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PHYTOCHEMISTRY AND ANTIBACTERIAL ACTIVITY OF *ENTANDA ABYSSINICA* LEAF EXTRACTS

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Abstract

The leaf of the plant *Entanda abyssinica* was studied to determine its antibacterial activity using the methanolic and ethyl acetate fractions. The two fractions were tested on *Staphylococcus aureus, Pseudomonas aeruginonsa* and *Escherichia coli* for their antibacterial activity using agar well diffusion assay method. The Minimum Inhibitory Concentration (MIC) was determined graphically by plotting the square of the diameter of the zones of inhibition against the logarithm of the fraction concentration. Its active constituents were determined using phytochemical analysis. The extraction was done with methanol and ethyl acetate of analytical grade as solvents using the Soxhlet extraction method. Acute toxicity test was also carried out. The phytochemical analysis revealed high content of alkaloids and flavonoids, with other active ingredients appearing in low concentration. Crude protein content of the leaf assayed revealed the presence of about 8.75% protein. The LD_{50} was 2154.1. The antimicrobial assay revealed a concentration-dependent level of activity on the test organisms. The mean IZD recorded for *Escherichia coli* on methanol and ethyl acetate fractions were 7.33 and 6 mm respectively while *Staphylococcus aureus* gave the mean IZD of 10.33 and 6mm for methanol and ethyl acetate fractions respectively. The Minimum Inhibitory Concentration (MIC) ranged from 3.54mg/ml (for the most susceptible test organism) to 7.08mg/ml. *Pseudomonas* showed no susceptibility pattern with methanol extract. The statistical results showed no significant difference (P=0.675) in the susceptibilities of the *E.coli* and *S. aureus* using methanol extract but ethyl acetate and methanol extracts susceptibilities of *S. aureus* and *E.coli* showed significant different (p =0.013). Analysis findings suggest that the leaf of *Entanda abyssinica*, though slightly toxic, have antibacterial activity and are potential source of alternative medication.

Keywords*: Entanda abyssinica*; Antibacterial; Toxicity; Phytochemistry

Introduction

In the ancient times mineral, plant and animal products were used as drugs. Since the origin of man, plants have been used to alleviate and treat diseases. In the recent past also, natural products from plants with therapeutic properties have become useful. [1]. Researches involving medicinal plants have yielded many valuable products and new compounds with good biological activity are discovered often, almost on a daily basis [2].

Notwithstanding, many problems were encountered by the use of plant materials to treat illness. One is that the knowledge regarding the plants and their specific use in treatment were reserved for only the traditional medicine-men and handed down only orally to the next generation. Another problem encountered in the ancient times is that of improper identification. Owing to the fact that many plants of the same genus generally look alike but are different when it comes to their chemical composition, plants of the same genus but different species can be misidentified and can cause severe problems due to differences in active compounds. Also, crude forms of these plants which were taken in arbitrary dosages and not in combination with other synthetic products were not so effective. Some plants could also cause irritations and allergic reactions to the processers and the users. Most of these plants were aimed at treating the symptoms and not the actual disease condition and because of this, some of them were considered safe only on the basis that they did not cause any apparent harm [1]. Parts of plants that have been implicated in therapeutic activities include the root, leaves, bark, seed, flower and fruits. Of the estimated 250,000 to 500,000 plant species, only a small percentage of phytochemical properties have been investigated and even a smaller percentage has been properly studied in terms of their pharmacological properties. It is estimated that only 5000 species have been studied for medical use [1]. But recently, there has been an interest in folk medicine.

The *Entanda* is a small to medium sized, low branching deciduous tree, up to 7-15m high, with a narrow open crown. The plant bark is fibrous and its leaves are large alternate, bi-pinnate up to 15 to 45 cm long, with 10-20 pairs of pinnate. Leaves are sometimes pubescent. Flowers are light-yellow or creamy in color and appear in racemes 7 to 15 cm long(Fig. 1). *Entanda abyssinica* produces pods containing 12 to 15 two-winged seeds per pod(Fig. 2). There is a resemblance of the tree with an acacia tree but it can be differentiated by its bi-pinnate leaves and also the absence of thorns. Leaves serve as food for animals, while the wood which is soft is easy to work on. Its fibers are used for making bands, ropes and storage baskets. The leaves have been employed for many medicinal uses in the past. It has been dried, ground and used as tonic tea, for wound healing, and possess antidotal effects against various toxic agents and fish poison. It is a tree widely spread in tropical Africa. It is mostly found in African countries including Nigeria and prefers sandy loam soils for its growth. Its flowering takes place during the rainy season, and the pods ripen towards the end of the rainy season but sometimes may extend into the dry season.

Entanda abyssinica has been studied in the past for its antimicrobial activity. Traditionally, it is used to treat ailments like rheumatism, bronchitis coughs, abdominal pains, diarrhoea and fever. It has also been claimed to prevent

miscarriage [3]. Some pharmacological properties of *E. abyssinica* have been previously reported, including anti-inflammatory, antimicrobial and antioxidant [4, 5, 6]. The leaves of *Entanda abyssinica* was screened for its inhibitory effect on ten strains of *Escherichia coli* 0157:H7 (EHEC) using the agar gel diffusion assay method. It was seen that ethanol extract of *Entanda abyssinica* inhibited all the ten strains used, showing variable antibacterial activities which is dependent on the extract concentration [7]. Another conducted revealed that the stem bark of *Entanda abyssinica* exhibited fungistatic and fungicidal activity against *Candida spp. and Aspergillus spp.* It also revealed that the extracts contain compounds with high antifungal potency [8].

Entanda abyssinica like other medicinal plants might be an alternative treatment in some cases of mild infectious diseases. They may also be a possible source for new potent antibiotics as alternatives to the ones the microorganisms have been resistant to [9]. The terpenoid and flavonoid compounds of *Entanda abyssinica* were studied and were found to possess good antibacterial activity against *S. typhimurium* [10]. Salim and his colleagues [11] also reported its use in the treatment of miscarriage in women of childbearing age. Methanol extract method has also been used to present the antioxidant properties of the plant [6]. This present work therefore is focused on the scientific study of the antibacterial properties of *Entanda abyssinica* leaf fractions while elucidating its toxicity and to see the urgent need for natural products as alternative for the use of antibiotics.

Methods

Plant Identification

The plant *Entanda abyssinica* was collected and confirmed by Mr. Ozioko of Department of Botany, University of Nigeria Nsukka. A large quantity of the plant leaf (Fig 1 and 2) was collected from Nsukka in Nsukka Local Government Area of Enugu State Eastern Nigeria.

