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Research paper

Phytochemistry and antibacterial activity of the methanol, petroleum ether and aqueous extracts of the leaves, stem bark and roots of bobgunnia fistuloides

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Abstract

Ethno-medicinally, *Bobgunnia fistuloides* parts (leaves, bark and roots) have been used by the Nupe communities in Niger State, Nigeria to treat various diseases for decades. The phytochemical and antibacterial activity of the methanol, petroleum ether and aqueous extracts of the leaves, roots and stem bark of *Bobgunnia fistuloides* were investigated using standard methods. The leaves, roots and stem bark of *Bobgunnia fistuloides* plant were collected from Binin village, Gbako Local Government Area (LGA) in Niger State Nigeria. The antibacterial activities of the plant extracts were determined using 5 pathogenic bacteria (*Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoneae, Salmonella typhi* and *Escherichia coli*). The result of the phytochemicals, in mg/100 g, revealed the values of 479.73±0.20, 465.98±0.14, 482.77±0.17; 1.71±0.06, 25.14±0.02, 23.90±0.03; 29.09±0.20, 36.77±0.06, 24.79±0.14; 22.40±0.02, 25.14±0.02, 23.90±0.03; 577.29±0.24, 586.52±0.11, 230.19±0.03 respectively for the phenols, flavonoids, alkaloids, tannins and saponnins contents of the roots; leaves and stem bark of the plant. In this study, the saponnins and phenol contents of the plant parts were relatively higher than the other parameters investigated. The leaves were found to contain more active principles, followed by the roots and stem bark. Based on the antibacterial activities, the result revealed that the respective zones of inhibition of the growths of the test microorganisms, with the exception of the aqueous leaves extract (against *Staphylococci aureus*; 25.00±1.00 mm) recorded highest values for the petroleum ether extracts of the stem bark (32.67±0.58, 33.00±1.00, 31,67±0.58 and 35.10±1.00 mm for *E. coli, B. subtilis, S. typhii* and *K. pneumonea* respectively). Statistically, *Klebsiella pneumoneae* (17.13±0.00 mm) recorded the highest average zones of inhibition based on the susceptibility of the pathogens to the extracts, followed by *Salmonella typhi* (16.92±1.00mm). The least zone of inhibition (12.00±0.50 mm) was recorded for *B. subtilis* using the stem bark aqueous extract of the plant while the highest value was recorded for the petroleum stem bark extract against *K. pneumonea* (35.00±1.00 mm). The MIC and MBC of the extracts ranged from 12.50 to 100.00 mg/cm3 respectively. The phytochemical components and antibacterial activities of the plant extracts in this study support the therapeutic value and the ethno-medicinal applications of this plant by the Nupe communities of Niger State Nigeria.

Keywords: *Antibacterial; Bioactive Components; Bobgunnia fistuloides; Inhibitors; Phytochemicals.*

1. Introduction

Plants are known to contain bioactive compounds such as tannins, phenols, glycosides, alkaloids, saponins, flavonoids, steroids and terpenes. Man has been using plant based medicines in the form of crude drugs such as teas, tinctures and other herbal concoctions since centuries. Some claim that it has less side effect, safe and less toxic compared to orthodox medicine (Misra, 2009). The continuous usage of plant based materials could be attributed to the functionality or the potency of bioactive compounds present in it (Misra, 2009).

Crude drugs used by man can be obtained from various parts of plants namely the bark, leaves, flowers, roots and seeds. The presence of bioactive components in plant parts promotes its therapeutic properties (Cragg and David, 2001). The bioactive components present in plant possess biological properties such as anti-carcinogen, anti-inflammatory, anti-apoptosis, anti-aging, anti-arthrosclerosis, as well as prevention and protection of cardiovascular diseases and the prevention of angiogenesis (Han *et al.*, 2007). Previous studies indicate that bioactive components present in plants part plays several physiological roles. For example, flavonoids are the rich source of colouring component in plants and it helps in combating oxidative stress and serves as growth regulators (Shuruq *et al.*, 2017). Flavonoids have been reported as cancer chemo-preventive agents. For example, consuming apples and onions, which are the main sources of flavonolquercetin, can reduce the incidence of breast, prostate, stomach, and lung cancer (Shuruq *et al.*, 2017). Report indicates that bioactive components in plant display antimicrobial properties to pathogens (Han *et al.*, 2007).

