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Effect of Iron, Copper and Zinc metal ions on the antibacterial activity of crude extract of *Aloe pulcherrima* And *Rumex abyssinicus* leaves

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EFFECT OF IRON, COPPER AND ZINC METAL IONS ON THE ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACT OF *Aloe pulcherrima* AND *Rumex abyssinicus* LEAVES

THESIS SUBMITTED TO SCHOOL OF GRADUTE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY (INORGANIC)

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Declaration

I declare that this is my original work, except where reference is made, and has never been submitted anywhere for award of any degree or diploma in any university.

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This M.Sc Thesis has been submitted with our approval as supervisors

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TABLE OF CONTENTS

CONTENTS PAGE

LIST OF TABLES

Tables Pages

LIST OF APPENDIX

LIST OF ABRIVATIONS

AMR	Antimicrobial Resistance
CPE	Crude Plant Extract
DMSO	Dimethyl Sulfoxide
LAF	Laminar Air Flow
MIC	Minimum Inhibitory Concentration
NA	Nutrient Agar
ZOI	Zone of Inhibition

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ABSTRACT

The aim of this study was to evaluate the antibacterial activity of the crude leaf extracts of *Aloe pulcherrima* and *Rumex abyssinicus* in the absence and presence of iron, copper and zinc metal ions from their salts $ZnSO_4$, $FeSO_4$ and $CuSO_4$ respectively with specific charges and fixed concentration on three bacterial species (*E. coli, Bacillus* and *S. aureus*). According to result basis the potency of chelated fractions showed averagely higher activity than that of unchelated fractions. Thus, the anti-bacterial activity of four solvent fractions was enhanced upon the addition of metal ions. Maximum ZOI (17 and 16.67 mm) was recorded in the case of *Aloe pulcherrima* for ethyl acetate extract up on of addition of zinc metal ion against gram positive bacteria (*Bacillus* and *S. aureus*) respectively; and for *Rumex abyssinicus* highest ZOI (15.33 mm) was recorded for ethyl acetate and methanol extract in the presence of Zn metal ion against *S. aureus*, which was done by agar disc diffusion method. This result was progressive as compared with the standard drug gentamycin (15.67 mm) and brought supportive information on the effect of metal ions on medicinal plants for future investigations. Also the phytochemical analysis and Uv-vis spectroscopic result showed the presence of secondary metabolites in both samples which were responsible for antibacterial activity.

1. INTRODUCTION

Bacterial infections are serious health and economic problems on the developing and developed countries. The severity of bacterial infection is due to the resistance of bacteria on currently available antibiotics [1]. An antimicrobial or antibiotics is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan's. Antimicrobial drugs either kill or prevent the growth of microbes. For a long period of time antibiotics drug are have been used as a preventive way to control the spread of bacterial infection disease. But, emerging bacterial infection and antimicrobial resistance against on the presently used antibiotics are some of most serious health threats to the world [2]. Antimicrobial resistance (AMR) is the development of resistance of a microorganism (bacteria, viruses, fungi and parasites) to an antimicrobial medicine to which it was previously sensitive. Due to the above reasons, the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance exhibited by pathogenic microbial agents search for plant products has increased for their potential antimicrobial activity. Thus, plants are used as a source of new drug discovery based on the traditional use of medicinal plants. Today over 40% of all modern clinical drugs are originated from natural products. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicines derived from medicinal plants for their health care [3]. Different parts of plants, specially herbs and rhizomes have medicinal property due to the presence of different active components like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols found in them [4]. Microbiological experiments and techniques have shown that medicinal plants exhibit antimicrobial properties against bacteria and other pathogens due to the presence of bioactive compounds [5].

The use of natural products for the prevention and treatment of different bacterial infection and other pathologies is continuously expanding throughout the world. [6]. A medicinal plant consists of natural compounds especially secondary metabolites which have been known to exhibit many therapeutic activities. Bioactive polyphenol compounds promote human health. Because due to their wide range of effects, including chelation of metals, besides direct free radical scavenging, polyphenol compounds exert antioxidant activity through interactions with the reduced form of transition metals, like copper, zinc, iron, which participate in free radical generating reactions bioactive polyphenol may sequester metal ions by chelation, thereby

preventing the metal-mediated generation of free radicals, and accordingly may protect the potential biological targets from oxidative stress [7]. Thus, the overall antioxidant action becomes a combination of a direct reaction with free radical and chelating metal. There is huge usage of herbal plants as a traditional health care in Africa countries. In Ethiopia, it is estimated that about 80% of the Ethiopian population is still dependent on traditional medicine, which essentially involves the use of plants. This implies that Ethiopia is a rich source of medicinal plants, for instance *Aloe pulcherrima* and *Rumex Abyssinicus* are some of the polyphenol containing Ethiopian medicinal plants which are commonly used as traditional medicine for the treatment of different ailments. Thus, polyphenol groups from *Aloe pulcherrima* and *Rumex abyssinicus* extracts may prove to be a vital source for antibacterial activity and developing lead molecules having various other biological activities.

Metal ions play a major role specially transition metal ions present in a wide choice for preparing antimicrobial substances in the development of new drugs [8]. Tannins, which are one of the natural products, exhibit strong antioxidant properties in comparison to low molecular weight phenolic compounds. The antioxidant properties of tannins can result from their free radical scavenging activity and from their ability to chelate transition metal ions like Fe (II) and Cu (II [9]. Thus, medicinal plants as well metal ions are used as a base for the development of new antibacterial drug discovery.

Many metals are used for preparing in organic drugs and also metal-polyphenol complexes have good biological and pharmacological activities, due to the effects of the number of *d*-electrons of the first transition metals on the formation of stable complexes. Antioxidant activity of bio-active compounds can result from their ability to chelate bivalent transition metal ions, like iron, copper and zinc. These ions can generate highly reactive •OH radicals by Fenton reaction. Chelating agents, which stabilize pro-oxidative transition metal ions by complexing them. Now a day the world looks for the use of traditional medicinal plants and metal ions complex as an alternative for drug resistant microbes and innovation of new antimicrobial substance [12].

Therefore, there is a need to develop effective and structurally diverse anti-bacterial drugs with new modes of action from medicinal plant by addition of metal ions. *Aloe pulcherrima* and *Rumex abyssinicus* are among the endemic plants in Ethiopia used as traditional medicines for

several diseases. The main objective of this thesis work is to evaluate the effect of metal ions on the crude extract of *Aloe pulcherrima and Rumex abyssinicus* species for antibacterial activity.

1.1 STATEMENT OF THE PROBLEM

Plant based medicines have been used to treat different disease, and plant bio-active compounds are dominant source of organic drugs. Now a day the infection of bacteria and resistance to antibacterial on currently available drug is a series health and economic problem of the world. Thus, new drug development and lead structure need a series attention to overcome these problems. Metal-polyphenol complexes have good biological and pharmacological activities due to these chelated medicinal plants are the sources for new drug discovery. *Aloe pulcherrima* and *Rumex abyssinicus* are among the traditional medicinal plants used for treating several infectious diseases due to the presence of secondary metabolites especially phenolic moieties. Although a number of investigations have been done on antibacterial activities of these plants extract and selected metal ions separately, but their common antibacterial effect has not yet done enough. Therefore, the aim of this investigation was to evaluate the common effect of Zn, Fe and Cu metal ions on the bacterial activity of crude extract of *Aloe pulcherrima* and *Rumex abyssinicus* for antibacterial activities and to lay a foundation for new drug discovery.

1.2. OBJECTIVES

1.2.1. General objective

- The main objective of the study is to evaluate the effect of zinc, iron and copper ions on the bacterial activity of crude extract of *Aloe pulcherrima* and *Rumex abyssinicus.*

1.2.1 Specific objectives

- To extract crude of A*loe pulcherrima and Rumex abyssinicus* using different solvents.
- To assess *in vitro* antibacterial activities (*bacillus, S. aureus,* and *E. coli*) of the crude leaf extracts of *Aloe pulcherrima* and *Rumex abyssinicus*,
- To evaluate the synergistic effect of crude extract and metal ion on antibacterial activities.
- To identify the phytochemical constituents in the plant extract by phytochemical analysis parameters.

SIGNIFICANCES OF THE STUDY

Many of the bioactive compounds are derived from plants and used for traditional healing. The bioactive phytochemical constituents present in the plant plays a significant role in the development of medicines and drug discovery, but resistance of bacterial infection on currently available antibacterial drug in the market is a series problem. Thus, new drug development and lead structure need a series attention to overcome these problems. Accordingly, the objective of this study was to evaluate the effect of metal ions (Zn^{2+}, Cu^{2+}, and Fe^{2+}) for antibacterial activity of crude extracts of *Aloe pulcherrima* and *Rumex abyssinicus*. The outcome of this will be useful to lay down the foundation for new drug discovery and helps to give basic information on metal-traditional medicinal plant interaction for further study.

2. REVIEW OF RELATED LITRATURE

2.1 *Aloe pulcherrima*

The genus *Aloe* is a succulent plant widely used as a medicine. There are at least 420 deferent plant species of *Aloe*. Among this *Aloe pulcherrima* is one of the endemic *Aloe* species traditionally used the treatment of wounds and burns, anti-oxidant. *Aloe Vera* specifically refers to the *Aloe barbadensis* Miller plant, which is the most common form used in Aloe-based products. Traditionally, the clear gel from the *Aloe* plant is rubbed on the skin as an ointment to treat wounds and burns. The green part of the leaf can be made into a juice or dried and taken orally as a laxative. *Aloe* species is used in many commercial products in various forms, including drinks, concentrates, capsules, powders and as a flavor [14].

Figure 1 Picture of *aloe* leaf image.

2.1.1 Medicinal value of *Aloe*

The genus *Aloe* has a long medicinal history since 1500 B C. The clear gel from the *Aloe* plant *(leaf part mostly)* is rubbed on the skin as an ointment to treat wounds and burns. Four species, specifically *Aloe barbadensis* Miller (commonly known as Aloe Vera), *Aloe ferox* (*Cape Aloe*), *Aloe arborescens* (*Candelabra Aloe*) and *Aloe Perry* baker (*Perry's Aloe*), have had their ethno-pharmacological usage particularly well documented. Of these, *Aloe Vera* and some others, such as *A. ferox* have been used for a long time in folk medicine for the treatment of constipation, burns and dermatitis [15].