Extraction

The leaves were dried under a shade during the dry season, ground into powder and packed into air tight containers. They were then taken to the laboratory for extraction.

The extraction of crude extract was done using the Soxhlet extraction procedure. The Soxhlet extraction procedure involves the extraction of fats and oil from solid materials by repeated washing (percolation) with organic solvents under reflux in special glassware. In this method, the sample was dried, ground into small particles and placed in a porous cellulose thimble. The thimble was placed in an extraction chamber, suspended above the flask containing the solvent and below the condenser. When the flask was heated the solvent evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the sample. When the solvent surrounding the samples exceeded a certain level, it overflowed and trickled back down into the boiling flask. At the end of the extraction the flask containing the solvent and lipid was removed, evaporated and the mass of the remaining lipid measured.

Phytochemical Analysis for the Active Ingredients

The test was carried out in order to reveal the presence and abundance of some active agents. The presence of the following active compounds was investigated according to the Harbone method [12].

Test for Carbohydrate (Molisch's Test)

An amount of 0.1g of the extract was mixed with 2ml of water and boiled and filtered., Few drops of naphtol solution in ethanol (Molisch's reagent) were added to the filtrate. Concentrated Sulphuric acid was then gently poured down the side of the test tube to form a lower layer below the aqueous solution. A purple ring indicates the presence of carbohydrate.

Test for Reducing Sugars (Fehling's Test)

To 1ml portion of the filtrate, equal volumes of Fehling's solution I and II was added and boiled on water bath for few minutes. The presence of reducing sugar was indicated by a brick red precipitate..

Test for Alkaloids

Wagner's reagent of 2ml quantities were added to 10mg of the extracts and allowed to dissolve. Presence of alkaloids was indicated by reddish brown precipitate.

Test for glycosides

About 5ml of dilute Sulphuric acid were added to about 0.1g of the extracts in test tubes and boiled for 15 minutes in a water bath, cooled and neutralized with 20% Potassium hydroxide solution. 10ml of a mixture of equal parts of Fehling's solution I and II were added and boiled for 5minutes. A more dense brick red precipitate indicates presence of glycosides.

Test for Saponins (Frothing Test)

About 10mls of the extracts were taken and diluted with 20 mL of distilled water and shaken vigorously for 15 minutes. A stable froth (foam) on standing indicates the presence of saponins.

Test for Tannins

About 10 mg of the extracts were dissolved in 45% of ethanol. The test tubes were boiled for 5 min and 1 mL of 15% ferric chloride solution was added to the mixture. The appearance of greenish -black color confirms the presence of tannins.

Test for Flavonoids

NaOH was added to 10 mg of each extract. A yellow colour which disappears or become colorless after adding few drops of diluted H_2SO_4 confirms the presence of flavonoids in the plant extracts.

Test for Resins

About 0.2g of the extract material was extracted with 15ml 96% ethanol. The extract solution was added into 20ml of distilled water in a beaker. Presence of precipitate indicates the presence of resins in the extracts.

Test for Proteins (Million's Test)

To a little portion of the extract in a test tube, two drops of Million's reagent were added. A yellow precipitate is indicative of proteins.

Test for Oils

About 0.1g amount of the extract poured on filter paper and the paper observed. Translucency on the filter paper indicates the presence of oils.

Test for Steroids

About 9 ml of ethanol was added to 1g of the extract, refluxed for few minutes and then filtered. The filtrate was concentrated by boiling it in a water bath. The filtrate was extracted with chloroform using a separating funnel. Then 1ml of concentrated Sulphuric acid (H_2SO_4) was carefully added to 0.5ml of the chloroform extract in a test tube form a lower layer. A reddish green interface indicates the presence of steroids.

Test for Terpenoids

The 0.5ml quantity of the chloroform extract was allowed to evaporate to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey color indicates the presence of terpenoids.

Test for Acidic Compounds

About 0.1g of the extract was placed in a clear dry test tube and sufficient water was added. This was put to warm in a hot water bath and then cooled. A piece of water-wetted litmus paper was dipped into the filtrate and color change from blue to red is indicative of the presence of acidic compounds.

Test for Crude Protein Content

Digestion:

The method as described by Pearson [13] , was used in the determination of crude protein content.

A 1g of minced sample was placed inside 100ml digestion flask. The following were then added into the flask: 2g anhydrous sodium sulphate, 1g hydrated cupric sulphate, a pinch of Selenium powder and 10ml conc. sulphuric acid

The flask was placed on an electric coil heater and gently boiled at first until lacking occurs; heat was then increased until the solution becomes clear. Heating was continued for at least one hour after solution has cleared. If black specks persist in the neck of the flask, it is an indication of incomplete digestion. The flask was then allowed to cool and the black specks rinsed down with distilled water and the content heated for further period until all specks disappear. After heating, distilled water was introduced into the flask up to the 100ml mark. The flask was shaken thoroughly.

Distillation:

Steam was passed through Microkjedahl distillation apparatus for about 10 minutes. Boric acid of 5ml quantiy was placed into 250ml conical flask and two drops of the indicator added. The conical flask was placed under the condenser such that the condenser tip is on the surface of the liquid. 5ml of the dilute digest was placed in the distillation apparatus and was rinsed down with distilled water. The cup was closed with the rod and 5ml of 40% NaOH was put in. This was let in carefully leaving behind a little to prevent Ammonia escaping. Steam was then let through for about 5 minutes until the amount of liquid in the conical flask is about twice what it was in the beginning of distillation. The Boric

acid indicator was titrated with 0.01ml HCl to the end point which was pinkish color. The titre volume was recorded, that was, volume of 0.01ml HCl that changed the indicator from green to pinkish color. Since 200mg/ml of the extract gave 1.75% of protein; 1g of the extract yielded a protein concentration of 8.75. The protein concentration of *Entanda abyssinica* was thus very low.

Acute Toxicity Test (LD_{50})

The Lorke procedure [14] of LD_{50} determination was used. With this method the following assumptions were made:

1 Substances more toxic than 1mg/kg are so highly toxic that it is not important to calculate LD_{50}.
2 LD_{50} values that are greater than 5,000mg/kg are insignificant.
3 An appropriate figure for the LD_{50} is usually adequate to estimate risk of acute intoxication.