Antimicrobial resistance is a tremendous global problem; these microorganisms show multiple resistances to a frequently used orthodox medicine, decreasing the drugs efficiency through multiple resistance or viral replication which has led great research into improving this challenging situation and finding new antimicrobials from different sources especially from medicinal plants (Panda and Dutta, 2011).

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According to Sofowora (2008) he stated that *S. obtusifolia,* and *S. alata* are used to treat skin infections caused by fungi while S. hirsuta are used medicinally for treating herpes caused by virus. Research shows that fungi and bacteria have displayed a high resistance to commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Panda and Dutta, 2011). Examples of these antimicrobial organisms are *Staphylococcus aureus, Escherichia Coli, Candida albicans, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus cereus* and many others. Plant materials contribute to human health care by which 80% of human population depends on traditional medicine to improve their health (Sofowora, 2008). Traditional medicine is cheaper and safer with minimum side effects (Panda and Dutta, 2011).

Bobgunnia fistuloides, also called Pao rosa in English is a leguminous plant belonging to the family of leguminosae-fabaceae. Report shows that the plant is found in Ghana, Gabon, Congo, Ivory coast, Cameroon and .Nigeria, in Cameroon the local name is Non Nsas, Ivory Coast is Boto, Mozambique Pau Ferro, and in Congo is Kisasamba, in Gabon is Oken (Oyen, 2012; Anwani *et al.,* 2020). In Nigeria, *Bobgunnia fistuloides* is known as Gbogi by the Nupe tribe, Dufai or Farau in Hausa, Udoghogho by the Igbos (Oyen, 2012; Anwani *et al.,* 2020). The plant is a tree that grows up to a height of 25-40 m tall (medium size), having a diameter of 80-120 cm (Alfonso and Miller, 2002). It has flowers in groups of 1-4. It undergoes bisexual reproduction and it is a sweet scented plant. The bark surface is slightly fissured, thus having watery exudates. The seed is in kidney-shaped form with grayish appearance. It undergoes epigeal germination and it has alternate leaves (Oyen, 2012). The leaves and the pods of this plant can be used as compost manure since they are rich source of nitrogen.

In traditional medicine, different parts of *Bobgunnia fistuloides* have been used to treat diseases in different countries. Report shows that in Gabon and Congo, the warm decoction of the bark are used to bath younger children to treat fever (Oyen, 2012). This indicates that there are certain phytochemicals (bioactive) components responsible for this action or treatment. The bark macerates are also used to treat filariasis of the eye and to cure skin diseases in Congo. A decoction of the bark mixed with sweet peppers is taken by nursing mothers to stimulate milk production.The crude extracts from the bark are also used by men to treat gonorrhea and to treat menstrual challenges by women as well as diarrhea (Groom, 2012).

Ethno-medicinally, *Bobgunnia fistuloides* parts (leaves, bark and roots) have been used by the Nupe communities, Niger State, Nigeria to treat various diseases for decades. Although, the introduction of orthodox medicine lowered the patronage of its herbal remedies, which hitherto was the only thriven means of effective medication in the olden days. Its herbal application still remain only alternative for a very large population of the communities, who are either far away from health centres or cannot afford the modern day drugs. This practice has been on without the beneficiaries knowing the active components as well as its side effect. Therefore, this research is aimed at analyzing the phytochemistry and antibacterial activity of the roots, leaves, and stems bark of *Bobgunnia fistuloides*.

2. Materials and methods

2.1. Plant collection, identification and preparation

The leaves, roots and stem bark of *Bobgunnia fistuloides* were collected at Binin village in Gbako Local Government area, Niger State, Nigeria. It was identified at the herbarium section of the Department of Botany, Federal University of Lafia, Nasarawa State, Nigeria. The materials collected were shade dried in an open air and pulverized into powdered form (Keta *et al.,* 2018).