Aloe pulcherrima has antibacterial activity due to the presence of anthraquinones such as aloin, emodin, saponins, and salicylic acid which increase blood flow to wounded areas and cell growth activity. Crude extract of *Aloe vera* had antibacterial effects against clinically isolated bacterial pathogens, and so does *Aloe pulcherrima* [16].

Recently, some species of *Aloe* such as, *A. trichosantha, A. pubescens, A. citrine, A. bertemariae, A. eumassawana* and *A. scholleri,* have been used in a wide range of skin and hair care products, and also form the basis of health drinks and tonics. The slimy gel inside the leaves consists of a complex mixture of polysaccharides, amino acids, minerals, trace elements and other biologically active substances, such as enzymes. *Aloe* species have been the source of laxative drugs, the main purgative principle being an anthrone-C-glycoside, aloin, which occurs at levels of between 18% and 30% of the dried product [17].

Aloe pulcherrima is an evergreen, succulent perennial shrub with a prostrate of pendant habit. It produces a rosette of leaves on top of a usually un branched stem that can be up to 1m long and 8 cm in diameter. *Aloe pulcherrima* is one of the endemic *Aloe* species traditionally used for the treatment of malaria and wound healing in central, Southern and Northern part of Ethiopia. [19]

2.1.2 Phenolic groups in Aloe species

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. For instance, anthraquinone (1) and anthraquinone derivatives are the main characteristic functional groups in Aloe species and biologically active. Some of them are Chrysophanol based anthraqunione (1) types are known to occur both in leaves and roots of Aloe species. Chrysophanol (2) and related anthraquinone derivatives such as helminthosporin (3) and isoxanthorin (4) are detected in both roots and leaves of most of the *Aloe* species. [20-22]. whereas, aloe-emodin (5), nataloe-emodin (6), nataloe-emodin-8-methyl ether (7), including its O-glycosides, O-α-L-rhamnopyranosylaloe-emodin (8) and nataloe-emodin-2-O-β-D-glucopyranoside (9) are the main constituents of the leaves of Aloe species [23-25].

Polyphenols are used for the prevention and cure of various diseases which is mainly associated with free radicals [26]. Aloesaponarin I (10), Laccaic acid di-methyl ester (11) and deoxyerythrolaccin (12) were reported to have anti-microbial activities [27], and occur in the

roots of many *Aloe* species including *A. saponaria* [28]. Different types of Phenolics are presented in figure 2 as follows.

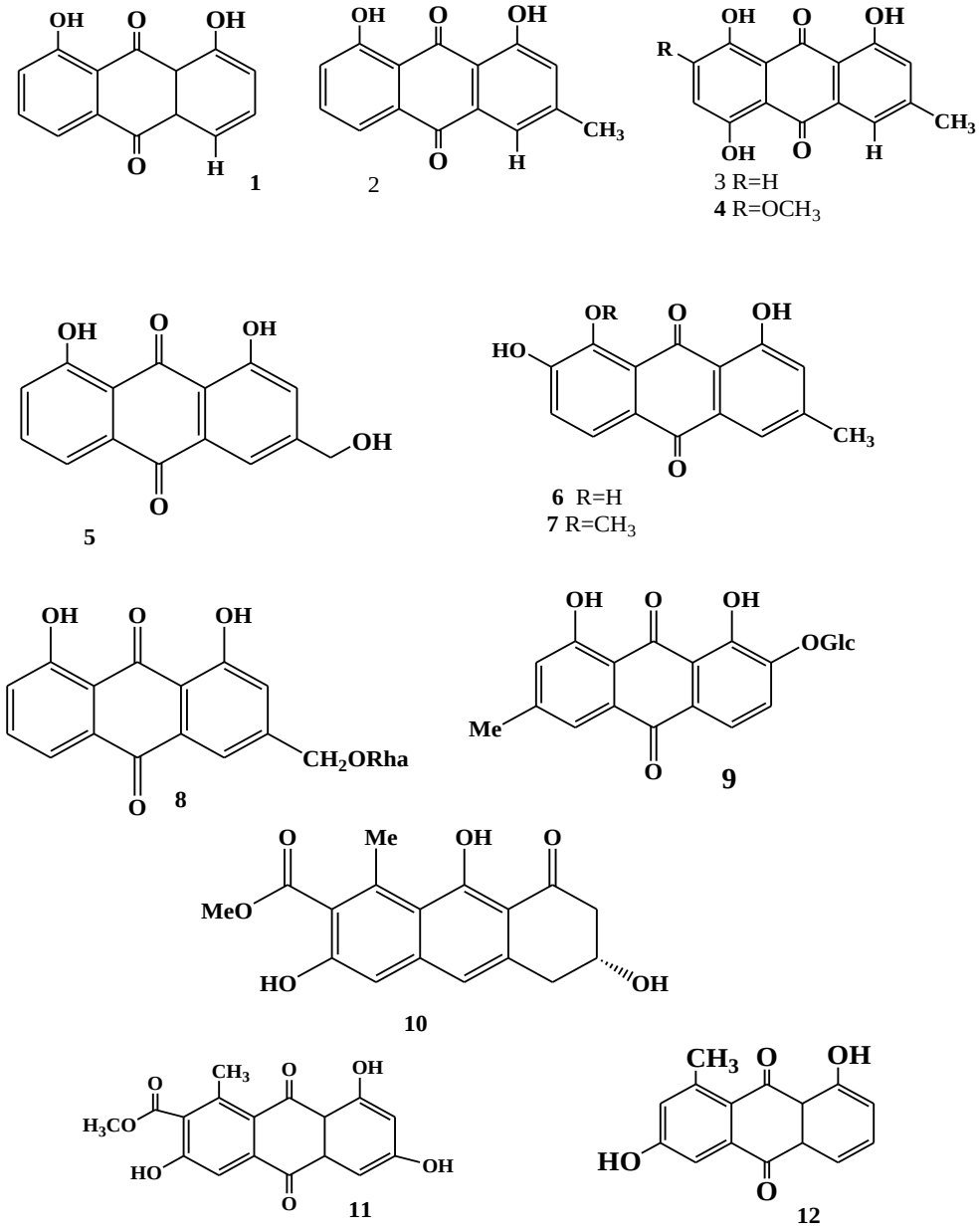


Figure 2 Some of phytochemicals isolated from aloe species

2.2 *Rumex abyssinicus*

The genus *Rumex* is found to be distributed worldwide. It belongs to the Polygonaceae family [31]. This genus includes more than 250 species. *Rumex abysinicus* is one of the endemic *Rumex* species found in Ethiopia. *Rumex abyssinicus* (polygonaceae) is a large annual herb up to 4 m high, leaves usually sagittate, inflorescence much branched, leafless panicle, nut light brown. These plants called in Amharic as Mekmako [32].

Figure 3 picture of *Rumex abyssinicus* plant.

This species is a perennial plant that grows abundantly in the wild. The fleshy to leathery leaves form a basal rosette at the root have minor leaf veins, and the leaf blade margins are entire [33]. It was reported that the whole plant is used as medicine such as for the treatment of gonorrhea, lung T.B., leprosy, fever, liver disease, hypertension, hemorrhoids, scabies, antiemetic, aphrodisiac, cough, rabies, rheumatism and migraine. If taken in large quantities then could produce toxic effect because of their oxalate contents [32]. There is no enough report about secondary metabolites and antioxidant potential of *Rumex* leaves. This feature, allied to the importance of the oxidative stress in the pathogenesis of various diseases, promoted us to better evaluate the potential antioxidant properties of this plant [34].

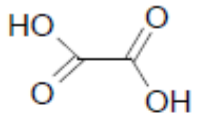
The polyphenolic content and antioxidant and antibacterial activity of the acetone, methanol, and water extracts were determined in different studies. The concentrations of the different classes of phenolic compounds were higher in the acetone and methanol extracts when compared with the other extracts [33]. Radical scavenging activity of the extract demonstrates that the methanol

extract has more significant antioxidant activity than the aqueous extract, and methanol extract of *Rumex abyssinicus* is more effective against gram-positive bacteria compared to gram-negative bacteria. [35]

2.2.1. Bioactive compounds from *Rumex* plants

Most of the species under the genus *Rumex* contain phyto-constituents like, flavonoids, anthraquinones and triterpenoids, and 80% methanolic extract of rhizome *Rumex abyssinicus* reported to have diuretic and analgesic activity [36]. Bioactive compounds are extracted from *Rumex abyssinicus*, characterized and checked for antioxidant and antibacterial activities. The phytochemical studies of the extracts revealed the presence of condensed tannins, flavonoid, and phenolic compounds [35]. As Teshale Mekonnen *et al.* (2010) reported 80% methanol extract of the rhizomes of *Rumex abyssinicus* possesses secondary metabolites such as tannins, saponins, flavonoids, steroids and anthraquinones. Flavonoids and tannins have been shown to be important for wound healing due to their antioxidant, anti-inflammatory and antibacterial activities.

Some of Previously isolated and reported chemical constituents from *Rumex abyssinicus* are oxalic acid (1), chrysophanic acid (2), emodine (3) and physcion (4).