Test animals used were albino mice weighing between 16-34g. The mice were placed in three groups of three animals per group. The first group (I) received 10mg/kg, the second group (II) received 100mg/kg while the third group (III) received 1000mg/kg each. The animals were constantly observed for the first two hours, intently for the next 4 hours and then overnight. The number of dead animals was recorded at the end of 24 hours. The second stage of the acute toxicity test was performed using doses of 1600mg/kg, 2900mg/kg and 5000mg/kg for the three groups having one animal per group.

Extract Dilution

1ml of 200mg/ml of the extract was taken using a syringe and put into a test tube and was covered with cotton wool. 2ml of Dimethyl Sulpur Oxide (DMSO) was added to six test tubes using a pipette to give a 100mg/ml concentration of the extract. This was followed by two fold serial dilutions using normal saline to get more dilutions- 50mg/ml, 25mg/ml 12.5mg/ml 6.25mg/ml and 3.125 mg/ml concentrations of the extract for potency test.

Preparation of Mcfarland's Standard

The equivalent turbidity of McFarland standard is a Barium Sulphate turbidity against which the turbidity of the test and control inoculum can be compared. When matched with the standard, the inoculum should give confluent or almost confluent growth. The mixture should be shaken immediately before use.

A 1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water and mixed well. A 1% w/v solution of Barium Chloride ($BaCl_2$. $2H_2O$) was mixed with 50ml of distilled water. Add 0.6ml of Barium Chloride solution to 99.4ml of the sulphuric acid solution and mix. Transfer a small volume of the turbid solution to a capped tube or screw cap bottle of the same type as used for preparing positive test and control inoculum.

Antimicrobial Activity of the Fractions using Agar Well Diffusion

Pure cultures of *Staphyloccocus aureus, Escherichia coli* and *Pseudomonas aeruginosa* used for the test were obtained from the Diagnostic Laboratory of the Department of Microbiology, University of Nigeria, Nsukka. The

organisms were sub-cultured in a sterile nutrient agar and incubated at 37°C for 24 hours. Discrete colonies were picked with a sterile loop and reconfirmed by standard biological methods and stock cultures were maintained in a refrigerator.

Nutrient agar medium was prepared according to the manufacturer's instruction for sub-culturing the bacteria while Muller- Hinton was also prepared and used for antimicrobial test of the extract on the bacteria. Pour-plate method of agar gel diffusion was preferred in checking antimicrobial activity of the extract. Sensitivity test medium was prepared in plates and checked for sterility by incubating the prepared plates at 37°C for 24 hours. A 24 hour broth culture of each of the test organisms was used for preliminary antimicrobial test. Holes of 8mm diameter were made on the plate after drying using a sterile glass borer. Six holes were made on each plate. After drilling the holes, 4-6 drops of different concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml) of the extract were put in each of the holes in each plate using a sterile Pasteur pipette. After that, the plates were incubated at 37°C for 24 hours. On observation, the extract was found to give zones of inhibition on the test organisms. The diameter of inhibition zones were measured using a meter rule.

Statistical Analysis

The statistical analysis was carried out with SPSS, version 15.0 using the T-test analysis to compare the activity of the two extracts respectively on the test organisms at 95% significance level using

Results

The phytochemical analysis of the methanolic and ethyl acetate fractions gave the constituents when analyzed. Alkaloids, flavonoids and steroids were present but in higher concentrations in the methanolic fraction than in the ethyl acetate while saponins were moderately present in the ethyl acetate extract but absent in the methanol extract. Steroids were moderately present in both extracts while reducing sugars and resins were absent in both fractions. Traces of glycosides, tannins, acidic compounds and proteins were also discovered in the leaf extracts. The results are shown in Table 1.

The minimum dose of the extracts that caused death of animal in 24 hours was 1600mg/kg and the LD_{50} was calculated to be 2154.066(Table 2). The antibiotic assay was carried out to determine the sensitivity of the test microorganisms to conventional antibiotics used for therapeutic uses None of the organisms were sensitive to Cotrimoxazole, Cloxacilin, Amoxicillin and Tetracycline. But there was some varying IZD by other antibiotics.

The antimicrobial test was carried out using the agar gel well diffusion. The zones of inhibition were recorded and reported in Tables 4 and 5 for the methanolic and ethyl acetate fractions respectively. The IZD increased with the concentrations of the extracts. But the size of inhibition zone produced against the *E.coli* by the ethyl acetate extract fraction varied inversely proportional to the concentration of the extract(Table 5). The results of the comparison of zones of inhibition produced by the test organisms against the methanolic and

ethyl acetate fractions are represented in bar chart figures 3 and 4 respectively.

The concentrations and IZD against which the MIC were calculated are shown in table 6 and 7. Here, a square of the IZD was plotted against the logarithm of the extract concentration. The MIC was determined by finding the antilog of the intercept on the x-axis. The results of the Minimum Inhibitory Concentration (MIC) are graphically represented. That of the methalonic fraction of *Escherichia coli shows* intercept on x-axis as 0.65, antilog of 0.55 and MIC of 3.54mg/ml (Fig 5). That of *Staphylococcus aureus* showed 0.55 intercept on x-axis, antilog of 0.55 and MIC of 3.55mg/ml (Fig 6). The MIC using Ethyl Acetate is demonstrated figure in as follows: *Escherichia coli* 0.7 intercept on x-axis, antilog 0.7, MIC 5.0mg/ml (Fig 7). *Staphylococcus aureus* intercept on x-axis 0.85, antilog of 0.85 and MIC of 7.08mg/ml (Fig 8). *Pseudomonas aeruginosa* intercept on x-axis was 0.6, antilog of 0.6 and MIC of 3.98 mg/ml (Fig 9). The MIC for *Pseudomonas aeruginosa* using methalonic fraction could not be determined since there was no IZD. Table 9 to 11 shows the concentrations and the IZD for the MIC determination using Ethyl Acetate extracts. The MIC plots of the three species of organism are seen in figure 7 to 9. The comparisons of MIC of the two fractions of the extracts are shown in figures 10.

