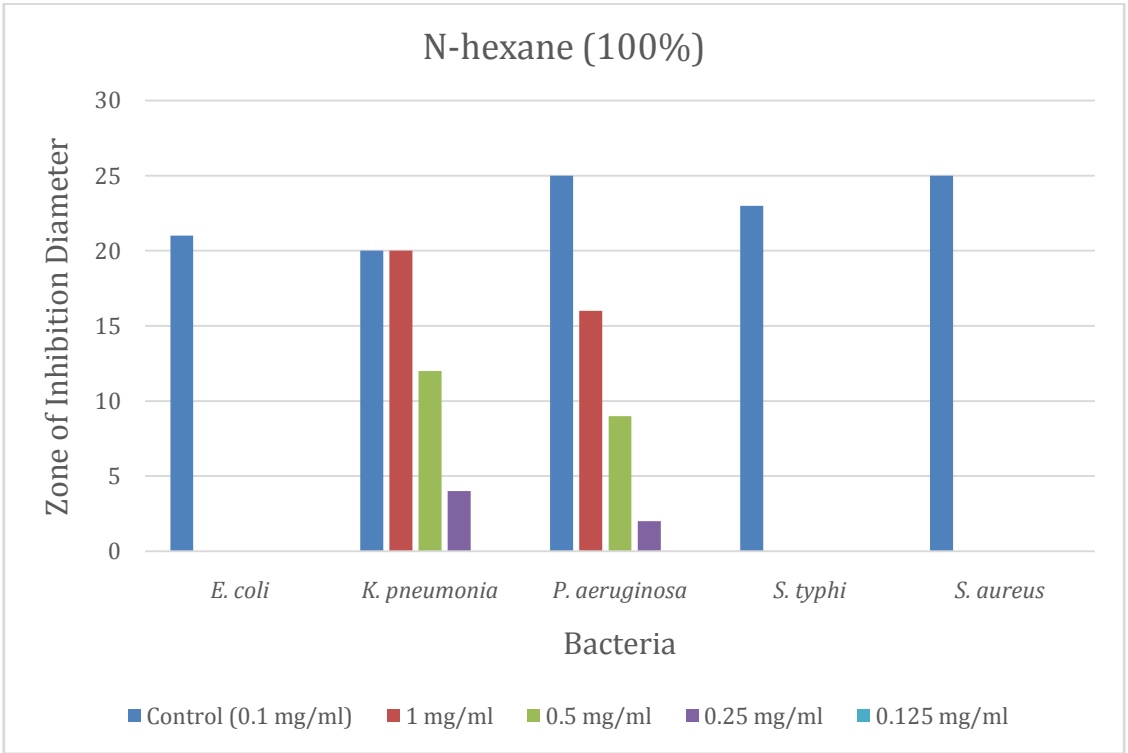

Figure 2. Effect of N-hexan(100%) fraction of O. speciosus on Bacteria

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poured into the corresponding Petri dish and allowed to solidify on the workbench. After the agar had solidified on the Petri dish, a sterile cork borer was used to remove a disc of agar from the agar layer in order to produce a well in each agar plate. The Wells were labelled with four stock concentrations of the crude Ethanol extract, and each fraction (VLC eluates) obtained from Oxyanthusspeciosus. Using a sterile Pasteur's pipette, 0.1ml of the different stock concentrations prepared from the crude extract and the fractions were carefully dropped into the well and then left on the work bench for 15 minutes for proper diffusion. The plates (Petri dishes) were incubated at 37° C for 24 hours. The diameter of the resulting Zones of inhibition were measured in millimeter (mm) through the base of the plate using a meter rule [12].