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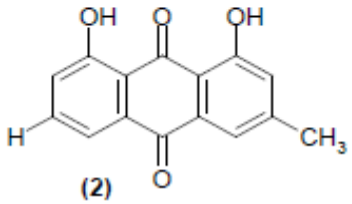

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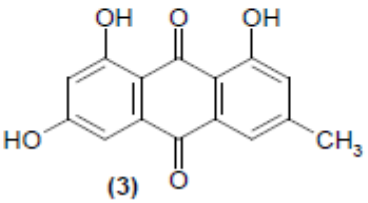

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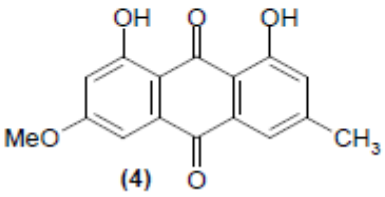

(4)

2.3 Biological activities of some of Essential metals

Metal ions play essential roles in about one third of enzymes activity in human being by modifying electron flow in a substrate or enzyme, thus effectively controlling an enzyme catalyzed reaction [38]. Iron plays an important role in forming complexes with molecular oxygen in hemoglobin and myoglobin. Inorganic iron contributes to redox reactions in the iron-sulfur clusters of many enzymes, such as nitrogenase (involved in the synthesis of ammonia from nitrogen and hydrogen) and hydrogenase [38]. Cobalt is one of the most important trace elements in animals and humans in the form of vitamin B-12 (cobalamin), this metal plays a number of crucial roles in many biological functions [38]. Copper is involved in connective tissue formation and maintenance. It serves as a catalytic component in many enzymes, e.g. it is an important constituent of metallo proteins (exhibiting oxidative reductive activity, e.g. oxidases or hydroxylases). Copper also influences specific gene expression in mammalian cells, nerve myelation and endorphin action with Cu deficiency impairing immunity [38]. The influence of zinc derives from its roles in enzymes, with functions that are both structural and catalytic. Indeed, there are approximately 300 zinc enzymes, with representatives known for each of the fundamental enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases) [38].

2.4 Effect of metal ions on bio-activity of medicinal plant extract

Anti-bacterial activity of bio active compounds can result from their ability to chelate bivalent transition metal ions like Fe^{2+}, Cu^{2+} and Zn^{2+}. These ions can generate highly reactive •OH radicals by Fenton reaction. Chelating agents, which stabilize pro-oxidative transition metal ions by complexing them and are regarded as secondary antioxidants. Metal-polyphenol complexes have good biological and pharmacological activities, due to the effects of the number of *d*-electrons of the first transition metals on the formation of stable complexes. [12]

The antibacterial activities crude plant extract increased up on addition of metal ions in varying ratios due to the formation of stable complex [28]. Accordingly if any drug scavenges the hydroxyl radical it may either scavenge the radical or may chelate metal ions like Fe^{2+} making it unavailable for the Fenton reaction. The ability of phenolic compounds to chelate metal ions depends on the availability of properly oriented functional groups. Many polyphenol compounds may chelate metal ions. The essentiality of trace metals for our health is well recognized but

metal compounds have been recognized as environmental and work-place carcinogens due to this, inorganic drugs are always going to be minor components of a pharmacopoeia. The interaction of metal ions with poly phenol compounds can lead to chelate formation which is crucial in the prevention of radical generation, which damage target biomolecules. Metal-poly phenol complexes have a much stronger free radical scavenging properties than the free poly phenol compound. Moreover, using of natural chelators like flavonoids, tannins and quinones is better than the synthetic ones due their less toxicity effects [6].

Therefore, the main aim of this project is to evaluate the antibacterial activities of crude leaf extract of *Aloe pulcherrima* and *Rumex abyssinicus* up on addition of different transition metal ions (Fe^{2+}, Zn^{2+} and Cu^{2+}) with specific charges. The antibacterial activities of bare metal ions, crude plant extract separately were evaluated and the differences in zone of inhibition after addition of the selected metal ions were evaluated.

3. MATERIALS AND METHOD

3.1. Plant material collection and preparation

The fresh plant sample were collected in July 2016 from natural habitats; South Western part of Oromia region Jimma Zone, Seka Chokorsa woreda, Buyo kebele which is far 376 km from Addis Ababa. Identification of the plant species was made by a plant taxonomist and voucher specimens was deposited at, botanical science laboratory, department of biology, Jimma University. The collected plant material was washed with distilled water, air-dried under shade and manually powdered to suitable size by using mortar and pistil to improve the subsequent extraction by increasing the surface area to facilitate the penetration of solvent into the cells of the plant powder.

3.2. Chemicals, Reagents and Culture Media

. All chemicals and solvents used were pure and analytical grade reagents. Metal ions in the form of $FeSO_4$, $CuSO_4$, and $ZnSO_4$ (Finkem products) were used. Petroleum ether, chloroform, ethyl Acetate, and ethanol (Hayman specialty products) were used to obtain plant extracts. DMSO is used for dissolving the plant extract and as control group for bioassay test, Gentamycin standard drug, Mueller Hinton agar (7101) and nutrient broth as culture media were used for bacteria culture in this study

3.3 Apparatus

Apparatus used are rotary vapor (Heidolph, USA), round bottom flask (250ml, 100 ml, and 50 ml), volumetric flask, measuring cylinder, pestle and mortar for grinding, filter papers, weighing balances, water bath, oven for drying purpose, Vertical Laminar Flow Cabinet (CLB-201-14) and incubator, were used for the study.

3.4 Test strains

In vitro antibacterial activity was examined on two gram-positive (*Bacillus Subtilis* and *Staphylococcus aureus* ATCC 25923) and one gram negative (*Escherichia coli* ATCC 25922) bacterial strains. The standard strains were obtained from, Medical Microbiology Research Laboratory of Jimma University.

3.5 Experimental Part

3.5.1 Plant Extract ion

Aloe pulcherrima and *Rumex abyssinicus* samples were soaked in 200 mL of each of the solvents namely petroleum ether, chloroform, ethyl acetate and ethanol for 24 h. The contents were then filtered through Whattman filter paper no. 1 and the filtrate were dried in the rotary evaporator to eliminate the extra solvent from extract and reserved for further use. The extraction was done in a similar manner for each solvent according to their increasing order of polarity. Stock solution of each crude extract was prepared by diluting 400 mg of each plant crude extract in 2 mL of DMSO to prepare a concentration of 200 mg/mL of plant extract and stock solution of the metal ions (10% of 5 mL metal ion solution) was prepared by mixing 0.5 gm of the powdered with 5 mL of distilled water, Sulfate salts of Zinc, iron and copper were used as a source of metal ions.

3.5.2 Anti-Bacterial activity

3.5.2.1 Agar disc diffusion method

Antibacterial activity of the extracts was evaluated by agar disc diffusion method. Agar disc diffusion and antibiotic optimization test is one of the methods to analyze the antimicrobial or antibiotic activity against various microorganisms. The activity was determined by measuring the diameter of zone of inhibition in mm [40]. The bacteria stock cultures were maintained on the NA slants which were stored at 4 ℃. Agar cultures of the test microorganisms were prepared according to manufacturer's instruction. The culture media (Mueller Hinton agar) were prepared. The petri plates and culture media were then autoclaved. After autoclaving, they were placed in laminar air flow. In laminar air flow, the media were carefully poured in to petri plates and allowed to get solidified. After the media solidified, freshly, grown liquid culture of the test pathogens solution (20 µL) of having similar turbidity with 0.5 McFarland were spreaded over the Müeller-Hinton Agar medium with sterile swab, and were marked with culture name. Disc having 6 mm in diameter was prepared and put on the Agar plates. The disc for plant extract was loaded with 50 µL from the prepared concentration (200 mg/mL) of plant extract and the disc for the combination of the plant extract and the metal ions were loaded with 25 µL of plant extract and 25 µL of the prepared 10% of 5 mL metal ion solution (5 mL distilled + 0.5 gm metal ion). Controls were set up in parallel using DMSO and standard drug gentamycin (10 µL). The petri

plates were then incubated at 37 ºC for 24 h. [45]. The growth or activity was observed after incubation, and the diameter of the zone of inhibition was measured around each disc for each extract.

3.6 Phytochemical Screening

Phytochemical analysis is a type of chemical assay which is used to identify the presence of various phytochemicals in the plant extract. The most of the phytochemicals are classified as secondary metabolites of the plant. The secondary metabolites are responsible for the antimicrobial activity of the plant [41]. The phytochemical screening is summarized in the following table

Table 1 phytochemical screening test

No.	Phytochemical test	Test performed	Observed result
1	Test for terpenoids and Sterols	Salkowski test	Formation of reddish brown color
2	Test for flavonoids	NaOH test	formation of yellow orange color
3	Test for tannins	Ferric chloride test	Formation of dark- green or a blue-black color
4	Test for phenols	Ferric chloride test	Formation of green or blue color
5	Test for quinones	KOH or NaOH test	Formation of blue green or red color

4. RESULTS AND DISCUSSION

4.1 Extraction and Fractionation

The air dried leaf of *Aloe pulcherrima* and *Rumex abyssinicus* were extracted sequentially by increasing polarity of solvents petroleum ether, chloroform, ethyl acetate and ethanol for the two plants separately after maceration process for 24 hr. Each fraction of the plants extracts were dried and subjected to phytochemical screening.

4.2 Phytochemical Screening

Different fractions of each of the plants leaves of *Aloe pulcherrima* and *Rumex abyssinicus* were subjected to phytochemical screening for various phyto-constituents. On phytochemical analysis for leaves of *Aloe pulcherrima* positive and/or negative results were revealed as shown in table 1 Positive result were observed for quinones in chloroform fraction, quinones, flavonoids and phenols in ethyl acetate fraction, tannins and phenols for ethanol fraction and there was no any positive result observed for the tested phytochemicals in petroleum ether fraction. Similarly, for leaf of *Rumex abyssinicus*, positive results of flavonoids; flavonoids and tannins; tannins, phenols and quinones were observed for chloroform, ethyl acetate and ethanol fractions respectively as shown in table 1 Whereas no any positive result were observed for petroleum ether fraction. These results were in agreement for leaves of *Aloe pulcherrima* with previous findings [42] that confirm the ethyl acetate and ethanolic extracts of *Aloe* were revealed for the presence of alkaloids, quinones, saponins, flavonoids, and tannins. In the same way phytochemical screening for leaf of *Rumex abyssinicus* revealed the presence of Flavonoids, tannins, phenols and quinones in the ethyl acetate and ethanol extract. These results were also in agreement with previous findings [43] the 80 % methanol extract of *R. abyssinicus* had been reported to possess secondary metabolites, including; tannins, saponins, flavonoids, steroids and anthraquinones. The active constituents of these two plants are the major source for the treatment of different kinds of bacterial diseases. The treating activity is due to the presences of different types of secondary metabolites or phytochemicals as observed from table 1 Thus, due to the active phytochemicals of traditional medicinal plants extracts; the *Aloe pulcherrima* and *Rumex abyssinicus* were used traditionally.