Discussion

The phytochemical analysis of the methanol and ethyl acetate extracts of *Entanda abyssinica* leaves revealed the presence of alkaloids, flavoiods, saponins, tannins and trepenoids. Resins and reducing sugars were totally absent in both extracts. This result agrees with that of Teke and his colleagues [6] who in addition obtained cardiac glycosides also. It also show similarities with that obtained from other plants of the same genus (*Entanda africana and Entanda phaseolides)* [15]. Flavonoids, saponins, tannins and alkaloids have been reported to possess antimicrobial activities [16, 17, 18]. Flavoniods are also strong antioxidants and are also found to be effective antimicrobial substances in vitro against a wide array of microorganisms by inhibiting the membrane bound enzyme [19]. They contained quercetin and its glycosides quercetin-3-O-α-l-arabinopyranoside and quercetin-3-O-β-d-arabinopyranoside. Quercetin-related flavonoid have been shown to demonstrate strong antibacterial activity against bot Gram-positive and Gram-negative organisms including S. aureus, *P. aeruginosa* and *E. coli* with MIC values ranging from 0.093 to 0.37 µg/mL [20]. They have been reported to possess substantial anti-carcinogenic and anti-mutagenic activities due to their anti oxidant and inflammatory properties [21]. They are also active in reducing high blood pressure [22]. They have the ability to kill tumors, inactivate and kill microorganisms [23]. Tannins are polymeric phenolic substances do precipitate gelatin from solutions. Alkaloids are naturally occurring nitrogen compounds that are commonly found to intercalate selectively with the DNA of microorganisms thus possessing antimicrobial properties [24]. Previous phytochemical screening of *E. abyssinica* indicated the presence of flavonoids, terpenoids and kolavic acid derivatives [25, 26]. For the toxicology studies the extract was slightly toxic. The death of the mice was observed on the second day of the inoculation. The LD_{50} of 2154.066mg/kg indicated that at a

concentration lower than 2154.066, the extract is not toxic but at a higher concentration, the extract is toxic. The LD_{50} obtained in this study fell within 500- 5000 in which category is considered as slightly toxic. This depicts the fact that the extract could be safe for human use. This does not, however, not exclude the fact that the value of the LD_{50} could possibly be influenced by factors such as the age diet, hosing (temperature, caging) and other environmental conditions [27]. The antimicrobial activities of the plant and its use for therapeutic purposes can thus be attributed the presence of these active compounds. The antibacterial activity against both Gram positive and negative organisms is indicative of the presence of broad spectrum antibacterial compounds in the leaf extract [28].

Methanol extract showed a higher activity because it was able to extract much constituents of the plant material than ethyl acetate. The extract also showed increasing activity on the test organisms with increasing concentrations except for *Pseudomonas aeruginosa* on which it showed no activity at all. This does not tally with a research which reported that the methanol extract had inhibitory activity on some test organisms including *Pseudomonas aeruginosa* [6]. The plant was also found *to* possess tremendous antibacterial activity against *S. typhimurium* which could be developed for the treatment of the bacterial diseases [10].

Entanda abyssinica leaf showed no dormancy but the solvent chosen for extraction should be correctly selected as to extract all active constituents. Although there is no standard extraction method recommended, extraction with methanol is mostly considered as being very efficient [29].

Both extracts showed high antimicrobial activity against *E. coli* than on other test organisms used. The plant extract showed higher inhibitory activity on the test organisms than as observed in the conventional antibiotics discs. The increase in the zones of inhibition with increasing extract concentration is as expected in the case of *E. coli* and S. aureus of the methanol extract. But in the case of ethyl acetate extract on *E. coli,* there was a decrease in the zones of inhibition with increasing concentration of the extract. This can be attributed to difficulty by the ethyl acetate extract to diffuse properly in the gel at higher concentration. Scientists have discovered that highly polar solvents, such as methanol are more useful in extraction [30].

The antibacterial activity of the *Entanda abyssinica* leaves has been established in this study. The activity of the extract varied with the solvent used for the extraction. Having determined the LD_{50}, the extract can be administered properly and maximized in the development of novel drugs. The antibacterial activities from the present investigation provide important baseline information for the use of *Entanda abysinnica* for the treatment of infections and the justification for its use as herbal medicine

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Figure 1: ***Entanda abyssinica*** **plant with flowers**

Figure 2: ***Entanda abyssinica*** **plant with pod**

Table 1: Phytochemical Analysis of the Plant Fractions

CHEMICAL CONSTITUENTS	RELATIVE ABUNDANCES	
	Ethyl acetate extract	**Methanol extract**
Alkaloids	-	+++
Flavonoids	-	+++
Glycosides	+	+
Steroids	++	++
Saponins	++	-
Tannins	+	-
Terpernoids	-	++
Carbohydrate	++	+
Proteins	+	-
Oils	-	++
Acidic compounds	+	+
Reducing sugars	-	-
Resins	-	-

KEY:

- Absent
+ Present in trace amount
++ Moderately present
+++ Abundantly present

Table 2: Acute Toxicity Test Determination of LD_{50} of the Methanol Extract of the Leaf on Mice

Animal Groups	Animal	mg/kg Administered	Weight of Animal	mg administered (100mg/ml)	No. of Death	Time of Death (24hrs after admin.)
1	Head	10	24	0.24	-	-
	Trunk	10	25	0.25	-	-
	Tail	10	32	0.32	-	-
2	Right hind	100	34	3.4	-	-
	Left hind	100	26	2.6	-	-
	Right foe	100	18	1.8	-	-
3	Left foe	1000	25	25	-	-
	Right ear	1000	22	22	-	-
	Left ear	1000	23	23	-	-
One animal group (2^{nd} day)	Tail	1600	20	32	1/1	-
	BF	2900	17	49.3	1/1	6h:42mins
	LH	5000	16	80	1/1	4h:5mins

Table 3: Antibiotic Sensitivity Profiling using Conventional Antibiotics

ANTIBIOTICS (ug)	ZONES OF INHIBITION (mm)		
	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
CXC (5)	-	-	-
ERY (5)	-	12	-
GEN (10)	-	9	21
AUG (30)	-	-	-
STR (10)	13	17	11
TET (10)	-	-	-
CHL (10)	15	15	-
AUG (30)	-	-	-
OFL (5)	-	-	18
GEN (10)	10	13	12
NAL (30)	-	-	-
NIT (20)	18	20	-
COT (25)	-	-	-
AMOX (25)	-	-	-
TET (25)	-	-	-

KEY:

GEN= Gentamicin NAL= Nalidixic Acid
NIT= Nitrofurantoin COT= Cotrimoxazole AMOX= Amoxicillin
TET= Tetracycline AUG= Augmentin OFL= Oflaxacin
CHL= Chloramphenicol CXC= Cloxacilin ERY= Erythromycin
STR= Streptomycin

Table 4: Antimicrobial Test using Methanol Extract

Organisms		Concentrations of the extract (mg/ml)					
		100	**50**	**25**	**12.5**	**6.25**	**3.125**
Escherichia coli	Inhibition	14	10	8	6	4	2
Staphylococcus aureus	Zone Diameter	17	14	12	9	6	4
Pseudomonas aeruginosa	(IZD) in mm	-	-	-	-	-	-

