Antibacterial Activity of the Ethanolic Root Bark Extract of *Plumbago zeylanica* (Linn.)

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Abstract: **-This study was carried out to investigate the antibacterial activity of the ethanolic root bark extract of *Plumbago zeylanica* against seven bacteria isolated from two dumpsites in the city of Akure. Agar well diffusion method was used for this study. It was revealed that the ethanolic root bark extract of *Plumbago zeylanica* seems promising since it showed antibacterial activity against the tested bacteria (both Gram positive and Gram negative). From this study, it was recorded that the antibacterial activity of the extract increased with increasing concentration i.e. the concentration of 0.5g/ml showed the highest antibacterial activity against the tested bacteria. The highest zone of inhibition of 12.57mm was observed in *Serratia* spat the concentration of 0.5g/ml. Upon completion of this study, it is therefore recommended that more research work should be carried out on this plant in order to develop alternative antibacterial drugs for the treatment of diseases caused by bacteria.**

***Keywords*- Antibacterial activity, Medicinal plants, dumpsites, *Serratia* spp, Bacteria, *Plumbago zeylanica,* Traditional, Ethanolic, Root bark extract.**

I. INTRODUCTION

Over the past few decades, there has been much interest in natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants have been tested. Many reports show the effectiveness of traditional herbs against microorganisms; as a result, plants have become one of the basis of modern medicine [1]. Medicinal plants are the local heritage with global importance. The world is endowed with a rich wealth of medicinal plants [2]. Medicinal plants are the main constituents of many of drugs of Indian system of medicine [3]. Natural products play an important role in drug development programmes in the pharmaceutical industry [4]. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, particularly from plants. Various medicinal plants have been used for years in daily life to treat diseases all over the world. Medicinal plants represent a rich source of antimicrobial in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties [5].

Traditionally, *P. zeylanica* is used as a stimulant digestant, expectorant, laxative and in the treatment of muscular pain and rheumatic diseases. In India, it is usually used to treat fever or malaria. According to [6], the flowers are used as digestant. The leaves are caustic, vesicant, aphrodisiac, good for scabies stimulant and are also used in treating sore and swelling [7]. They are used to treat infections and digestive problems such as dysentery. [8] recorded that in West Africa the root or the leaves crushed with lemon juice, are used as a counter-irritant and vesicant. In Nigeria, the roots pounded with vegetable oil are used as a treatment for rheumatic swellings. Powdered bark, root or leaves are used as a conventional method to treat gonorrhoea, syphilis, tuberculosis, rheumatic pain, swellings and wounds treatment system in Ethiopia. In other regions of Africa a paste of the root in vinegar, milk and water is used to treat influenza and black water fever; root infusion is taken orally to treat shortness of breath; root decoction with boiled milk is swallowed to treat inflammation in the mouth, throat and chest. In Mauritius and Rodrigues, a root decoction is also used to treat diarrhoea and dyspepsia. The roots and leaves of *P. zeylanica* are widely used medicinally in India and China. Traditionally, *P. zeylanica* is believed to kill intestinal parasites, and it is used clinically to treat rheumatism, intestinal parasites, anaemia due to "stagnant blood", external and internal trauma, toxic swelling and malignant furunculous scabies [9].

The study aims to test the antibacterial activity of *Plumbago zeylanica* ethanolic root bark extract against the bacteria isolated from two dumpsites in the city of Akure.

II. MATERIALS AND METHODS

The fresh roots of *Plumbago zeylanica* were collected from Lujomu, Ayeyemi street in Ondo town after proper identification.

Preparation of Extract

The ethanolic root bark extract of *Plumbago zeylanica* was prepared by properly washing the roots and then allowing them to air dry for thirty minutes. The bark of these roots were removed, air dried and then pulverized into fine powder using a blender, after which 60g of the fine powder was soaked in 300ml ethanol for 3 days. The extract was concentrated in rotary evaporator, exposed to the air in the laboratory for 3 days in order to allow for the complete evaporation of the ethanol present and then stored in glass vials. Appropriate concentrations of the extract were made in Dimethyl Sulphoxide (DMSO) for the experiments.