Based on screened phytochemical, the two plants mostly contains polar constituents. The reason is on both two plants, there were no any positive and indicative results for petroleum ether fraction whereas phyto-constituents such as flavonoids, tannins, phenols, quinones which are more polar phytochemicals observed in more polar solvents such as chloroform, ethyl acetate and ethanol. The result of phytochemical screening is summarized in table 1.

Table 2 Phytochemical screening result for the petroleum ether, chloroform, ethyl acetate and ethanol fraction of leaves of *Aloe pulcherrima* and *Rumex abyssinicus*

No.	Phytochemicals	Color Indication (+ve or - ve)							
		Aloe pulcherrima				*Rumex abyssinicus*			
		Pet. Ether	Chloro form	E/Ac.	Ethan ol	Pet. Ether	Chloro form	E/Ac.	Etha nol
1	Terpenoids & Sterols	_	_	_	_	_	_	_	_
2	Flavonoids	_	_	+	_	_	+	+	_
3	Tannins	_	_	_	+	_	_	+	+
4	Phenols	_	_	+	+	_	_	_	+
5	Quinones	_	+	+	_	_	_	_	+

(-) Stands for negative result and (+) stands for positive results

4.3. Antibacterial Activity Test

4.3.1. Antibacterial activity of leaf of *Aloe pulcherrima* fractions compared with combined metal ions.

Traditional healers use only the gel part of *Aloe* leaves. Here, the investigation took places to observe the effects of three metal ions (Cu^{2+}, Fe^{2+} and Zn^{2+}) on the bioactivity of phytochemicals from *Aloe pulcherrima* leaf fractions. Three bacteria test organisms *Bacillus*, *S. aureus*, *E. coli*; four fractions of *Aloe pulcherrima* (petroleum ether, chloroform, ethyl acetate and ethanol); and each three metal ions combined (chelated) with each of the four fractions of *Aloe pulcherrima,* the positive control antibiotic Gentamycin and negative control DMSO were used to evaluate the influence of metal ions on the bioactivities of each fractions. The diameter of Zone of Inhibition (ZOI) measured in millimeter (mm). The result is summarized in Table 2 below.

Table 3 Effect of Cu^{2+}, Fe^{2+} and Zn^{2+} metal ions on antibacterial activity of four fractions of *Aloe pulcherrima* leaf on three bacterial strains (*B. Subtilis, S. aureus, E. coli)*

Test organism	Extract (Ext)/Control	Zone **of inhibition (mm)**			
		Ext/control	**Ext + Cu**	**Ext + Fe**	**Ext + Zn**
		Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
B. Subtilis	Petroleum	8.00±0.00	9.00±0.00	14.67±0.47	9.33±0.47
	Chloroform	9.33±0.57	8.33±0.47	11.00±0.00	14.00±0.00
	Ethyl acetate	9.67±0.57	14.3±0.47	14.00±0.00	17.00±0.00
	Ethanol	8.67±0.57	9.33±0.47	9.67±0.47	14.30±0.47
	Gentamycin	11.00±1.00	-	-	-
	DMSO	6.33±0.57	-	-	-
S. aureus	Petroleum	6.67±0.57	8.67±0.47	8.67±0.47	10.00±0.00
	Chloroform	8.67±0.57	8.67±0.47	8.67±0.47	12.70±0.94
	Ethyl acetate	10.00±1.73	16.30±0.47	16.33±0.47	16.70±0.47
	Ethanol	8.67±2.88	12.00±0.47	12.00±0.00	15.00±0.00
	Gentamycin	15.67±2.08	-	-	-
	DMSO	6.33±0.57	-	-	-
E. coli	Petroleum	8.00±0.00	13.30±0.47	13.33±0.47	10.00±0.00
	Chloroform	9.00±0.00	12.30±0.47	12.33±0.47	10.00±0.00
	Ethyl acetate	8.67±0.57	13.30±0.47	13.67±0.47	15.30±0.47
	Ethanol	8.33±1.52	13.00±0.47	13.00±0.00	15.3±0.47
	Gentamycin	15.67±2.08	-	-	-
	DMSO	6.00±0.00	-	-	-
Total average ZOI		**8.64±0.86**	**11.54±2.65**	**12.27±2.39**	**13.3±2.8**

As seen from table 2 the activity of different fraction had different bioactivity. The average zone of inhibition were found to be 8.64, 11.54, 12.27, and 13.3 mm for the extract, extract + Cu^{2+}, extract + Fe^{2+} and extract + Zn^{2+} respectively, but in all cases the potency of chelated fractions showed averagely higher activity than that of unchelated fractions or the anti- bacterial activity of the crude extracts were increased after addition of metal ions. Thus, the anti-bacterial activity of four solvent fractions was enhanced upon the addition of metal ions. Comparing to each chelated fractions, Zn^{2+} ion chelated with ethyl acetate fraction exhibited highest ZOI (17± 0.00 mm) for *B. Subtilis,* (16.70±0.47 mm) for *S. aureus and* (15.3 ±0.47 mm) for *E. coli*. This implies that the zinc metal ion increased the bioactivity of all ethyl acetate fractions rather than

that of iron and copper metal ions. For the ethyl acetate chelated, copper show maximum ZIO against gram positive bacteria (*B. Subtilis*, and *S. aureus*) with ZOI 14.3±0.47 and 16.33±0.47 mm respectively, even better than the standard drug gentamycin (15.67±2.08 mm). This result was in agreement with the finding [44] using four different solvents; ethyl acetate, ethanol, chloroform, petroleum ether; of the four solvents, ethyl acetate and ethanol extract give the best result against gram positive bacteria except *E. coli.* In case of methanol chelated fraction on *Staphylococcus aureus* (11 mm) showed highest zone of inhibition, also according to [45] the antimicrobial activity of the crude extract of aloe leaf was increased in the presence of metal ion is in close agreement with this work.

4.3.2 Antibacterial activity of leaf of *Rumex abyssinicus* fractions compared with combined metal ions.

Rumex abysinicus, which belong to the family polygonaceae, is a perennial herb, up to 4 m tall, the leaf of the plant is usually sagittate, and inflorescence and much branched. The family polygonaceae comprises around 250 species, *Rumex abysinicus* is one of the endemic *Rumex* species found in Ethiopia and traditionally used for the treatment of sexually transmitted disease, diabetes, lung TB and leprosy, the rhizomes are used to refine butter and give it a rich yellow color.

As it was done for *Aloe pulcherrima* the investigation took places to observe and compare the anti-bacterial activity of the crude extracts and effects of Cu^{2+}, Fe^{2+} and Zn^{2+} metal ions for the anti-bacterial activity of phytochemicals from *Rumex abysinicus* leaf fractions. Three bacteria test organisms *Bacillus*, *S. aureus*, *E. coli*; four fractions of *Rumex abysinicus* (petroleum ether, chloroform, ethyl acetate and ethanol); and each three metal ions combined (chelated) with each four fractions of *Rumex abysinicus*, were used. The positive control antibiotic Gentamycin and the negative control DMSO were also used to evaluate the influence of metal ions on the anti-bacterial activity of each fraction. The diameter of Zone of Inhibition zone of inhibition measured in millimeter (mm). The result is summarized in Table 3 below.

Table 4 Effect of Cu^{2+}, Fe^{2+} and Zn^{2+} metal ions on antibacterial activity of four fractions of *Rumex Abyssinicus* leaf on three bacterial strains (*B. Subtilis, S. aureus, E. coli)*

Test organism	Extract (Ext)/Control	Zone of inhibition (mm)			
		Ext/control	Ext + Cu	Ext + Fe	Ext + Zn
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Bacillus	Petroleum ether	6.33±0.57	8.00±0.00	10.33±0.57	10.33±1.52
	Chloroform	6.30±0.57	10.67±0.57	6.33±0.57	13.33±0.57
	Ethyl acetate	7.00±0.00	10.33±0.57	8.33±0.57	13.00±0.00
	Ethanol	8.33±0.57	10.00±0.00	10.67±0.57	12.67±0.57
	Gentamycin	11.00±1.00	-	-	-
	DMSO	6.33±0.57	-	-	-
S. aureus	Petroleum ether	8.67±0.57	10.00±0.00	11.00±0.00	10.33±0.57
	Chloroform	6.67±0.57	15.00±1.00	6.67±0.57	14.33±0.57
	Ethyl acetate	9.00±1.00	13.00±0.00	9.33±2.30	15.33±0.57
	Ethanol	11.00±1.00	14.33±0.57	11.67±1.52	15.33±0.57
	Gentamycin	12.30±4.93	-	-	-
	DMSO	6.00±0.00	-	-	-
E. coli	Petroleum ether	6.33±0.57	7.00±0.00	9.67±0.57	11.33±0.57
	Chloroform	9.67±1.15	12.33±0.57	6.67±0.57	12.67±0.57
	Ethyl acetate	7.00±1.00	11.66±0.57	7.33±0.57	12.33±0.57
	Ethanol	7.33±1.15	11.33±1.15	8.00±0.00	12.00±0.00
	Gentamycin	16.00±1.73	-	-	-
	DMSO	6.00±0.00	-	-	-
Total average zone of inhibition		**7.80±1.52**	**11.14±2.34**	**8.83±1.86**	**12.77±1.72**

From table 3, the bioactivity of *Rumex Abyssinicus* of different solvent extract in the presence of metal ion against the three pathogens (*B. Subtilis, S. aureus, E. coli*) has shown. The total average zone of inhibition were found be 7.80±1.52, 11.14±2.34, 8.83±1.86, and 12.77±1.72 mm for the extract, extract + copper, extract + iron, and extract + zinc respectively. The bioactivity of the combined complex showed averagely higher activity as compared with the crude extracts. Comparing to each chelated fractions, Zn^{2+} ion chelated with ethyl acetate and ethanol fraction exhibited highest zone of inhibition (15.33 ±0.57 mm) against *S. aureus,* which is much better than the standard gentamycin (12.33±1.93 mm). Zn^{2+} ion combination with chloroform extract also show good potency of antibacterial activity against *Bacillus* and *S.*

aureus with zone of inhibition 13.33±0.57 mm and 14.33±0.57 mm respectively. This result strengthen the traditional use of medicinal plants, and they are used as a source for new drug discovery and lead structure developments This result is in agreement with the finding [46] the antibacterial activities of ethyl acetate extract of *Rumex* leaf had maximum antibacterial activity with zone of inhibition range 12.1 to 19.5 mm. The growth of *Staphylococcus aureus* was strongly inhibited by ethyl acetate extract. Phytochemical analysis also reveals the presence of bio active compounds like tannins, phenols and quinones in the ethanol extract and flavonoid and tannins in the ethyl acetate fraction of *Rumex abyssinicus*.