2.6 Determination of Minimum Inhibitory Concentration (MIC) of Extracts on Various Micro-organisms

Agar layers containing susceptible microorganisms from the above test for activity of extracts were prepared in various Petri dishes and labeled. Duplicate plates (dishes) were made for each organism. Using sterile cork borers, 5 wells were made and labeled for various concentrations of the serial dilutions made. A drop of each of the respective concentrations of the extracts and standard antibiotics was added to each well labeled for each extract and kept for 15 min to allow diffusion of the extracts into the agar layer. The plates were incubated at 37 °C for 24 h. The diameter of any resulting zone of inhibition was measured in millimeter. MICs of extracts on various microorganisms were obtained equal to the concentrations of the extracts which on further dilution produced no zone of inhibition (activity) on the microorganisms [12].

III. RESULTS AND DISCUSSION

Antibiotic resistance has formed the bane of antimicrobial treatment and has made the researcher in the area to be astute in drug discovery especially from the plant kingdom [13]. The extraction process resulted in a low yield of 0.61%, indicating a modest quantity of extractable constituents from the aqueous plant material [14]. This indicates that the leaves probably contain mostly of non-polar constituents. However, the phytochemical screening of the crude extract identified several bioactive compounds, such as alkaloids, flavonoids, saponins, and phenolics. The presence of these compounds supports the extract's potential for medicinal applications, particularly due to their known roles in antimicrobial activity. Interestingly, alkaloids were confirmed in both samples through tests like Dragendorff and Hager's, while flavonoids were also consistently detected, although the $AlCl_3$ test returned a negative result [10].

The observed antibacterial activity of the crude extract against all the organisms except S. typhi in Figure 1, prompted further separation of the crude extract on VLC. Consequently, out of the seven (7) fractions obtained from VLC, three fractions (N-hexane: ethyl acetate (70:30), ethyl acetate (100%), and ethyl acetate: ethanol (30:70)) were completely inactive against all the organisms. The N-hexane 100% fraction showed activity against K. pneumonia and P. aeruginosa with MIC of 0.25 mg/ml each as presented in Figure 2. N-hexane: ethyl acetate (30:70) fraction in Figure 3 showed activity against E. coli and S. typhi with MIC of 0.25 and 1 mg/ml respectively. The fraction in Figure 4 exhibited a high MIC (1 mg/ml) against P. aeruginosa and S. aureus while inactive in others tested organisms. Similarly, the fraction in figure 5 was only active against S. aureus with an MIC of 0.25 mg/ ml. The antibacterial activity observed across different solvent fractions highlights the role of solvent polarity in extracting specific phytochemicals and their effectiveness against the tested microorganisms. Obviously, the active part O. speciosus leaf extract lies in the extremely end of nonpolar and polar part of the extract [15]. The observed activity could also be supported by the phytochemical constituents documented on the phytochemical screening result in Table 1 as they are metabolites that are very polar and nonpolar.

The results confirm that phytochemical composition and solvent polarity significantly influence antibacterial activity, with MIC values providing a quantitative measure of potency. Furthermore, isolation and identification of the compounds in the fraction would be beneficial in understanding and optimizing their potential for medicinal use.