Phytochemical Screening

Phytochemical is a natural bioactive compound found in plant that is formed during plant's normal metabolic process. These chemicals are often referred as "Secondary metabolities". They include alkanoids, flavonoids, carbohydrates, tannins, terpenoids, phenols and so on.

Plants are considered as bioreactors or biosynthetic laboratories as they synthesize a wide range of characteristic therapeutically important molecules in the form of secondary metabolites. Thus, a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation. For preliminary phytochemical analysis, the extract was subjected for preliminary screening using the standard procedure for identifying various phytoconstituents. The phytochemical screening was carried out as described by [10].

Determination of the Antibacterial Activity of the ethanolic root bark extract of Plumbago zeylanica

Agar well diffusion method was employed to ascertain the antibacterial activity of the extract against the bacteria that were isolated from the dumpsites. The pure culture of the bacteria strains were each picked with a sterile inoculating loop into the test tubes containing 10ml nutrient broth and labelled appropriately. The test tubes were incubated at 37ºC for 24 hours. After incubation, pour plate method was carried out by transferring 1ml broth from the test tubes into each Petri dish, after which Mueller Hinton agar was poured. Five wells were then bored in each Petri dish using ethanol sterilized cork borer (8mm) and then the extract (i.e. in varying concentrations of 0.1g/ml, 0.2g/ml, 0.3g/ml, 0.4g/ml and 0.5g/ml) was added into the four wells. Chloramphenicol (0.5g/ml) which is the positive control was added to the fifth well, the Petri dishes were incubated at 37°C for 18-24h. DMSO was used as negative control.

Measurement of the Zones of Inhibition

After incubation, the zones of inhibition on each Petri dish was measured in millimetres (mm).

Statistical Analysis

Statistical Package for Social Sciences (SPSS) was used to analyze the data obtained, and where significant differences occur, the means were separated using Duncan's New Multiple Range Test (DNMRT).

III. RESULTS

Phytochemical Screening of the extract of Plumbago zeylanica

The result of the phytochemical screening of the ethanolic root bark extract of *Plumbago zeylanica* is presented in Table 1. Six of the phytochemicals that were tested for were present in the ethanolic extract namely Saponins, Tannins, Flavonoids, Steroid, Terpenoids and Alkaloids while Phlobatannins and Anthraquinone were absent. Also the tests carried out for the presence of cardiac glycoside gave positive results.

Table 1: Phytochemical Screening of the extract of *Plumbago zeylanica*

CONSTITUENTS	
Saponins	+
Tannins	+
Phlobatannins	_
Flavonoids	+
Steroids	+
Terpenoids	+
Alkaloids	+
Anthraquinone	_
CARDIAC GLYCOSIDE	
Legal test	+
Keller kiliani test	+
Salkwoski test	+
Lieberman test	+

(+ represents present while – represents absent).

Antibacterial Activity of the Root Bark Extract of Plumbago zeylanica against the Bacteria Isolates.

The result of the antibacterial activity of the root bark extract of *Plumbago zeylanica* against the bacteria isolates is presented in Table 2. *Pseudomonas aeruginosa* exhibited the highest resistance to the extract at the highest extract concentration of 0.5g/ml i.e. *P. aeruginosa* yielded the lowest zone of inhibition. The zones of inhibition obtained using the control (20.87±0.24mm) and the different concentrations of the extract are significantly different from one another at $p<0.05$ for *P. aeruginosa.*

For *Serratia* sp, the zone of inhibition obtained at 0.1g/ml (2.60±0.10mm) was significantly different from that obtained at 0.2g/ml (8.60±0.06mm) at $p<0.05$. The zones of inhibition obtained at concentrations 0.3g/ml (11.35±0.06mm) and 0.4g/ml (11.28±0.03mm) are not significantly different at $p>0.05$. Also, the zone of inhibition obtained at 0.5g/ml (12.57±0.07mm) is significantly different from the zone obtained when the control, Chloramphenicol (23.73±0.15mm) was used at $p<0.05$. Likewise, this similar result was obtained for *Escherichia coli.*

The zones of inhibition obtained for *Staphylococcus aureus* at 0.1g/ml (1.63±0.09mm) and 0.2g/ml (1.57±0.07mm) are not significantly different at $p>0.05$, whereas there was a significant difference in the zones obtained using the extract concentrations of 0.3g/ml (2.78±0.03mm), 0.4g/ml (3.82±0.07mm), 0.5g/ml (11.13±0.13mm) and the control (22.20±0.53mm) at $p<0.05$.

