PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACITVITY

OF

CRASSOCEPHALLUM VITELLINUM LEAF EXTRACTS

BY

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A DISSERTATION SUBMITTED TO SCHOOL OF PHARMACY IN PARTIAL FULFILLMENT OF A REQUIREMENT FOR THE AWARD OF BACHELORS DEGREE OF PHARMACY

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ABBREVIATIONS

Mg.....Milligrams

Ml.....Milliliter

MI.....Mean zone of inhibition

Mm.....Millimeter

ME.....Methanol extract

HE.....Hexane extract

EA.....Ethyl acetate extract

PC.....Positive control

NC.....Negative control

SD.....Standard deviation

CERTIFICATION

I certify that this work is an original work which was carried out by Mr.Wangoye khalim, a final year pharmacy student under my supervision.

Signature.....

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DEDICATION

This research work is dedicated to my lovely wife Kampi zainab and son Kalondo Abdulrazaaq who have tolerated my absence throughout the entire period of study.

DECLARATION

I hereby declare that this dissertation entitled "Phytochemical analysis and antibacterial activity of *carssocephallum vitellinum* leaf extracts'' is a bonafide and genuine research work carried out by me under the guidance of Dr.Fahad Muhammad Mukhtar.

Signature.....

Wangoye Khalim

ACKNOWLEDGEMENT

The completion of this work is not only a fulfillment of my dreams but also the fulfillment of the dreams of my wife and son who have taken lots of pain and patience for me in completion of this program.

Iam indebted to my esteemed supervisor and Lecturer, Dr. Fahad Muhammad Mukhtar. An impressive personality for his valuable discussion and encouraging guidance throughout the duration of this work. His words of advice have been etched in my heart and I will always Endeavour to hold up his ideals.

Iam also thankful to the Government of Belgium and Belgium technical cooperation (BTC) whose scholarship has enabled to realize this dream.

Finally, thanks goes to my classmates Mutumba Muzamiru and Jibi James for their invaluable contribution and support during my stay at Kampala international university.

ABSTRACT

Objectives: The objectives of this study to carry out qualitative analysis of phytochemical constituents present in hexane, ethylacetate and methanolic extracts of *crassocephallum vitellinum* leaves and thereafter evaluate the antibacterial activity of the leaf extracts of this plant against clinical pathogens of human origin.

Methods: Extraction of phytochemicals was performed by successive solvent extraction technique as explained by Hossamani.P.A (2012). After the extraction process, phytochemical analysis was performed using qualitative methods explained by Evans (2002) and Harbone (1998).

Antibacterial activity of the extracts was tested against *staphylococcus areus, Escherichia coli* and *Klebsilla pneumonia*. The extracts were prepared at three varying concentrations of 300mg/ml, 150mg/ml and 75mg/ml. The antibacterial activity was determined by Agar well diffusion method as described by Sharmavaishale et al (2013).

Results: Preliminary phytochemical investigation revealed the presence of alkaloids, reducing sugars, tannins, cardiac glycosides in all the three extracts used. Quinones were only present in the hexane extract where as diterpenoids, steroids and terpenoids were present in hexane and methanolic extracts only.

Hexane extract demonstrated antibacterial activity against *staphylococcus aureus* at the highest concentrations. The maximum mean zone of inhibition was 13.5±2.12mm

In addition, hexane demonstrated maximum zone of inhibition (18.5±4.95) against Escherichia coli at the highest concentration of the extract but remained inactive at the lower concentrations of this extract against Escherichia coli. Similarly, at 300mg/ml, hexane extract inhibited the growth of *Klebsilla pneumonia*e with a min zone of inhibition of 13.5mm but also remained inactive at the lower concentrations of 150mg/ml and 75mg/ml.

The methanolic extract was only active against *staphylococococcus aureus* at 300mg/ml with a mean zone of inhibition of 18±2.83mm. Methonolic extract did not have activity against Klebsilla pneumonia and *Escherichia coli* at all the three varying concentration. Furthermore, ethyl acetate showed no antibacterial activity against all the test microorganisms at all the three concentrations of the extracts.

Conclusion: Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, reducing sugars steroids, terpenoids, diterpenoids tannins quinines in ether hexane, ethylacetate or methanolic extract. Finally, the present screening results demonstrated that hexane extract of *crassocephallum vitellinum* leaves has potent antibacterial activity and this plant may be a new source for novel antibacterial compound discovery for treating drugs resistant clinical human pathogens.

CHAPTER ONE: INTRODUCTION.

BACKGROUND:

Globally, over 80% of the world's population use herbal medicinal remedies to treat and manage different ailments (WHO 2005)Although statistical information regarding the use of herbal medicinal products in Uganda are scanty; there is an increased demand for herbal remedies among patients especially those with chronic diseases such as hypertension and diabetes mellitus.

Crassocephalum vitellinum belongs to the family Astereceae, they are widely distributed in Uganda especially in the western part of Uganda and bear green leaves with small yellow flowers and According to one survey conducted among the traditional herbalists in western Uganda, the plant products are used in the treatment of gonorrhea, syphilis, urinary tract infections and skin infections among others (Monik 2009).