Generally the potency of bio activity of plant phytochemicals was increased up on addition of transition metal ions metal ions, this is due to the effects of the number of *d*-electrons of the first transition metals on the formation of stable complexes; and due to this anti-bacterial activity of bio active compounds can result from their ability to chelate bivalent transition metal. This confirms the ability of Phenolic compounds to chelate metal ions which increase the anti-bacterial activity of plant bioactive compounds, which is discussed in the literature above [6, 12, and 28].

4.4. UV-Vis Spectrophotometric analysis of ethyl acetate and ethanol extract combined with Zn^{2+} ion

The UV-Vis profile of the plant extract was measured at a wavelength range of 200 to 800 nm. Accordingly the UV-Vis Spectrophotometric analysis were done for the ethyl acetate fraction of *Aloe pulcherrima* and for ethyl acetate and ethanol fractions of *Rumex abyssinicus* due to their progressive antimicrobial activities alone and up on addition of metal ions. Even though it is difficult to interpret spectra for solutions containing more than a single compound, I supposed to discuss the spectra of compounds of my interest.

Chlorophyll are universal constituents of wild vascular plants and classified as chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*). Both chlorophylls show absorption maxima at wavelengths corresponding to blue and red region, but chlorophyll assay in crude extracts, which inevitably contain carotenoids as well, is routinely based on absorption maxima in red light to avoid overlap with these accessory pigments that show strong absorption below 500 nm. Absorption maxima at 659nm and 642nm for Chl *a*, and Chl *b* respectively, but these peaks will

shift slightly according to solvent system, and such shifts must be taken into account for precise measurement [46].

Table 5 Uv-vis spectra analysis results

Ser. No.	Uv- vis spectroscopy result for ethyl acetate extract of *Aloe pulcherrima* leaf				Uv- vis spectroscopy result for ethanol extract of *Rumex abyssinicus* leaf				Uv- vis spectroscopy result for ethyl acetate extract of *Rumex abyssinicus* leaf			
	Plant extract only		Plant ext. + Zn^{2+} ion		Plant extract only		Plant ext. + Zn^{2+} ion		Plant extract only		Plant ext. + Zn^{2+} ion	
	Wave length (nm)	Absorbance Value	Wave length (nm)	Absorbance Value	Wave length (nm)	Absorbance Value	Wave length (nm)	Absorbance Value	Wave length (nm)	Absorbance Value	Wave length (nm)	Absorbance Value
1	260	0.206	237	0.190	232	0.135	235	0.126	240	0.286	215	0.237
2	320	0.681	265	0.180	253	0.145	270	0.113	320	0.756	250	0.290
3	415	1.848	312	0.486	346	1.377	342	1.335	410	1.174	335	1.456
4	505	0.141	419	1.794	663	0.049	660	0.060	475	0.615	400	1.211
5	605	0.126	607	0.089					605	0.049	610	0.072
6	660	0.486	653	0.386					665	0.226	655	0.302

From table 4 (Appendix 4A and 4B) of Uv-vis spectra of ethyl acetate extract of *Aloe pulcherrima* leaf peaks at 260, 320, 405, 505, 605 and 660 nm with absorbance value of 0.206, 0.681, 1.848, 0.141, 0.126 and 0.486 respectively; from the Uv-vis spectra of the combination of ethyl acetate extract and Zn^{2+} ion (Appendix 4C and 4D) wave length 237, 265, 312, 419, 607 and 653 nm respectively; from the Uv- vis spectroscopy result for ethanol extract of *Rumex abyssinicus* leaf peaks at wave length 232, 253, 346 and 663 nm with absorbance value of 0.135, 0.145, 1.377 and 0.049 respectively; and from Uv-vis spectra result of combination of ethanol extract of *Rumex abyssinicus* leaf and Zn^{2+} metal ion peaks at 235, 270, 342 and 660 nm with absorbance value of o.126, 0.113, 1.335 and 0.060 were recorded.

The spectra for phenolic compounds (tannins) and flavonoids typically lie in the range of 230-290 nm [47], and the $n \rightarrow \pi^*$ transition of C = O group typically occurs at 275 – 295 nm. On $\alpha\beta$–alkyl conjugation, the absorption peak suffers a bathochromic shift, becomes broadened and shifts to 300– 320 nm [49]. Also poly phenol compounds causes both the ($\pi \rightarrow \pi^*$) and ($n \rightarrow \pi^*$) transition to longer wave length to the region 220-260 and 310-330 nm respectively, due to the conjugation of ethylenic group with the carbonyl functional group.

Due to this we can conclude the data of Uv-vis spectra of the plant extracts confirms the presence of phenolic compounds like flavonoids, quinones and tannins. The presence of conjugation, delocalization occurred by lone pairs and the formation of new complex formed between ligands and metal ions cause shift in wave length, change in intensity and also may cause large bathochromic shift. The change of the above parameters also confirms the formation of complex between the plant phytochemicals and metal ion.

Generally the peaks appears on the spectra lies in Uv-vis range of organic compounds so, we can conclude that the crude extract of *Aloe pulcherrima* and *Rumex abysinicus* leave composed of the phytochemical compounds which were identified by phytochemical screening test.

5. CONCLUSION AND RECOMENDATION

It was concluded that the crude extracts of both *Aloe pulcherrima* and *Rumex abyssinicus* leaves are rich in secondary metabolites like quinones, flavonoids, tannins, and phenols. The phytochemical analysis and Uv-vis spectroscopic data reveals the presence of these secondary metabolites which are responsible for the antibacterial activity. Most of the bioactive components were extracted in the ethyl acetate and ethanol extracts. So the ethyl acetate and ethanol extracts are more essential as traditional medicines. The potency of antibacterial activity of crude extracts was increased up on addition of three metal ions (Cu, Fe, and Zn) due to ability of these bioactive compounds to chelate metal ions, especially Zinc metal ion chelated to the phytochemicals show strong anti-bacterial activity against gram positive bacteria. The ZOI is greater when chelated with these metal ions even greater than that of the standard drug gentamycin. Generally good antibacterial activity was recorded for the combined complex formed from the plant extract and the metal ions, this leads to increase the use of *Aloe pulcherrima* and *Rumex abyssinicus* for medicinal purpose that makes the future drug cheap and easily accessible. Finally, based on my work the following points are put as recommendation for other researchers.

- Further research has been required on isolation and characterization to confirm specificity of the compounds responsible for antimicrobial activity.
- Other part of plants (roots, steams) should be studied.
- Other metal ions should be checked for their effect.
- MIC test for the determination of the minimum concentration of antibacterial to inhibit or kill the microorganism has to be done.

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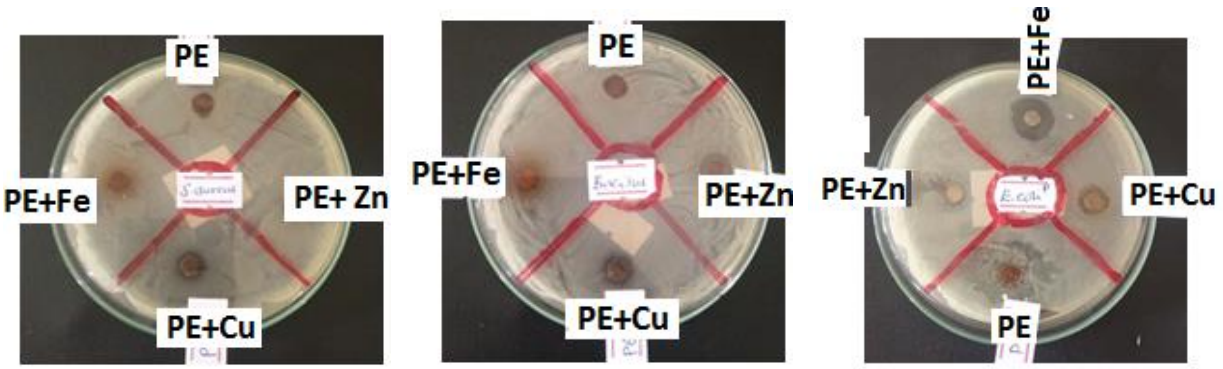
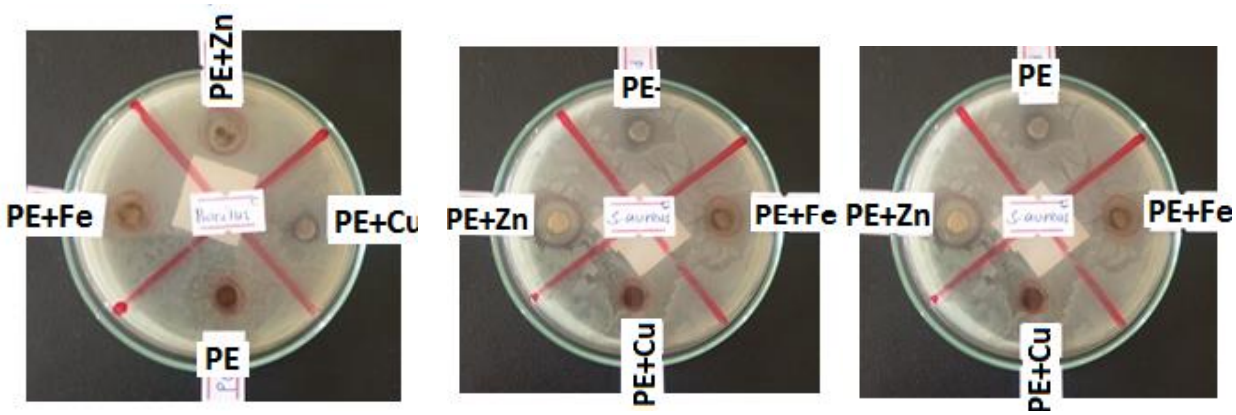
APPENDIX