For *Streptococcus* sp, there was a significant difference in the zones of inhibition obtained using extract concentrations of

0.1g/ml (5.82±0.07mm), 0.2g/ml (6.78±0.03mm), 0.3g/ml (11.10±0.06mm) and the control (24.07±0.23mm) at $p<0.05$. The zones of inhibition obtained at 0.4g/ml (11.78±0.03mm) and 0.5g/ml (12.00±0.00mm) are not significantly different at $p>0.05$.

The zones of inhibition obtained using the control and the different concentrations of the extract are significantly different from one another at $p<0.05$ for *Klebsiella* sp.

Lastly, there was no significant difference in the zones of inhibition obtained for *Bacillus* sp using the extract concentrations of 0.1g/ml (8.28±0.03mm) and 0.2g/ml (8.82±0.07mm) at $p>0.05$, whereas there was a significant difference at $p<0.05$ when extract concentrations of 0.3g/ml (9.28±0.03mm), 0.4g/ml (9.82±0.07mm), 0.5g/ml (11.00±0.00mm) and the control (21.37±0.61mm) were used.

Table 2: Antibacterial Activity of the Root Bark Extract of *Plumbago zeylanica* against the bacteria isolates.

Concentration	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Control	20.87 ± 0.24^{f}	23.73 ± 0.15^{e}	23.00 ± 0.58^{e}	22.20 ± 0.53^{e}	24.07 ± 0.23^{e}	24.80 ± 0.95^{f}	21.37 ± 0.61^{e}
0.1g/ml	2.10 ± 0.06^{a}	2.60 ± 0.10^{a}	2.10 ± 0.10^{a}	1.63 ± 0.09^{a}	5.82 ± 0.07^{a}	2.82 ± 0.07^{a}	8.28 ± 0.03^{a}
0.2g/ml	5.53 ± 0.03^{b}	8.60 ± 0.06^{b}	4.07 ± 0.07^{b}	1.57 ± 0.07^{a}	6.78 ± 0.03^{b}	4.03 ± 0.03^{b}	8.82 ± 0.07^{ab}
0.3g/ml	6.85 ± 0.06^{c}	11.35 ± 0.06^{c}	5.32 ± 0.07^{c}	2.78 ± 0.03^{b}	11.10 ± 0.06^{c}	5.53 ± 0.03^{c}	9.28 ± 0.03^{d}
0.4g/ml	7.50 ± 0.00^{d}	11.28 ± 0.03^{c}	5.32 ± 0.07^{c}	3.82 ± 0.07^{c}	11.78 ± 0.03^{d}	9.32 ± 0.07^{d}	9.82 ± 0.07^{c}
0.5g/ml	7.85 ± 0.06^{e}	12.57 ± 0.07^{d}	11.13 ± 0.09^{d}	11.13 ± 0.13^{d}	12.00 ± 0.00^{d}	10.57 ± 0.07^{e}	11.00 ± 0.00^{d}

Note: Means followed by the same letter in column are not significantly different ($p>0.05$) from each other using Duncan's New Multiple Range Test (DNMRT).

A- *Pseudomonas aeruginosa,* B- *Serratia* sp, C- *Escherichia coli,* D- *Staphylococcus aureus,* E- *Streptococcus* sp, F- *Klebsiella* sp, G- *Bacillus* sp

Effect of different concentrations of the ethanolic root bark extract of P. zeylanica on the bacterial isolates.