2.1.1. Preparation of the crude extracts

A 50 g portion of the crushed samples (leaves, stem bark and roots) were neatly wrapped separately inside Whatman filter paper and mounted in the extractor unit of Soxhlet apparatus. Different parts of the plant were extracted separately using 350 cm^3 of methanol and petroleum ether until the extraction processes were completed. The extracts obtained were transferred into a beaker, heated in a water bath for 4 hours until the solvents evaporated, leaving behind the pure extract. After that, the methanol and petroleum ether extracts of the parts were kept in an air tight container for antibacterial study (Method 945.16 of AOAC, 2005).

2.1.2. Preparation of the aqueous extracts

A 50 g portion of the crushed samples (leaves, stem bark and roots) were measured and were soaked in 500 cm^3 of water in 1000 cm^3 beaker. The solution mixtures were allowed to stand for 3 days. The sample solutions obtained were freeze dried using freeze dryer (model, 316L). The extracts obtained were kept in air tight container for antibacterial activity (Nas *et al.,* 2018).

2.2. Phytochemical screening

2.2.1. Alkaloid determination

The method described by Oloyed (2005) was used to determine the total alkaloid in the crude extract. Half grams of the plant parts was weighed into a test tube containing a mixture of 5 cm^3 96 % ethanol and 5 cm^3 H_2SO_4 and then filtered. After, 1 $cm^3$of the filtrate was added into another test tube containing 5 cm^3 of 60 % H_2SO_4 and was allowed to stand for 5 minutes. Thereafter, 5 mL of 0.5 % formaldehyde was added and was allowed to stand at room temperature for 3 hours. The absorbance values were recorded at the wavelength of 565 nm. Vincristine extinction coefficient (E_{296}, ethanol {ETOH} = 15136$M^{-1}cm^{-1}$) was used as reference alkaloid.

2.2.2. Flavonoid determination

A 0.5 grams of the plant parts (roots, leaves and stem bark) was weighed into a test tube followed by the addition of 5cm^3distilled water. A 0.50 cm^3portion of the aqueous extract was added into a test tube containing 1.5 cm^3 absolute methanol, 0.1 cm^3 of 10 % aluminum chloride, 0.10 $cm^3$of 1 M sodium acetate and 2.8 cm^3 of distilled water and were incubated at ambient temperature for 30 minutes. Double Beam shimadzu UV-spectrophotometer (UV-1800) were used to measure the absorbance at 415 nm. The calibration curves were obtained using quercetin as the standard (Chang et al., 2002).

2.2.3. Tannin determination

The amounts of tannins in the plant parts were determined using spectrophotometric method as described by Chang *et al.* (2002). A 0.2 g portion of each of the crude extracts was weighed into a 50 cm^3 beaker followed by the addition of 20 cm^3 of 50 % methanol, covered with paraffin film and was heated in a water bath at 80 ^{0}C for 1 hour. The mixture was thoroughly shaken to obtain uniformity. The extract obtained was filtered into a 100 cm^3 volumetric flask followed by the addition of 20 cm^3 of distilled water, 2.50 cm^3 of Folin-Denis' reagent, and 10 cm^3 of sodium carbonate solution. The mixtures were thoroughly shaken and allowed to stand for 20 minutes at room temperature. The absorbance was recorded at 760 nm using double beam Shimadzu UV-spectrophotometer (UV-1800). Standard tannic acid solutions were used to obtain the calibration curve.

2.2.4. Total phenol determination

The method described by Singleton *et al.* (1999) was used to determine the total phenol in aqueous extracts of the roots, leaves and stem bark of The crushed samples (0.01 g) each was weighed using electronic weighing balance (FA1004 N) and were transferred into *Bobgunnia fistuloides* different test tubes. The weighed samples were dissolved in 10 mL distilled water. Aqueous extract (0.5 cm^3) was oxidized using 2.5 $cm^3$of 10 % Folin-Ciocalteu's reagent which was neutralized by the addition of 2 mL of 7.5 % sodium carbonate. The reaction mixture was incubated at 45 ^{0}C for 40 minutes for colour development. The absorbance of the solution was recorded using double beam Shimadzu UV spectrophotometer (UV-1800) at765 nm. Standard garlic acid was used to prepare the calibration curve.