Table 5: Result of the antimicrobial test using Ethyl Acetate extract

Organisms		Concentrations of the extract (mg/ml)					
		100	**50**	**25**	**12.5**	**6.25**	**3.125**
Escherichia coli	Inhibition	-	2	4	6	8	10
Staphylococcus aureus	Zone Diameter	7	5	-	-	-	-
Pseudomonas aeruginosa	(IZD)in mm	8	7	6	5	2	-

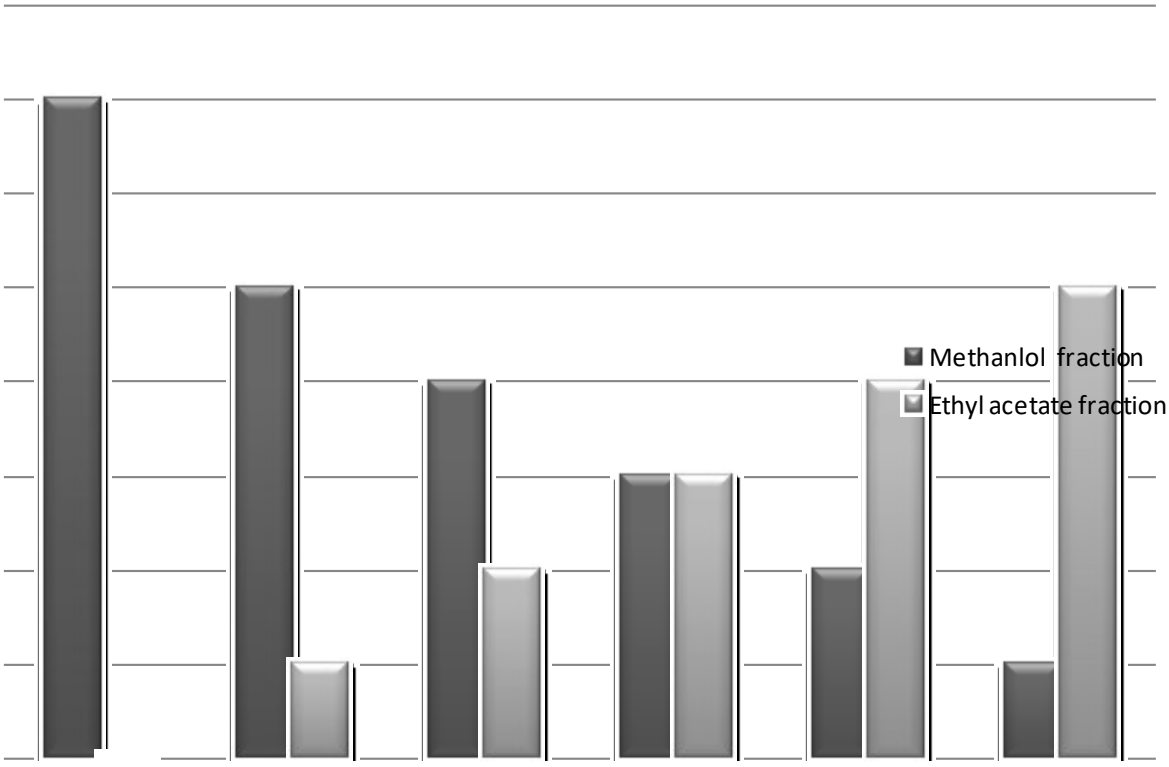

Figure 3: Comparison of the zones of inhibition of methanol and ethyl acetate extracts on *Escherichia coli*

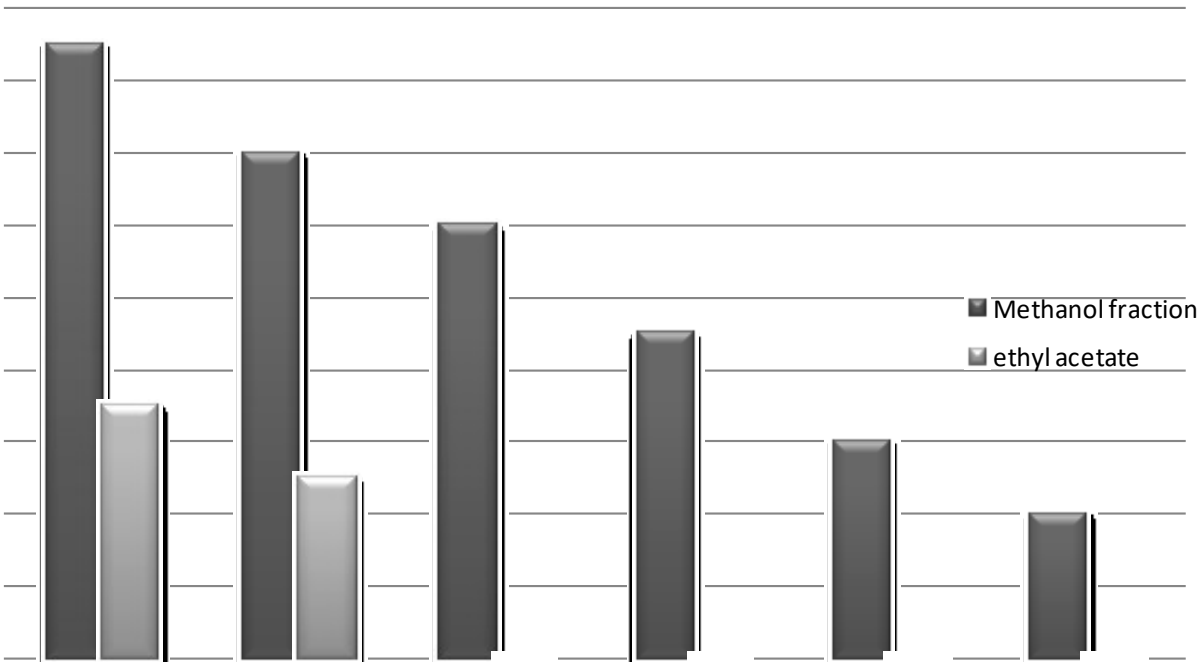

Figure 4: Comparison of the zones of inhibition of methanol and ethyl acetate extracts on *Staphylococcus aureus*

Table 6: Determination of Minimum Inhibitory Concentration (MIC) of Methanol extract on *Escherichia coli*

Concentration (mg/ml) C	Log C	Inhibition zone diameter IZD (mm)	IZD^2
100	2	14	196
50	1.69	10	100
25	1.39	8	64
12.5	1.09	6	36
6.25	0.79	4	16
3.125	0.49	2	4