The effect of different concentrations of the ethanolic root bark extract of *P. zeylanica* on the bacterial isolates is represented in Table 3. As the concentration of the extract increased, there was a corresponding increase in the zone of inhibitions exhibited by the different bacteria isolates.

At 0.1g/ml, the plant extract was more effective against *Streptococcus* sp and *Bacillus* spproducing zones of inhibition of 5.82±0.07mm and 8.28±0.03mm respectively. At the extract concentration of 0.2g/ml, the plant extract was more potent against *Serratia* sp, *Streptococcus* spand *Bacillus* sp with zones of inhibition of 8.60±0.06mm, 6.78±0.03mm and 8.82±0.07mm respectively. Similarly at 0.3g/ml, the ethanolic root bark extract of *P. zeylanica* was most effective against *Serratia* sp, *Streptococcus sp* and *Bacillus sp* with zones of inhibition of 11.35±0.06mm, 11.10±0.06mm and 9.28±0.03mm respectively. The extract concentration of 0.4g/ml was very effective against *Pseudomonas aeruginosa, Serratia* sp, *Streptococcus* sp, *Klebsiella* sp and *Bacillus* sp which yielded zones of inhibition of 7.50±0.00mm, 11.28±0.03mm, 11.78±0.03mm, 9.32±0.07m and 9.82±0.07mm respectively. Lastly at the highest extract concentration of 0.5g/ml, the extract was very effective against all the tested bacteria with *Pseudomonas aeruginosa* having the lowest zone of inhibition of 7.85±0.06mm and *Serratia* sp had the highest zone of inhibition of 12.57±0.07mm.

Table 3: Effect of different concentrations of the ethanolic root bark extract of *P. zeylanica* on the bacterial isolates

Concentration	A	B	C	D	E	F	G
Control	20.87 ± 0.24^{a}	23.73 ± 0.15^{cde}	23.00 ± 0.58^{bcd}	22.20 ± 0.53^{abc}	24.07 ± 0.23^{de}	24.80 ± 0.95^{e}	21.37 ± 0.61^{ab}
0.1g/ml	2.10 ± 0.06^{b}	2.60 ± 0.10^{c}	2.10 ± 0.10^{b}	1.63 ± 0.09^{a}	5.82 ± 0.07^{d}	2.82 ± 0.07^{c}	8.28 ± 0.03^{e}
0.2g/ml	5.53 ± 0.03^{c}	8.60 ± 0.06^{e}	4.07 ± 0.07^{b}	1.57 ± 0.07^{a}	6.78 ± 0.03^{d}	4.03 ± 0.03^{b}	8.82 ± 0.07^{f}
0.3g/ml	6.85 ± 0.06^{d}	11.35 ± 0.06^{g}	5.32 ± 0.07^{b}	2.78 ± 0.03^{a}	11.10 ± 0.06^{f}	5.53 ± 0.03^{c}	9.28 ± 0.03^{e}
0.4g/ml	7.50 ± 0.00^{c}	11.28 ± 0.03^{f}	5.32 ± 0.07^{b}	3.82 ± 0.07^{a}	11.78 ± 0.03^{g}	9.32 ± 0.07^{d}	9.82 ± 0.07^{e}
0.5g/ml	7.85 ± 0.06^{a}	12.57 ± 0.07^{e}	11.13 ± 0.09^{c}	11.13 ± 0.13^{c}	12.00 ± 0.00^{d}	10.57 ± 0.07^{b}	11.00 ± 0.00^{c}

Note: Means followed by the same letter in row are not significantly different ($p>0.05$) from each other using Duncan's New Multiple Range Test (DNMRT).

A- *Pseudomonas aeruginosa,* B- *Serratia* sp, C- *Escherichia coli,* D- *Staphylococcus aureus,* E- *Streptococcus* sp, F- *Klebsiella* sp, G- *Bacillus* sp

Figure 1 shows the comparative analysis of the antibacterial activity of the ethanolic root bark extract of *P. zeylanica* with the control at concentration of 0.5g/ml. From the graph, it was revealed that the extract had the least antibacterial activity against *Pseudomonas* aeruginosa. The control had a greater antibacterial activity against all the bacteria, although the extract was also effective in inhibiting the growth of all the bacteria.