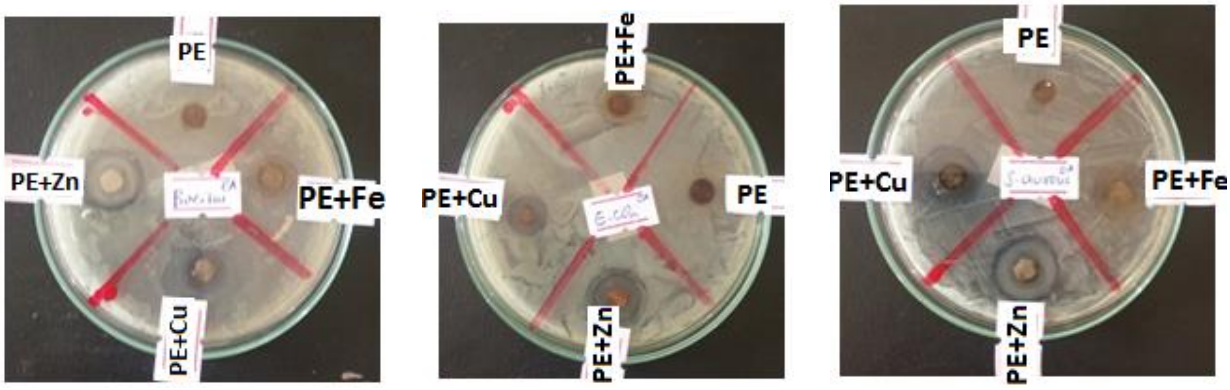
Appendix1A: Antibacterial activity of petroleum ether extract of *Aloe pulcherrima* leaves.



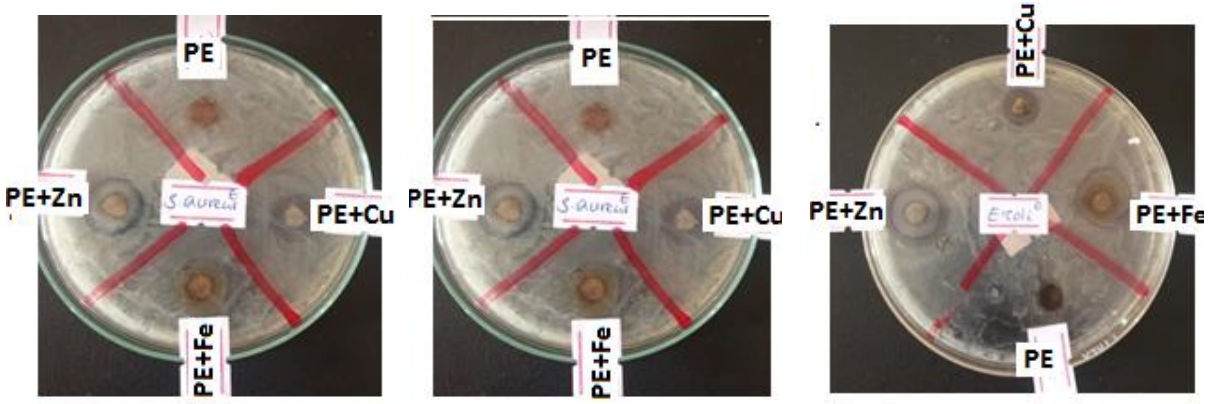
Appendix1B: Antibacterial activity of chloroform extract of *Aloe pulcherrima* leaves.



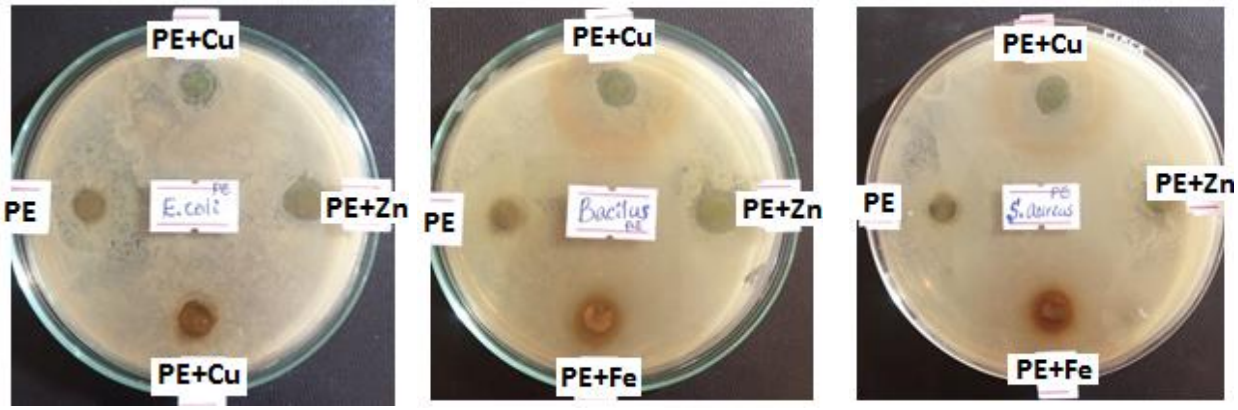
Appendix1C: Antibacterial activity of ethyl acetate extract of *Aloe pulcherrima* leaves.



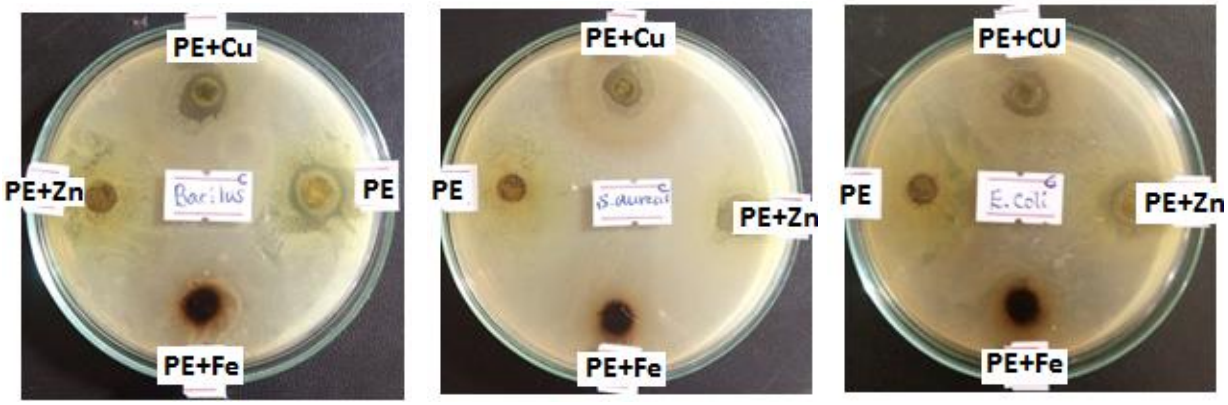
Appendix1D: Antibacterial activity of ethanol extract of *Aloe pulcherrima* leaves.



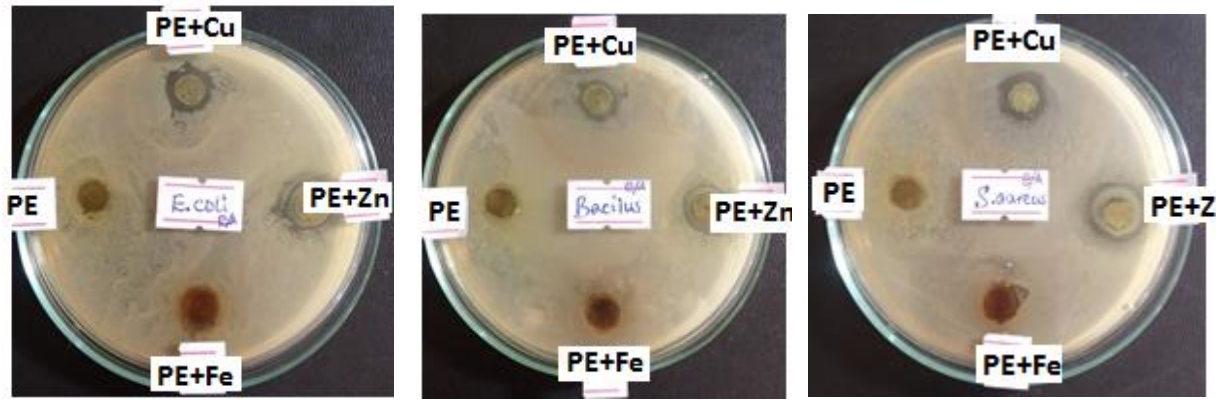
Appendix2A: Antibacterial activity of petroleum ether extract of *R. abyssinicus* leaves.