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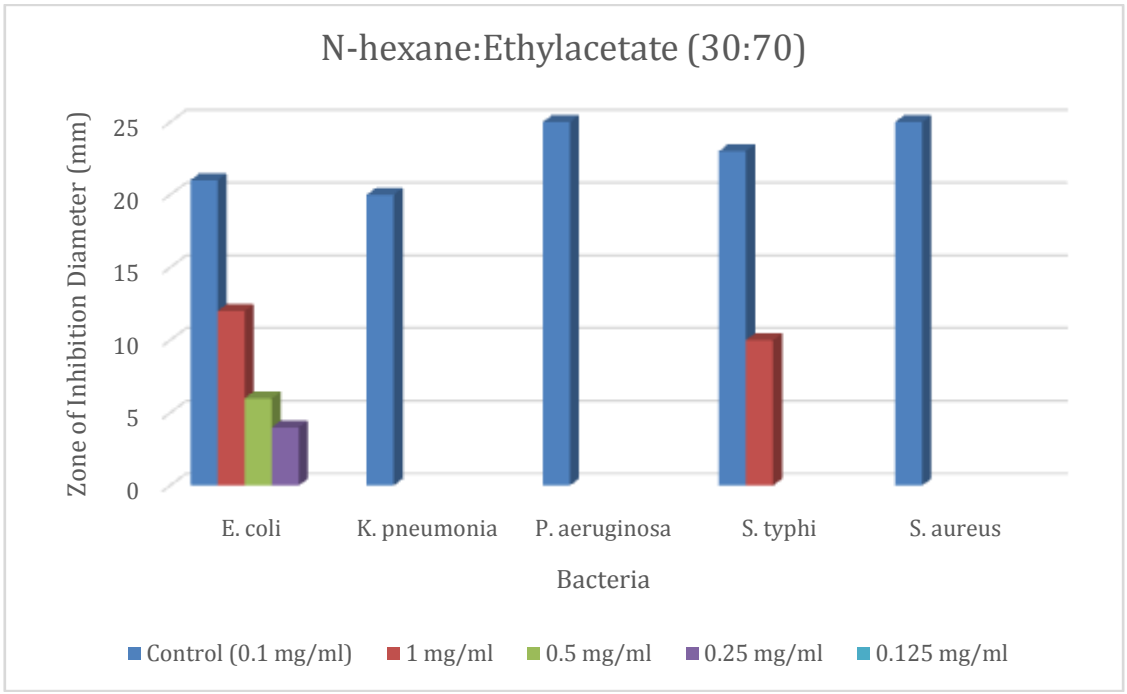

Figure 3. Effect of N-hexane:Ethyl actate (30:70) fraction of O. speciosus on Bacteria