2.2.5. Determination of saponnins

A 0.5 grams of the crude extract was measured using analytical weighing balance (FA1004 N). The weighed samples were transferred into test tubes, followed by the addition of 20 cm^3 of 1 N HCl. The mixture was boiled in water bath at 80 ^{0}C for 4 hours. The reaction mixture was allowed to cool in a desiccator and then filtered into a beaker. Petroleum ether (50 cm^3) was added to the filtrate. Thereafter, the ether layer was collected and was evaporated to dryness. Equal volumes of acetone and ethanol (5 cm^3) were added to the filtrate, followed by the addition of 6 cm^3 of ferrous sulphate and 2 cm^3 of concentrated sulphuric and the mixture was allowed to stand for 10 minutes. The absorbance was recorded at 490 nm. Standard saponins solutions were used to prepare the calibration curve (Oloyed, 2005).

2.3. Antibacterial screening of root, stem bark and leaves extracts of *Bobgunnia fistuloides*

2.3.1. Standardization of extracts

The methanol, petroleum ether and aqueous extracts (1 g) were dissolved in 5 cm^3 of 10 % dimethylsulphoxide (DMSO). Ampiclox was used as the positive standard with a concentration of 40 mg/cm^3 for all bacterial isolates.

2.3.2. The test microorganisms

Five different bacteria were clinically isolated and were identified from the Department of Microbiology, Federal University of Technology, Minna. The isolated bacteria include *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis* and *Klebsiella pneumonia.* The isolated bacteria were properly maintained on sterile Mueller Hinton nutrient agar at -4 ^{0}C.

2.3.3. Standardization of the inoculums

The bacteria isolated were sub-cultured into 20 cm^3 of nutrient broth and incubated for 24 hours. A 0.2 cm^3portion ofthe broth containing the isolates was taken into another freshly prepared nutrient broth and incubated for 2-3 hours which is equivalent to 1.5×10^6 CFU/cm^3 of Mcfarland standards (Kawo and Kwa, 2011).

2.3.4. Susceptibility test using plant extracts and control

Muller Hinton agars (28.0 g) was suspended in 1000 cm3 distilled water in a conical flask and were sterilized using autoclave at 121 ^{o}C for 15 minutes. The agar was poured into six different petri-dishes and was allowed to gel. The plates were oven dried before use to get rid of excess water on the surface and to avoid microbial attack. A sterile cork borer of 5 mm diameter was used to create six wells on the nutrient broth agar and was properly labeled for the well diffusion test. From the 200 mg/mL concentration of the extracts prepared, 2.0 cm^3 each were introduced into the wells, as well as 2.0 cm^3 of 40 mg/cm^3 of Ampiclox (control) with the aid of micropipette. After which, the test organism were inoculated on the nutrient agar to test for the susceptibility. The Petri-dish was incubated for 24 hours at 37 ^{o}C. The different zones of inhibition were measured using a transparent ruler. The sensitivity of the pathogens towards the extracts was recorded (Sani *et al.*, 2016).

2.3.5. Determination of minimum inhibitory concentration (MIC)

The MIC of the methanol, petroleum ether and aqueous extracts of the roots, leaves and stem bark extracts were determined using the method described by Adelanwa *et al.* (2016). A 2.0 cm^3 of the freshly prepared nutrient broth was measured into ninety one test tubes different tubes. The test tubes were grouped into six and labeled according to the extracts that showed activity toward the pathogens. A 2.0 cm^3 of the extracts were added into the test tubes containing the nutrient broth. Serial dilutions of 100, 50, 25, 25, 12.5 and 6.25 mg/cm^3 were made from the test tubes. The pathogens were inoculated into the various test tubes and incubated at 37 ^{0}C for 24 hours. The test tubes were examined for microbial growth. The least concentration that inhibits microbial growth was considered to be the MIC or the lower concentration of any extract (Adelanwa *et al.*, 2016).

2.3.6. Determination of bacterial concentration (MBC)

The MBC of methanol, petroleum ether and aqueous extracts of the roots, stem bark and leaves extracts were determined using the method described by Adelanwa *et al.* (2016). The test tubes that showed no visible growth in the MIC assay were sub-cultured on a freshly

prepared nutrient agar plate and were incubated at 37 ^{0}C for 48 hours. The MBC were recorded on the lowest concentration of the extract that did not show any growth on new set agar plate (Adelanwa *et al.*, 2016).