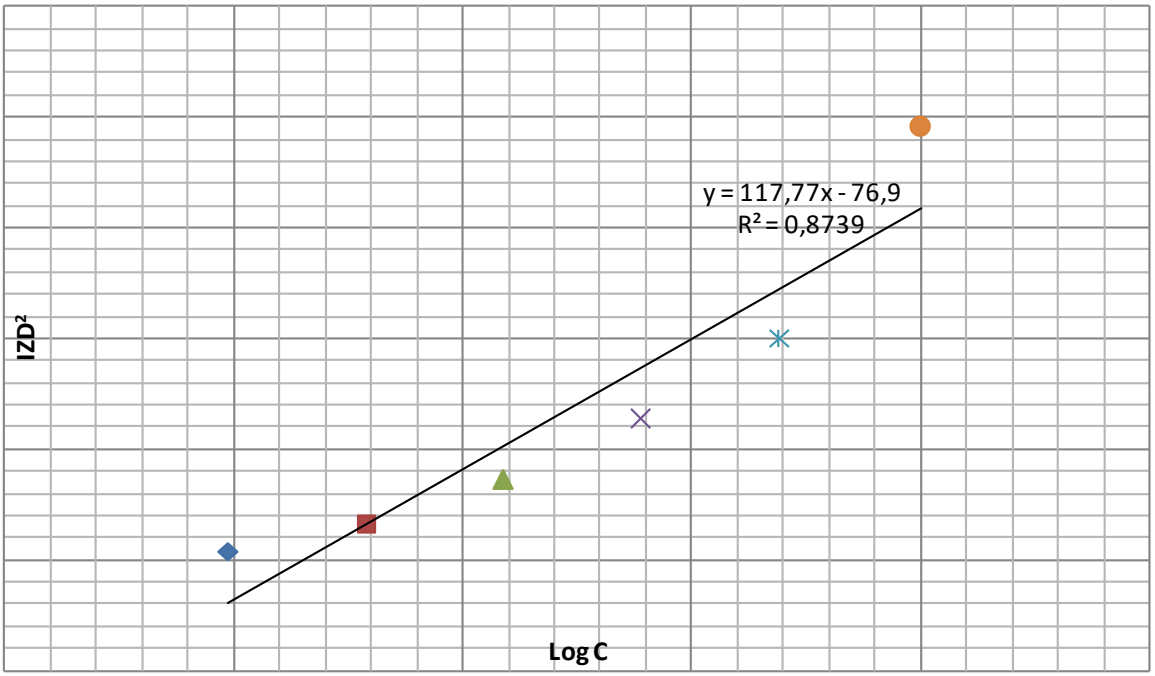

Figure 5: Minimum Inhibitory Concentration for Methanol Extract *Escherichia coli*

Table 7: Determination of Minimum Inhibitory Concentration (MIC) of Methanol extract on *Staphylococcus aureus*

Concentration (mg/ml) C	Log C	Inhibition zone diameter IZD (mm)	IZD^2
100	2	17	289
50	1.69	14	196
25	1.39	12	144
12.5	1.09	9	81
6.25	0.79	6	36
3.125	0.49	4	16

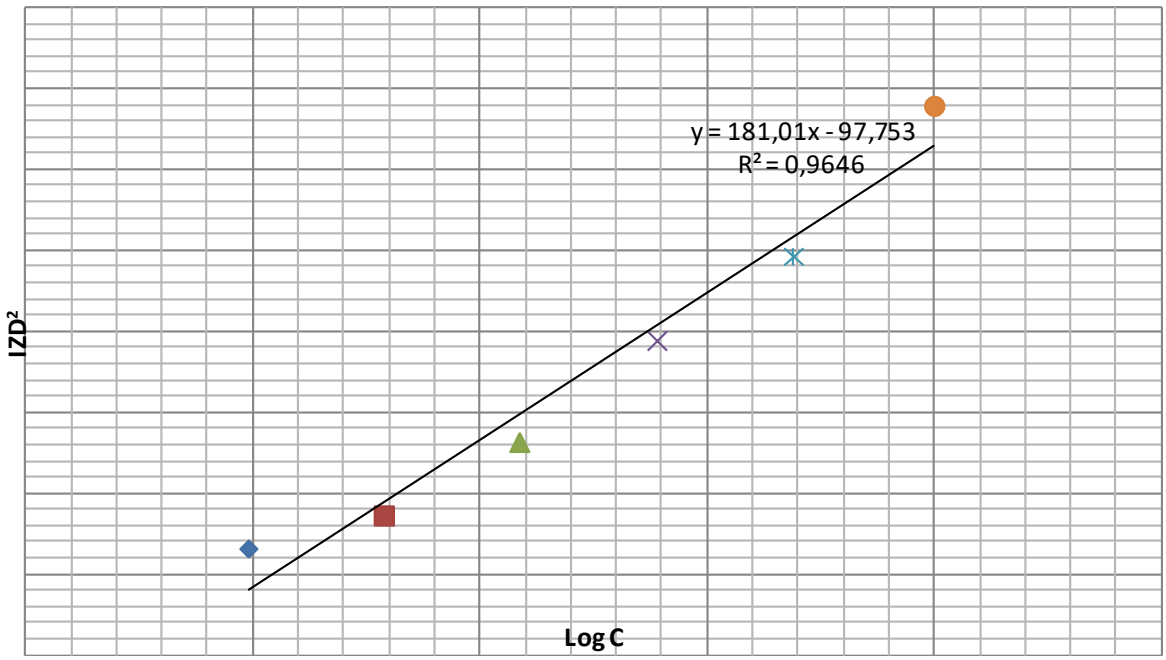

Figure 6: Minimum Inhibitory Concentration for Methanol Extract *Staphylococcus aureus*

Table 8: Determination of Minimum Inhibitory Concentration (MIC) of Methanol extract on *Pseudomonas aeruginosa*

Concentration (mg/ml)	C	Log C	Inhibition zone diameter IZD (mm)	IZD^2
100		2	-	-
50		1.69	-	-
25		1.39	-	-
12.5		1.09	-	-
6.25		0.79	-	-
3.125		0.49	-	-

Table 9: Determination of Minimum Inhibitory Concentration (MIC) of Ethyl Acetate extract on *Escherichia coli*

Concentration (mg/ml)	C	Log C	Inhibition zone diameter IZD (mm)	IZD^2
100		2	-	-
50		1.69	2	4
25		1.39	4	16
12.5		1.09	6	36
6.25		0.79	8	64
3.125		0.49	10	100