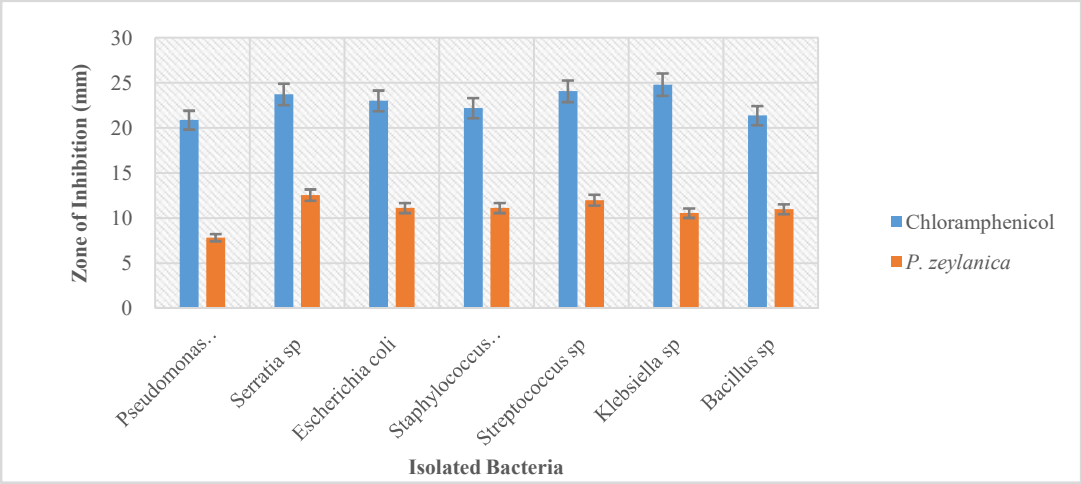

Figure 1: Comparative analysis of the antibacterial activity of the ethanolic root bark extract of *Plumbago zeylanica* at concentration 0.5g/ml with the Control (Chloramphenicol).

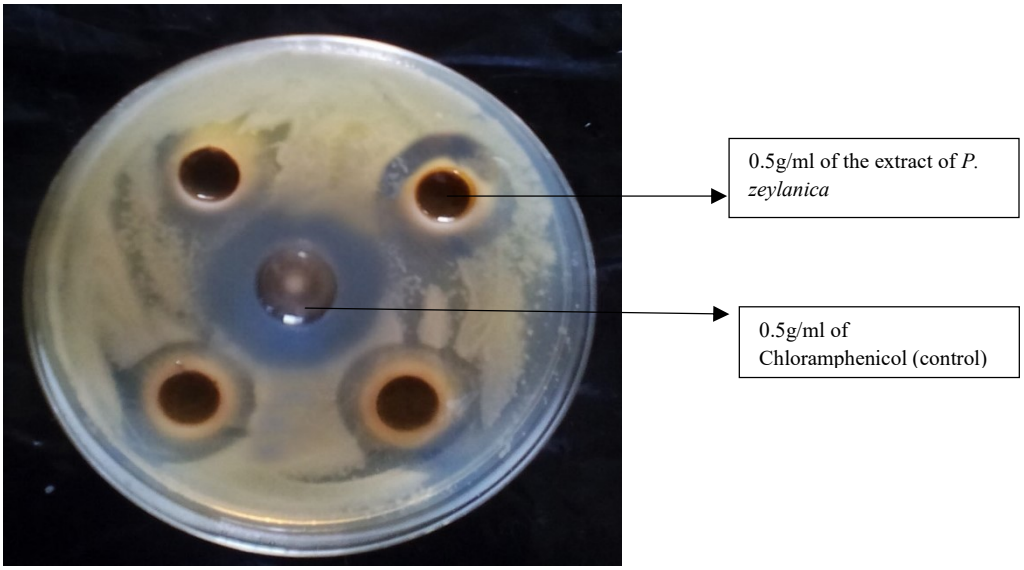

Plate 1: Zone of inhibition on *Bacillus* sp at 0.5g/ml of the extract in replicates