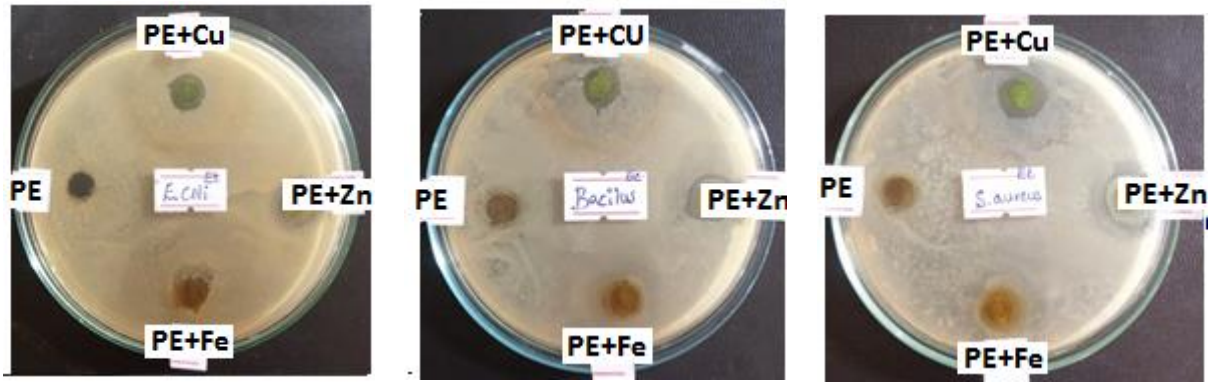
Appendix2B: Antibacterial activity of chloroform extract of *R. Abyssinicus* leaves.



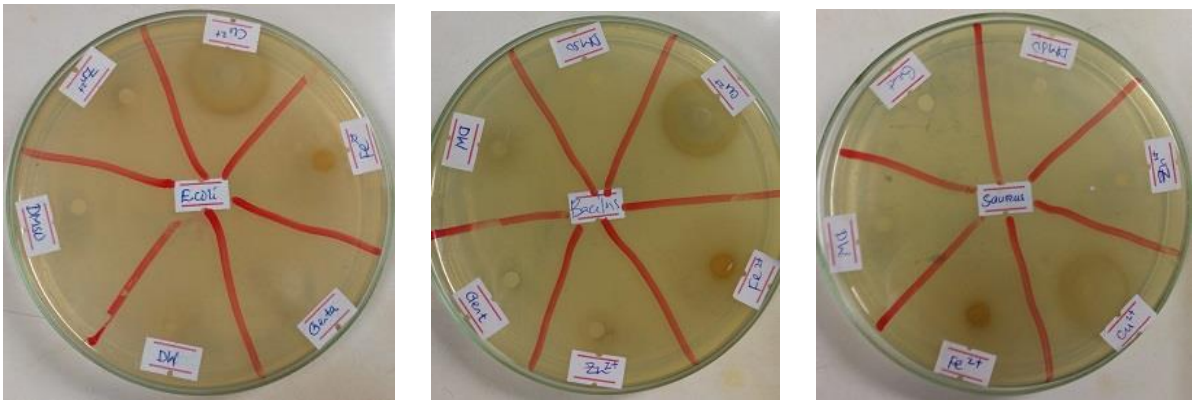
Appendix2C: Antibacterial activity of ethyl acetate extract of *R. Abyssinicus* leaves.



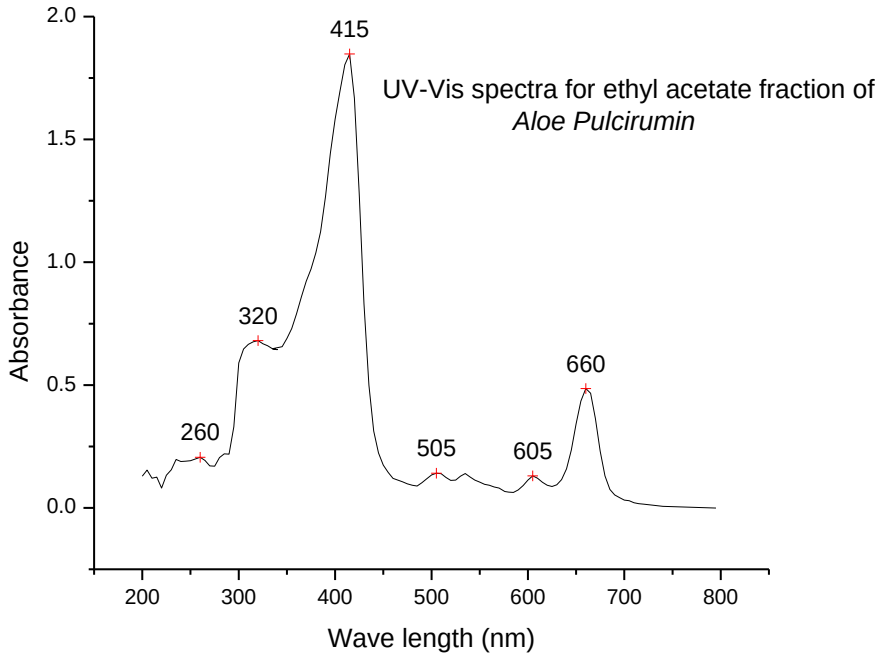
Appendix2D: Antibacterial activity of ethanol extract of *R. Abyssinicus* leaves.



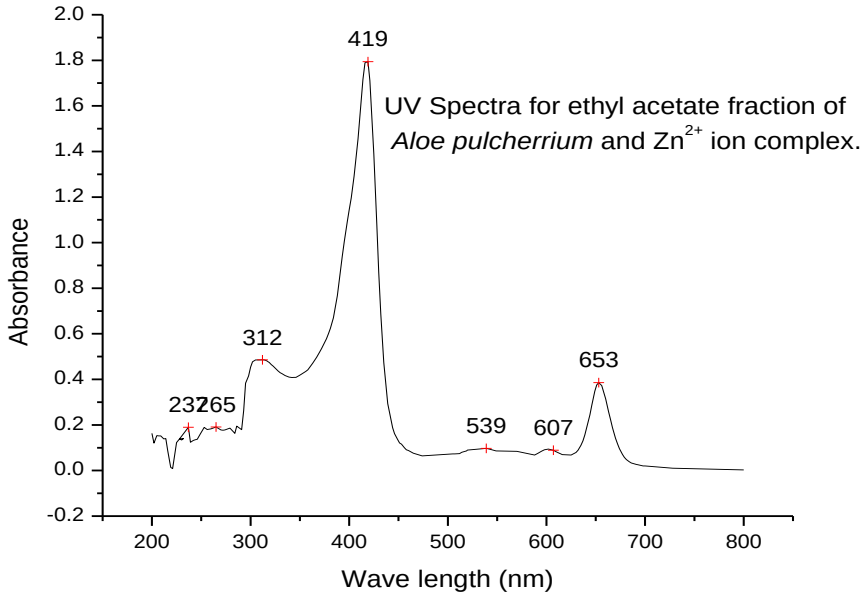
Appendix3A: Antibacterial activity of the Control group



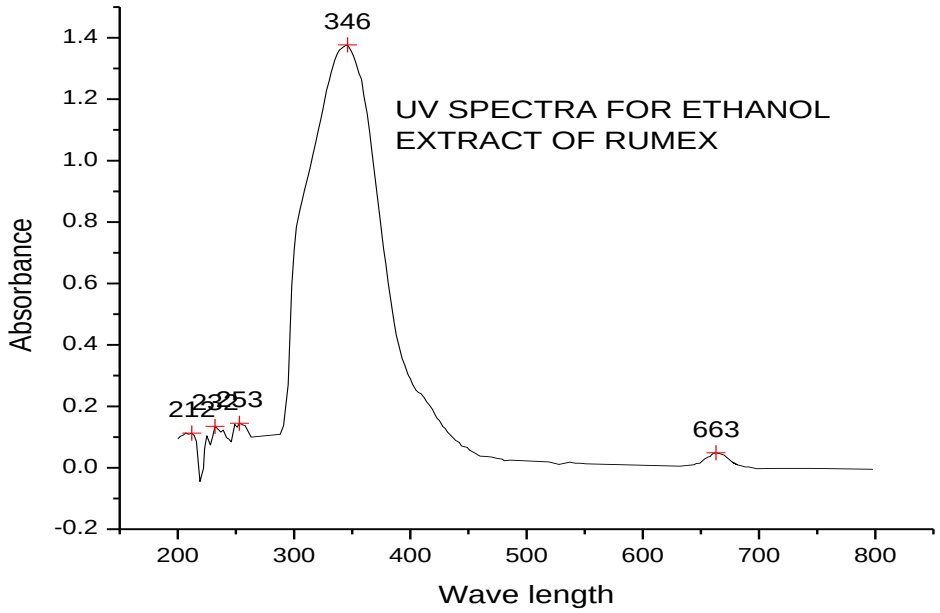
Appendix4A: Uv-vis spectra for ethyl acetate extract of *Aloe pulcherrima*



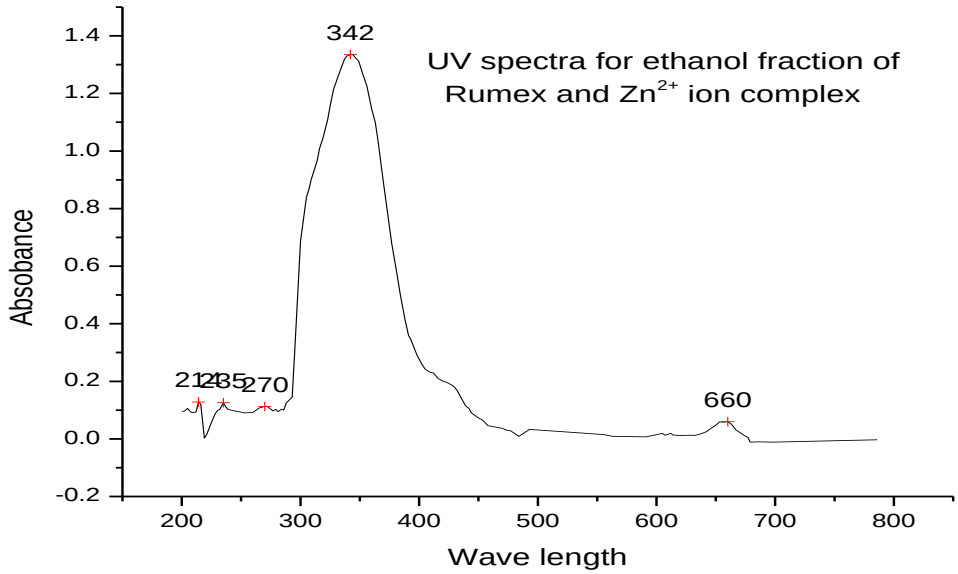
Appendix4B: Uv-vis spectra for ethyl acetate extract of *Aloe pulcherrima* and zinc metal ion.