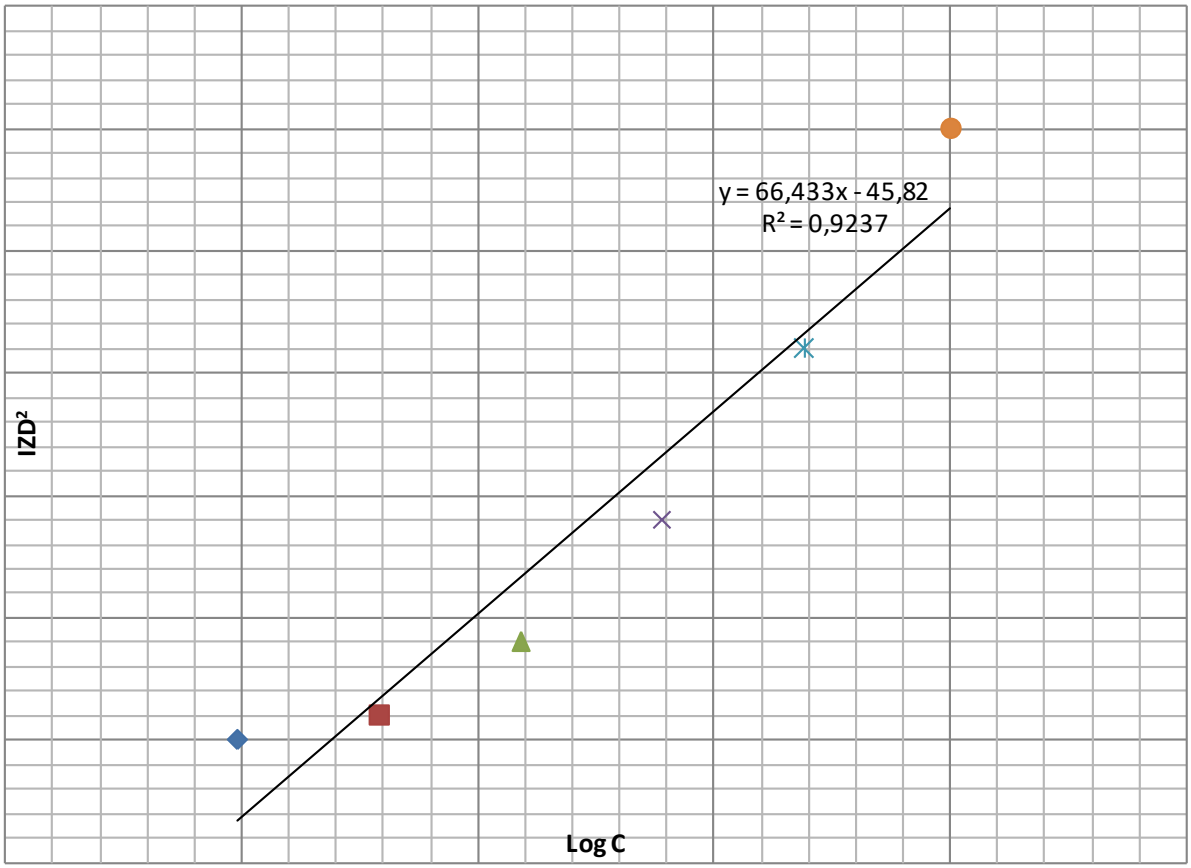

Figure 7: Minimum Inhibitory Concentration for Ethyl acetate extract *Escherichia coli*

Table 10: Determination of Minimum Inhibitory Concentration (MIC) of Ethyl Acetate Extract on *Staphylococcus aureus*

Concentration C (mg/ml)	Log C	Inhibition zone diameter IZD (mm)	IZD^2
100	2	7	49
50	1.69	5	25
25	1.39	-	-
12.5	1.09	-	-
6.25	0.79	-	-
3.125	0.49	-	-

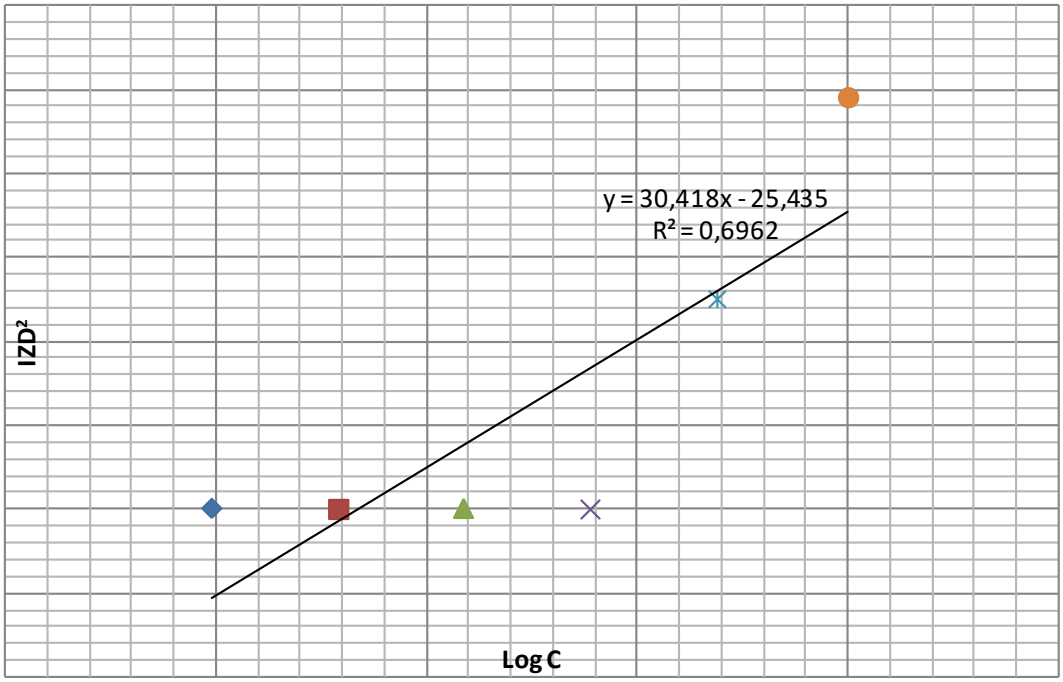

Figure 8: Minimum Inhibitory Concentration for Ethyl Acetate Extract *Staphylococcus aureus*

Table 11: Determination of Minimum Inhibitory Concentration (MIC) of Ethyl Acetate Extract on *Pseudomonas aeruginosa*