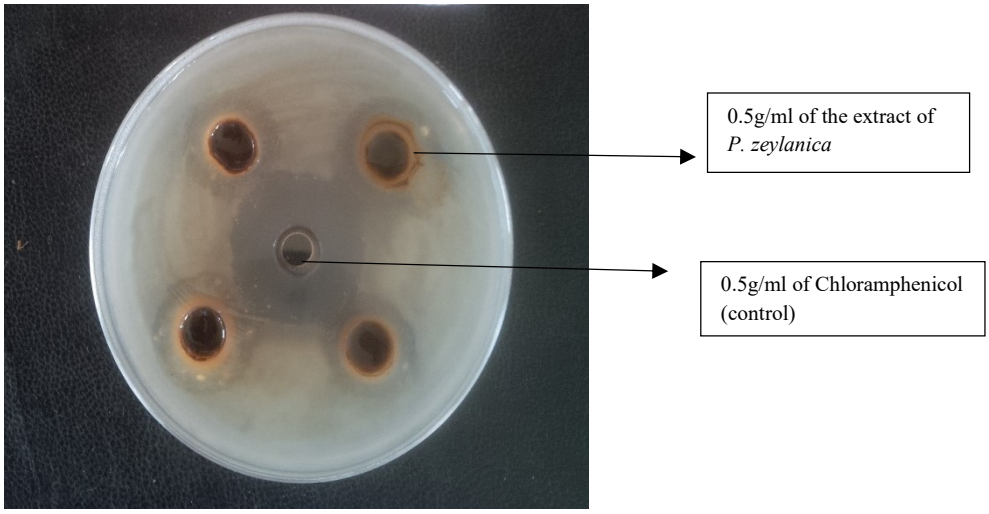

Plate 2: Zone of inhibition on *Klebsiella* sp at 0.5g/ml of the extract in replicates

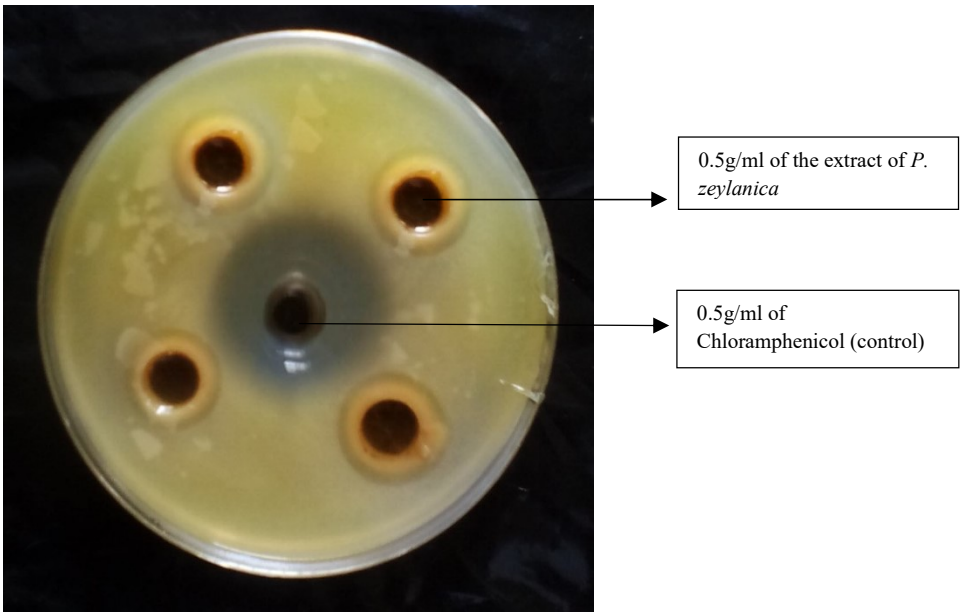

Plate 3: Zone of inhibition on *Pseudomonas aeruginosa* at 0.5g/ml of the extract in replicates