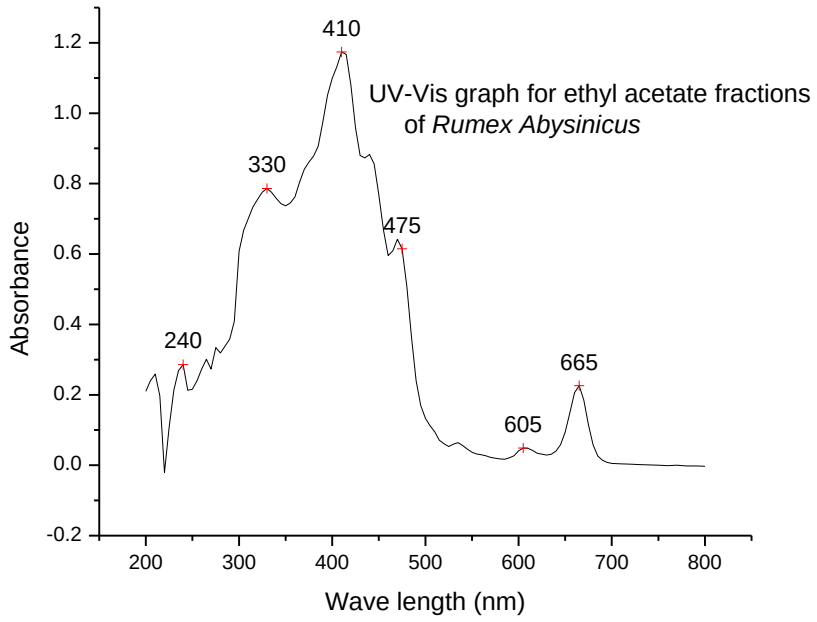
Appendix4C: Uv-vis spectra for ethanol extract of *Rumex abysinicus*



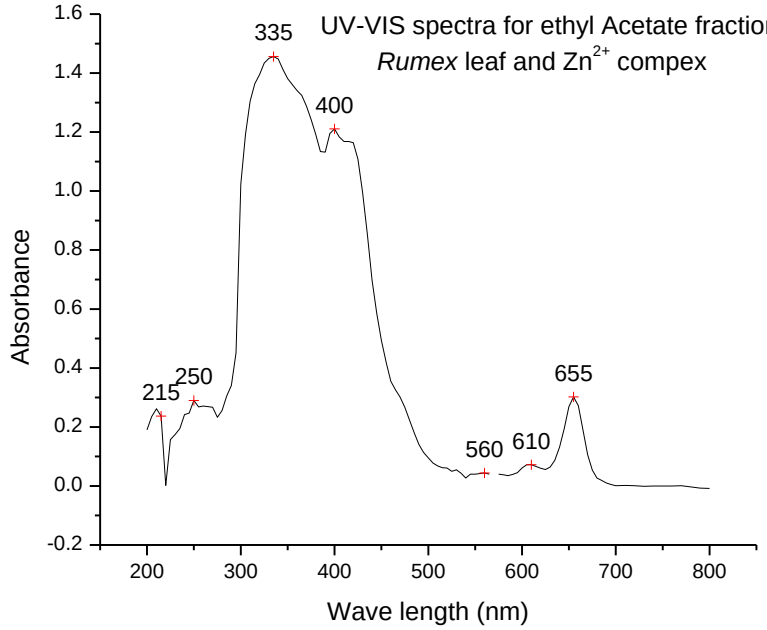
Appendix4D: Uv-vis spectra for ethanol extract of *Rumex abysinicus* and zinc metal ion complex



Appendix4E: Uv-vis spectra for ethyl acetate extract of *Rumex abysinicus*



Appendix4F: Uv-vis spectra for the combination of ethyl acetate extract and Zn^{2+} ion of *Rumex abysinicus*



Appendix 5A: Anti-bacterial activity of *Aloe pulcherrima* leaf extract

Test organism	**Extract (Ext)/Control**	Zone **of inhibition (mm)**															
		Ext/control				**Ext + Cu**				**Ext + Fe**				**Ext + Zn**			
		1	2	3	Av	1	2	3	Av	1	2	3	Av	1	2	3	**Mean ± SD**
Bacillus	Petroleum ether	8	8	8	8.00±0.00	9	9	9	9.00±0.00	15	15	14	14.67±0.47	9	10	9	9.33±0.47
	Chloroform	9	10	9	9.33±0.57	9	8	8	8.33±0.47	11	11	11	11.00±0.00	14	14	14	14.00±0.00
	Ethyl acetate	10	10	9	9.67±0.57	15	14	14	14.3±0.47	14	14	14	14.00±0.00	17	17	17	17.00±0.00
	Ethanol	9	8	9	8.67±0.57	9	10	9	9.33±0.47	9	10	10	9.67±0.47	14	15	14	14.30±0.47
	Gentamycin	12	10	11	11.00±1.00	-	-	-	-	-	-	-	-	-	-	-	-
	DMSO	6	7	6	6.33±0.57	-	-	-	-	-	-	-	-	-	-	-	-
S. aureus	Petroleum ether	6	7	7	6.67±0.57	9	9	8	8.67±0.47	11	12	11	8.67±0.47	10	10	10	10.00±0.00
	Chloroform	9	9	8	8.67±0.57	9	9	8	8.67±0.47	11	11	11	8.67±0.47	12	14	12	12.70±0.94
	Ethyl acetate	9	9	12	10.00±1.73	17	16	16	16.30±0.47	15	15	14	16.33±0.47	16	17	17	16.70±0.47
	Ethanol	12	7	7	8.67±2.88	12	12	12	12.00±0.47	14	15	14	12.00±0.00	15	15	15	15.00±0.00
	Gentamycin	15	18	14	15.67±2.08		-	-	-	-	-	-	-	-	-	-	-
	DMSO	6	6	7	6.33±0.57		-	-	-	-	-	-	-	-	-	-	-
E. coli	Petroleum ether	8	8	8	8.00±0.00	13	14	13	13.30±0.47	14	11	14	13.33±0.47	10	10	10	10.00±0.00
	Chloroform	9	9	9	9.00±0.00	12	13	12	12.30±0.47	11	11	10	12.33±0.47	10	10	10	10.00±0.00
	Ethyl acetate	8	9	9	8.67±0.57	13	14	14	13.30±0.47	15	15	14	13.67±0.47	15	16	15	15.30±0.47
	Ethanol	8	7	10	8.33±1.52	13	13	13	13.00±0.47	15	16	16	13.00±0.00	15	16	15	15.3±0.47
	Gentamycin	15	18	14	15.67±2.08		-	-	-	-	-	-	-	-	-	-	-
	DMSO	6	6	6	6.00±0.00		-	-	-	-	-	-	-	-	-	-	-

Appendix 5B: Anti-bacterial activity of *Rumex abyssinicus* leaf extract

Test organism	Extract (Ext)/Control	Zone of inhibition (mm)															
		Ext/control				Ext + Cu				Ext + Fe				Ext + Zn			
		1	2	3	Mean ± STDEV	1	2	3	Mean ± STDEV	1	2	3	Mean ± STDEV	1	2	3	Mean ± STDEV
Bacillus	Petroleum ether	6	6	7	6.33±0.57	8	8	8	8.00±0.00	11	10	10	10.33±0.57	12	10	9	10.33±1.52
	Chloroform	6	6	7	6.30±0.57	10	11	11	10.67±0.57	7	6	6	6.33±0.57	13	14	13	13.33±0.57
	Ethyl acetate	7	7	7	7.00±0.00	10	11	10	10.33±0.57	8	8	9	8.33±0.57	13	13	13	13.00±0.00
	Ethanol	8	8	9	8.33±0.57	10	10	10	10.00±0.00	11	10	11	10.67±0.57	12	13	13	12.67±0.57
	Gentamycin	12	10	11	11.00±1.00	-	-	-	-	-	-	-	-	-	-	-	-
	DMSO	6	7	6	6.33±0.57		-		-	-	-	-	-	-	-	-	-
S. aureus	Petroleum ether	9	9	8	8.67±0.57	10	10	10	10.00±0.00	11	11	11	11.00±0.00	10	11	10	10.33±0.57
	Chloroform	7	6	7	6.67±0.57	14	15	16	15.00±1.00	7	6	7	6.67±0.57	14	14	15	14.33±0.57
	Ethyl acetate	10	9	8	9.00±1.00	13	13	13	13.00±0.00	12	8	8	9.33±2.30	16	15	16	15.67±0.57
	Ethanol	12	10	11	11.00±1.00	15	14	14	14.33±0.57	12	10	13	11.67±1.52	15	15	16	15.33±0.57
	Gentamycin	10	9	18	12.30±4.93	-	-	-	-	-	-	-	-	-	-	-	-
	DMSO	6	6	6	6.00±0.00		-	-	-	-	-	-	-	-	-	-	-
E. coli	Petroleum ether	6	6	7	6.33±0.57	7	7	7	7.00±0.00	10	9	10	9.67±0.57	11	11	12	11.33±0.57
	Chloroform	9	11	9	9.67±1.15	12	12	13	12.33±0.57	7	6	7	6.67±0.57	13	13	12	12.67±0.57
	Ethyl acetate	8	7	6	7.00±1.00	12	12	11	11.66±0.57	8	7	7	7.33±0.57	13	12	12	12.33±0.57
	Ethanol	8	6	8	7.33±1.15	12	10	12	11.33±1.15	8	8	8	8.00±0.00	12	12	12	12.00±0.00
	Gentamycin	15	18	15	16.00±1.73	-	-	-	-	-	-	-	-	-	-	-	-
	DMSO	6	6	6	6.00±0.00		-	-	-	-	-		-	-	-	-	-