2.3.7. Procedure for the minimum inhibition control using antibiotics

After preparing the Muller Hinton agar plates, the same test organisms used for the plant extracts were inoculated on the agar plate. Fourty milliliter of the selected antibiotics (Ampiclox) was placed on the agar containing the test organism and was incubated at 37 ^{0}C for 18-24 hours. The zones of inhibition (in mm) were measured with a transparent ruler. The results obtained were compared with the results obtained with the plant extracts (Etonihu *et al.*, 2011).

2.4. Statistical analysis

All the determinations were carried out in triplicates and the data obtained were expressed as mean±standard deviations. All data were analyzed using Statistical Package for the Social Science (SPSS 25.0). The results were expressed using one way ANOVA and Duncan's multiple to compare the significant differences at $p<0.05$.

3. Results and discussion

Table 1 and Figure 1 show the amounts of phytochemical contents such as tannins, phenols, alkaloids, flavonoids and saponinsof the roots, leaves and stem bark of *Bobgunnia fistuloides*

Table 1: Phyto-Chemical Constituent of the Roots, Leaves and Stem Bark of Bobgunnia Fistuloides Expressed in Mg/100g

	Plant parts		
Phytochemicals	Roots	Leaves	Stem bark
Phenols	479.733±0.196^b	465.983±0.136^a	482.773±0.172^c
Flavonoids	11.707±0.064^a	25.143±0.023^c	23.903±0.029^b
Alkaloids	29.090±0.202^b	36.767±0.055^c	24.790±0.144^a
Tannins	22.400±0.020^a	25.143±0.023^c	23.903±0.029^b
Saponnins	577.293±0.235^b	586.517±0.097^c	230.187±0.025^a

Values are expressed in mean ±SD of triplicate determinations. Superscript values labeled a, b, c along the rows are significantly different at $p<0.05$. SD= standard deviation.

3.1. Phytochemical constituents

From Table 1, the result of the analysis revealed that *B. fistuloides* is a potential source of phenolic compound, the stem bark having 482.773 mg/100g which recorded the highest phenol content, followed by the roots (479.733 mg/100g) and the leaves were found to be the lowest in phenol content (465.983 mg/100g). The phenolic content in this study was found to be higher than the phenolic content reported by Hong et al. (2015) in the leaves of *Helicteres hirsute* which values ranged from 3.99 to 8.33 mg/100g. The phenol content in *B. fistuloides* is higher compared to other medicinal plants analyzed by Kadiri *et al.* (2015). It has been reported that plant rich in Phenolic compound have the ability to fight against diseases such as cancer, diabetes, urinary tract infection and heart diseases (Gnanaraja *et al.*, 2014; Osuntokun, 2018). The high phenolic content in the parts investigated, is an indication that *B. fistuloides* can also help to improve the health of any individuals who solemnly depends on it as the only alternative medication for the treatment of diseases caused by bacteria studied.

The leaves of *B. fistuloides* recorded the highest value of flavonoids content (25.143 mg/100g), followed by the stem bark (23.903 mg/100g) while the root (11.707 mg/100g) was the least. The flavonoid values obtained in this study is in agreement with the values reported by Gnanaraja *et al.* (2014) on some medicinal plants such as *Dalbergia sissoo* (16.02 mg/100g), and *Delonix regia* (28.29 mg/100g). Several researches have revealed that flavonoids have the ability to modify allergens, act against carcinogens and eliminate free radicals and reactive oxygen species (Deepak *et al.*, 2016). Ebuna and Ifemeje (2015) reported that flavonoids possess antioxidant properties, ability to fight acute and chronic inflammation, anti-viral and antimicrobial properties. Therefore, the presence of flavonoids in *B. fistuloides* parts shows that the plant can contribute to the elimination of carcinogens, free radicals and could serve as antibacterial agent against some pathogens.

Alkaloids play significant roles in both plant and animal. It contributes to plant survival against any microbial attack. The alkaloid content found in the leaves (36.767 mg/100 g) is relatively higher compared to the roots (29.090 mg/100g) and the stem bark (24.790 mg/100g). The result is lower compared to the alkaloid content found in *Ficus capensis* (422.12 mg/100g) as reported by Achi *et al.* (2017). Plants that contain alkaloids have the ability to reduce blood pressure and to calm nervous system with regards to mental deformity (Alexander, 2016). The presence of alkaloids in plant contributes to its usefulness in the treatment of skin diseases, cure of snake bite and to palliate pains. Alkaloids are pertinent in the production of analgesic drugs. They have anti-malaria, anticancer and anti-arrhythmic properties (Arpita, 2017). Thus, high alkaloid content in *B. fistuloides* could serve as a major reason while it is used to treat fever in traditional medicine.