Concentration (mg/ml)	C	Log C	Inhibition zone diameter IZD (mm)	IZD^2
100		2	8	64
50		1.69	7	49
25		1.39	6	36
12.5		1.09	5	25
6.25		0.79	2	2
3.125		0.49	-	-

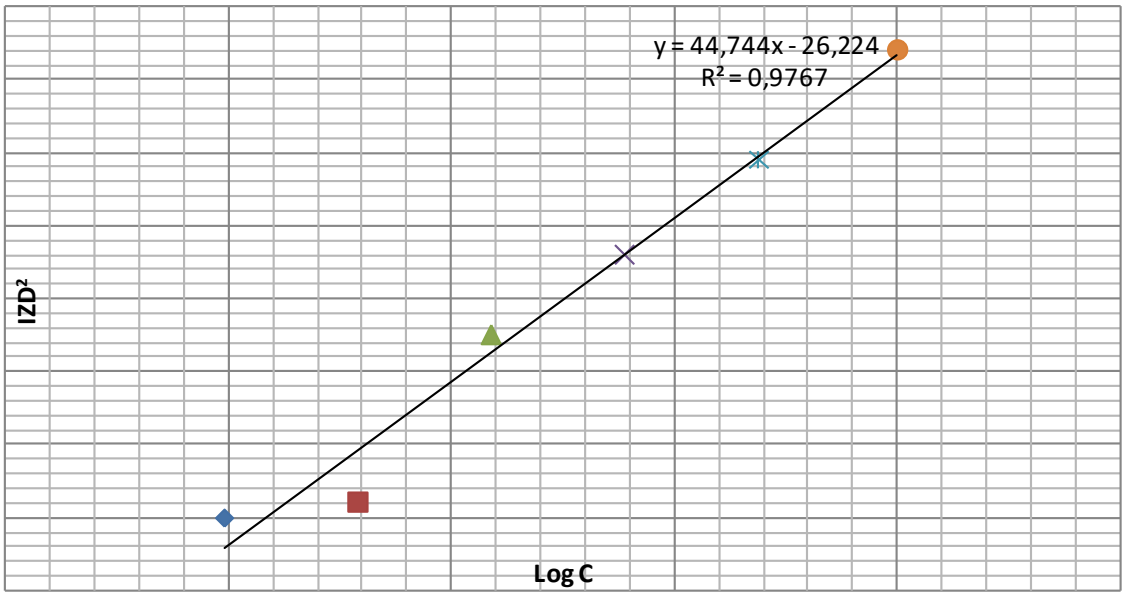

Figure 9: Minimum Inhibitory Concentration for Ethyl Acetate Extracts *Pseudomonas aeruginosa*

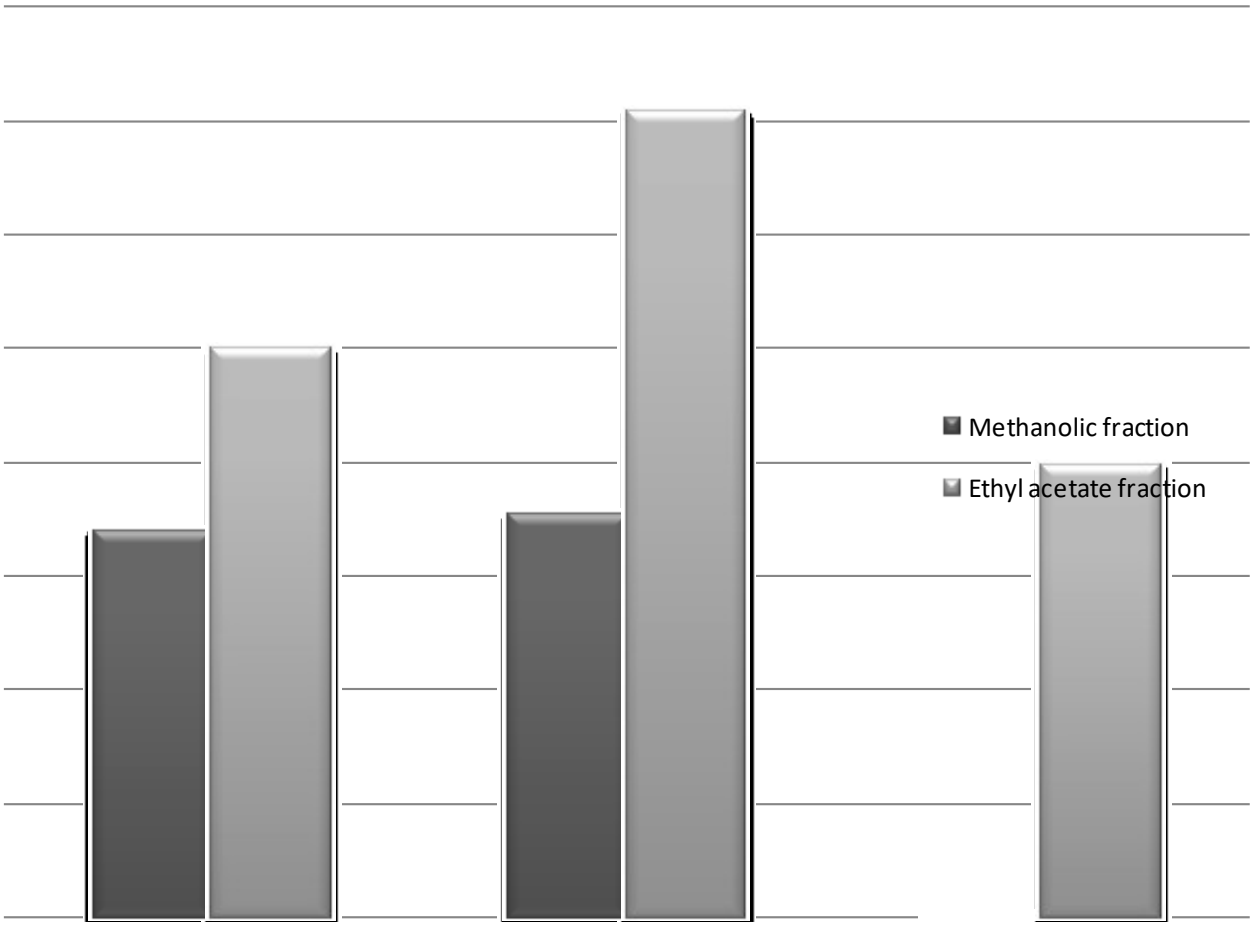

Figure 10: Comparison of MIC for the methanolic and ethyl acetate fractions on the test organisms.