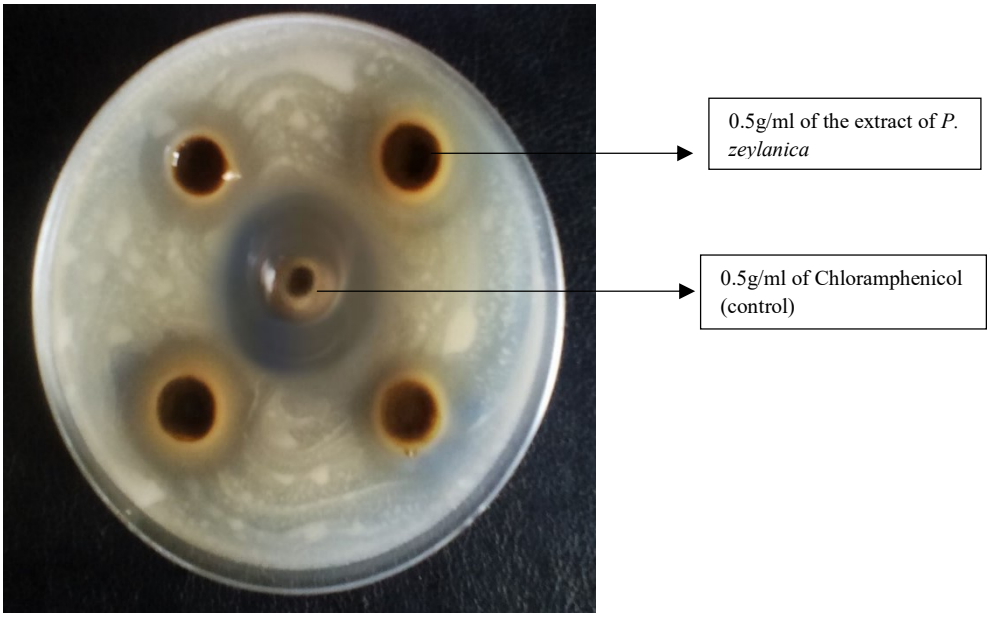

Plate 4: Zone of inhibition on *Serratia* sp *aeruginosa*at 0.5g/ml of the extract in replicates

IV. DISCUSSION

The result of the phytochemical analysis carried out on the root bark extract of *Plumbago zeylanica* revealed the presence of Saponins, Tannins, Flavonoids, Steroids, Terpenoids and Alkaloids, while Phlobatannin and Anthraquinone were absent. This result correlates with that of[11] who also revealed the presence of Saponins, Tannins, Flavonoids, Steroids, Terpenoids and Alkaloids in the ethanolic root bark extract of *P. zeylanica.*

Results obtained in this present study revealed that the ethanolic root bark extract seems promising since it showed antibacterial activity against the tested bacteria (both gram positive and gram negative). It was recorded that the antibacterial activity of the extract increased with increasing concentration i.e. the concentration 0.5g/ml showed the highest antibacterial activity against the tested bacteria. The highest zone of inhibition of 12.57mm was observed in *Serratia* spat the concentration of 0.5g/ml. [12] observed in his experiment that *Bacillus sp, Escherichia coli*, *Klebsiella* spand *Staphylococcus aureus* were sensitive to the ethanolic root bark extract of *Plumbago zeylanica*. In a study by [13],he observed that the ethanolic root bark extract of *Plumbago zeylanica* exhibited strong antibacterial against *Escherichia coli* and *Staphylococcus aureus.* Also, [14] also observed the effectiveness of this extract against *Escherichia coli*. Likewise

in the antimicrobial studies on *Plumbago zeylanica* L. carried out by [11], it was observed that this extract inhibited the growth of *Staphylococcus aureus and Bacillus sp.*

V. CONCLUSION

The ethanolic root bark extract of *Plumbago zeylanica* had a potential antibacterial activity against the majority of the bacteria found at the dumpsites. It is therefore recommended that the use of medicinal plants should be further encouraged as they possess various antibacterial properties.

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