The leaves of *Bobgunnia fistuloides* recorded the highest amount of tannins (25.143 mg/100g), followed by the Stem bark (23.903 mg/100g), whereas the roots had the least amount of tannins (22.400 mg/100g).The tannin content in *B. fistuloides* is higher when compared to tannin content in *Tephrosia purpurea* (0.77 mg/100g) as reported by Gnanaraja *et al.* (2014), but very low compared to tannin content in *Ficus capensis* leaves (687.64 mg/100g) as stated by Achi *et al.*, (2017). These differences could be attributed to ecological factors of where the plants are grown. Several studies indicated that tannin extract have the potency to reduce cancer tumor, decrease blood pressure, reduce the level of serum lipid and to hasten blood clotting (Gnanaraja *et al.*, 2014).

Saponins in plants are known to protect plants against any microbial attack. The plant parts are rich in saponins contents. From the results obtained, the leaves recorded the highest saponnin content (586.517 mg/100g) followed by the roots ((577.293 mg/100g) and the least saponin content was found in the stem bark (230.187 mg/100g). The results indicated that the saponins content in *B. fistuloides* is higher compared to the saponin content in some medicinal plants such as *Sesanum indicum* (0.74 mg/100g), *Alafia barteri* (1.67 mg/100g) and *Clausena anisata* (1.01 mg/100g) as reported by Kadiri *et al.*, (2015). Other researchers showed that saponins possess antimicrobial and

antibacterial activity. An intake of medicinal plant that contains saponins improves easy migration of micro molecules into the cell membrane (Alexander, 2016). According to Bolanle *et al.* (2014), it was stated that saponins have the ability to prevent the growth of cancerous cells, prevent cardiac attack and can also act against excess cholesterol in the blood. Saponins are also beneficial in treating yeast and fungal infections (Ejikeme *et al.*, 2014). Hence, the presence of saponins in *B. fistuloides* parts can promote its anti-fungal, anti-cancer and antimicrobial properties (Ejikeme *et al.*, 2014).

3.2. Antibacterial activity

Figure 2 below shows the susceptibility of the methanol and petroleum ether extracts of the roots, and stem bark of *Bobgunnia fistuloides* on some Gram positive and Gram negative bacteria which include *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi,* and *Klebsiella pneumonia*. From the results obtained, the methanol and petroleum ether leaves extracts had not activity on any of the selected pathogens, whereas the aqueous leaves extract were more susceptible against *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Klebsiella pneumonae* except *Bacillus subtilis.* The inability of the leaves extracts to show no activity could be attributed to the solvents (methanol and petroleum ether) used. However, the non sensitivity of the methanol and petroleum leaves extract to the pathogens do not demonstrate the absence of bioactive compounds, rather it depends on the solubility of the active components (Tsobou *et al.*, 2015). Hence, the bioactive components were more soluble in the aqueous extracts than the methanol and petroleum ether leaves extract. Furthermore, the inability of the methanol and petroleum ether leaves extract could also be attributed to the presence of other substances which served as antagonist and thus inhibited the effectiveness of the bioactive compounds (Tsobou *et al.*, 2015).