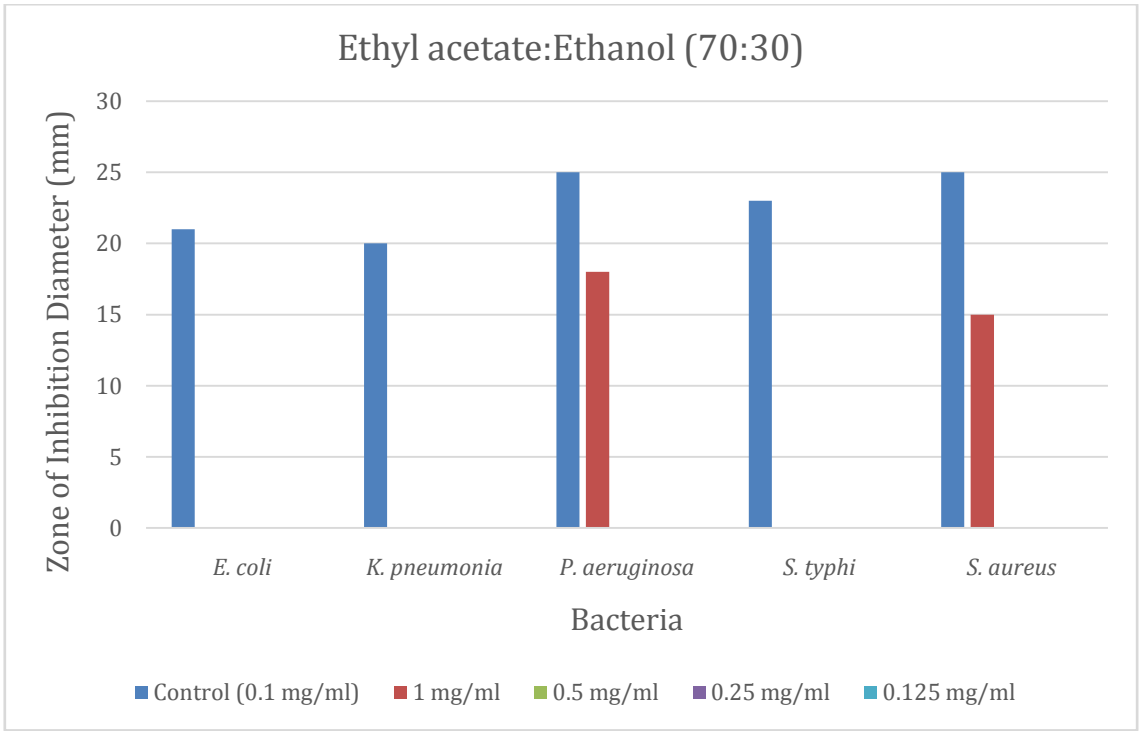

Figure 4. Effect of Ethyl actate:Ethanol (70:30) fraction of O. speciosus on Bacteria

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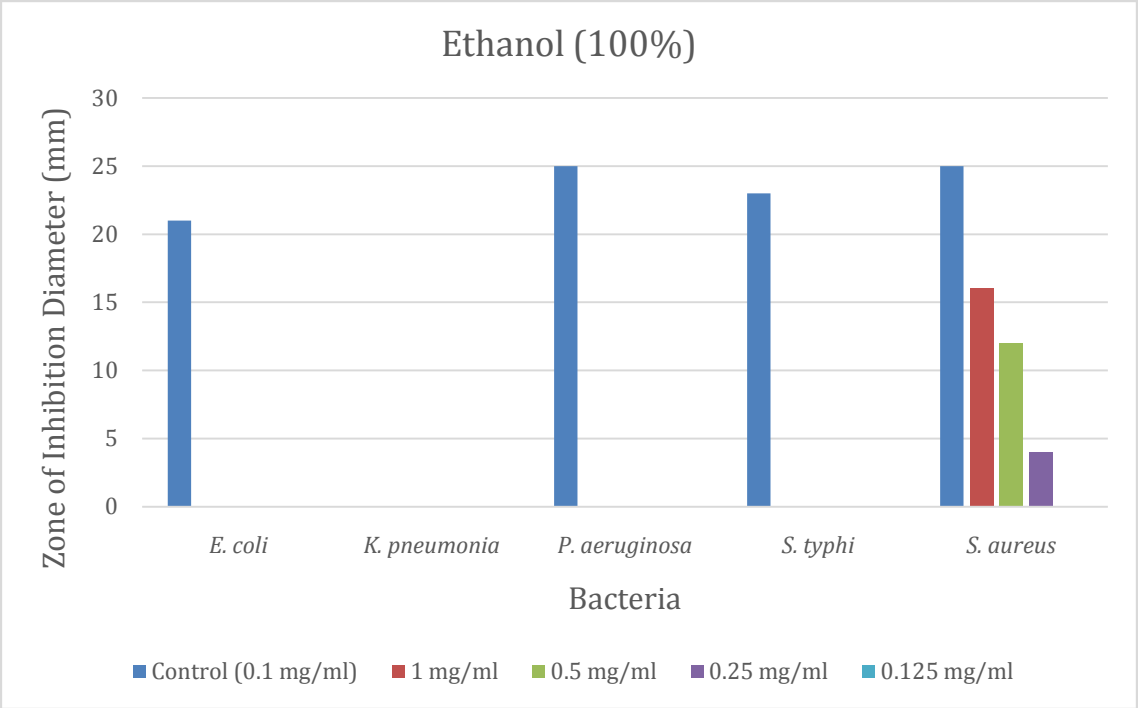

Figure 5. Effect of Ethanol (100%) fraction of O. speciosus on Bacteria

IV. CONCLUSION

The study confirms the potential of the aqueous ethanol extract of Oxyanthusspeciosus leaves in treating pulmonary conditions in folk medicine. It further shows the fractions that are active against the test organisms with specificity for further isolation, characterization and optimization of the lead constituent.

Conflict of Interest

The authors declare no conflict of interest

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