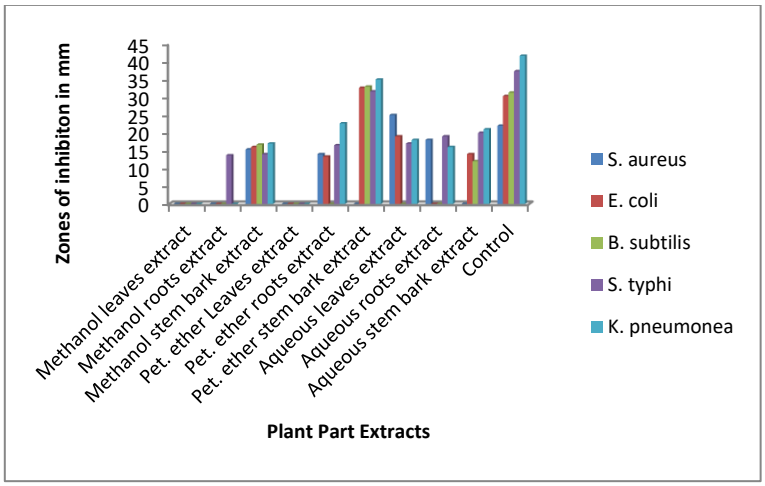

Fig. 1: Antibacterial Activity of the Methanol, Petroleum Ether and Aqueous Extracts of the Leaves, Stem Bark and Roots of *Bobgunnia fistuloides* and Control (Ampiclox) on the Selected Pathogens.

Salmonella typhi was found to be susceptible to the methanol root extract (13.67±0.58 mm) while *Staphyloccous aureus, Bacillus subtilis, Klebsilla pneumonia* and *Escherichia coli* had no antibacterial activity. Petroleum ether roots extract recorded zones of inhibition against *Staphyloccous aureus*(14.00±1.00mm), Salmonella typhi (16.50±0.50 mm), *Klebsiella pneumonia* (22.67±0.58mm) and *Escherichia coli* (13.33±0.57 mm) except *Bacillus subtilis*. The aqueous root extract was found to inhibit *S. aureus* (18.00±1.00mm), *S. typhi* (19.00±0.60 mm) and *K. pneumonia* (16.00±1.00 mm).

The methanol stem bark extract showed antibacterial activity against all the tested isolates. The result indicated that *Klebsiella pneumonea* (17.00±0.00 mm) recorded the highest zone of inhibition while the least was *Salmonella typhi* (14.00±1.00 mm). The aqueous stem bark extracts were susceptible to all the pathogens except *Salmonella typhi*. The zones of inhibition of the control (Ampiclox 40 mg/ml) ranges from 22.00 to 41.67 mm.

Statistically, *Klebsiella pneumonia* (17.13±0.00 mm) recorded the highest average zones of inhibition based on the susceptibility of the pathogens to the extracts, followed by *Salmonella typhi* (16.92±1.00mm), E. coli (12.33±0.57mm), *S. aureus* (9.40±0.50mm) while least average zone of inhibition was found in *B. subtilis* (9.30±0.52mm). From the results obtained, gram positive bacteria (*S. aureus* and *B. subtilis*) were less susceptible than the gram negative bacteria.

The differences observed in the antibacterial activity could be the sensitivity of the pathogens towards the plant extracts and morphological constitution of these microorganisms (Murnisyazwani and Rabeta, 2019). Chaghaby (2014) reported that gram-negative bacteria consist of outer phospholipidic membrane encompassing lipoprotein and lipopolysaccharide that make the cell wall impermeable to plant extracts. The differences in the membrane of bacteria investigated in this study attributed to the reasons why gram negative bacteria (*Klebsiella pneumonia, Salmonella typhi* and *E. coli*) were more susceptible than gram positives (Chaghaby, 2014).

3.3. MIC and MBC of the extracts

Figure 2show the results of the minimum inhibitory concentration (MIC) of the plant part extracts. The results showed the various concentrations of methanol, petroleum ether and aqueous extracts which could inhibit or kill the isolates. The control had the lowest MIC (2.50 mg/ ml) against all the tested isolates.

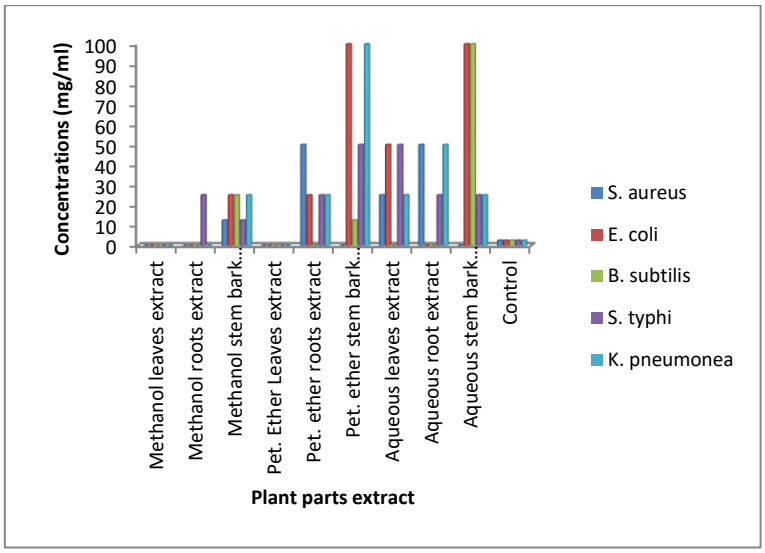

Fig. 2: Minimum Inhibitory Concentration (MIC) of the Methanol, Petroleum Ether and Aqueous Extracts of the Leaves, Roots and Stem Bark of *Bobgunnia fistuloides* and Control on the Selected Pathogens.

Lower MIC (12.50 mg/ml) was observed mostly by the methanol extracts than the petroleum ether extracts and aqueous extracts. MBC of methanol extract ranged between 12.50 and 50mg/ml, petroleum ether extract ranged from 12.50 to 100.00 mg/ml and the aqueous extract ranged from 25 to 100 mg/l as shown in figure 3 below. However, *Staphylococcus aureus* and *Bacillus subtilis* showed no activity for MBC against the petroleum stem bark and root extracts. The MBC result reveals that the methanol extracts have more bactericidal effect compared to the aqueous and petroleum ether extracts. The values obtained for the MIC and MBC shows that *B. fistuloides* has the ability to kill bacteria.

Generally, the MIC and MBC of the methanol extracts had the lowest inhibitory and bactericidal effect compared to the aqueous and petroleum ether extracts. This indicates that methanol extracts contain more active components that can inhibit or kill the tested bacterial than other solvent used in this study. The appreciable amount of bioactive components present in the extracts contributed to inhibitory and bactericidal effect against the microorganisms investigated in this study (Murnisyazwani and Rabeta, 2019).

The inhibitory actions of the extracts against the selected isolates shows that *B. fistuloides* could serve as a potential active ingredient in the development of orthodox drugs for the treatment of ailment caused by these bacteria investigated.

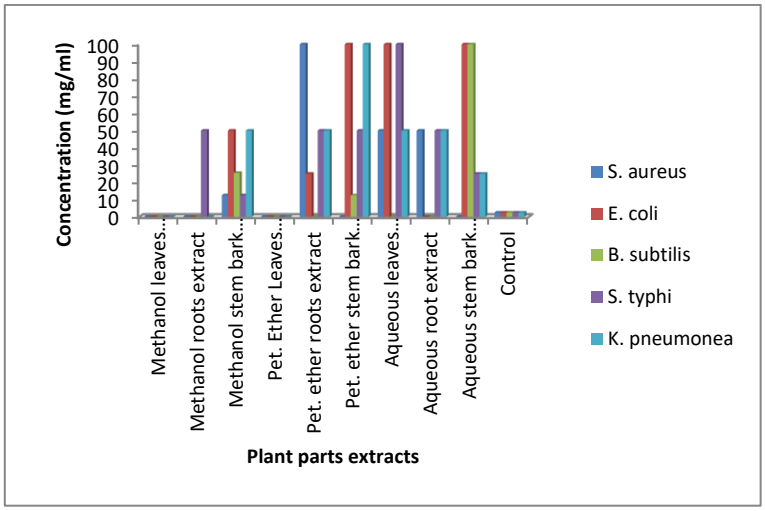

Fig. 3: Minimum Bactericidal Concentration (MBC) of the Methanol, Petroleum Ether and Aqueous Extracts of the Leaves, Roots and Stem Bark of *Bobgunnia fistuloides* and Control on the Selected Pathogens.

4. Conclusion

Quite a number of both positive and negative gram bacteria are available to be tested against plants extracts. a total of 5 bacteria were used. All the tested organisms had their growth inhibited by most of the extracts from the leaves, stem backs and roots of *Bobgunnia fistuloides.* The results obtained presented the efficiency of the bioactive constituents of the plants to the field of herbal medicine. These findings, therefore, provide information on the bioactive constituents and medicinal potentials of *Bobgunnia fistuloides.*

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