However, within the practice of phyto-therapy, there are plants that lack ethnomedical validity of efficacy or safety and moreover there is no proper regulatory system that ensures that any of these plant remedies do what they claim (MemoryM, 2001).The National drug authority of Uganda reported that one of the greatest challenges faced with herbal medicine regulation in Uganda is widespread misconception amongst herbalists that documentation requested for by NDA is intended to steal their indigenous knowledge, adulteration of products with western medicines, peddling of products with no therapeutic benefits and making unsubstantiated medicinal claims(NDA, Uganda 2009).

Consequently; many patients develop health complications due prolonged use of non efficacious medicinal preparations. The continued use of herbal remedies with unproven efficacy in the management of STDs is likely to increase the morbidity of STDs and this in turn will increase HIV among the people because of the inter-relationship between STDs and HIV (Torre et al 2009).

According to one survey in Uganda, 67% of the households who were close to the public health facilities believed that medicines were not available in the health facilities and the length of stock out in the public facilities was at 792.9 days (MOH Uganda 2010).It is the thesis of this study that research into available medicinal plants in the local communities around Bushenyi would provide alternatives for primary health.

Furthermore, over the last decade, there has been a rise in numerous resistant strains of pathogenic micro-organisms to already known potent antibiotics which were formally efficacious, posing a public health threat, yet research into new antibiotic compounds has stagnated (Mouokew et al 2011).Natural products of higher plants may possess a new source of agents with possible novel mechanism of action (Sawsan 2013).

One way to prevent antibiotic resistance of pathogenic organisms is using new compounds that are not based on existing synthetic antimicrobial agents (Himal et al 2008).Therefore research into medicinal plants is important in generating evidence based information that may be of value in disclosing disclose new sources of therapeutically active compounds that can be used as lead compounds in the development of potent drugs (G. Senthilmurugan et al 2013).

In addition, the World Health Organization supports the use of traditional medicine provided they are proven to be efficacious and safe (WHO 2005). Therefore it is necessary to evaluate on a scientific point of view, the potential of traditional medicines for the treatment of infectious diseases (John et al 2006.).

This present work is therefore intended to screen photochemical in the leaf extracts of *crassocephalum vitellinum* and evaluates the antimicrobial activity of the leaf extracts of *Crassocephalum Vitellinum* against *Klebsiella pneumonia*, *Staphylococcus aureus* and *Escherichia Coli.*

1.0 STATEMENT OF THE PROBLEM

Most Ugandans do not have access to essential medicines provided by the public health facilities to treat their primary health care problems. As a result, most communities resort to use of herbal remedies which are readily and cheaply available as alternatives. Unfortunately, several herbalists in Uganda are still treating patients with herbal medicines that lack ethno-medical validity of efficacy and safety.

Lastly, the growing rise of resistant microorganisms against the formally potent antibiotics is threatening public health yet research into new antibiotic compound is stagnated. Therefore there is an urgent need to scale up new antibiotic search.

1.1 RATIONALE OF THE STUDY.

The rationale for this study is three-dimensional. The first one is the apparent mismatch between Uganda's high health needs and the limited access to conventional medicines in the public health facilities. The second justification for this study is the apparently high level of antibiotic resistance which is threatening public health. Natural antimicrobial therapeutics may constitute a reservoir of new antimicrobial substances to be discovered. The third concern is the wide spread use of herbal products with unsubstantiated medicinal claims.

1.2 OBJECTIVES:

1.2.1 BROAD OBJECTIVES.

The purpose of the study is to analyze the phytochemicals in three different leaf extracts of *Crassocephalum vitellinum*, and evaluate the antimicrobial activity of these extracts against *Klebsilla pneumoniae*, *staphylococcus aureus* and *Escherichia coli.*

1.2.2 SPECIFIC OBJECTIVES.

- To carry out qualitative tests for the identification of phytochemicals in the leaf extracts of *Crassocephalum Vitellinum*.
- To evaluate the qualitative antibacterial activity of the leaf extracts of *Crassocephalum Vitelinum* against *Klebsiella pneumoniae, staphylococcus aureus* and *Escherichia coli.*

1.4 RESEARCH QUESTIONS:

- What is the phytochemical profile of the leaf extracts of *Crassocephalum Vitellinum* in methanol, n-hexane and ethyl acetate ?
- Do the leaf extracts of *Crassocephalum Vitellinum* have antimicrobial activity against *Klebsiella pneumoniae*, *staphylococcus aureus* and *Escherichia coli*?

1.5 SIGNIFICANCE OF THE STUDY:

This study will provide scientific evidence that will;

(i) Validate the ethno medicinal knowledge of the local community in western Uganda through antibacterial screening methods.

(ii) Support new antibiotic search, in an attempt to overcome the burden of the rapidly emerging resistant microorganism and unlock the opportunities for discovering and development of new plant based drugs that are less expensive but equally or more potent.

(iii) Support the development of locally available primary health care alternatives so that the communities can meet their health needs without necessarily relying on the inadequate and expensive conventional medicine.

(iv) Generate scientific information that will contribute to the development and documentation of phytochemicals in the monograph of medicinal plants used in Uganda as well as supporting the improvement in the regulatory system of herbal medicinal products in Uganda.

CHAPTER TWO: LITERATURE REVIEW

2.1 *Crassocephalum vitellinum:*

*Crassocephalum vitellinum (Benth.) S. Moore.*Belongs to the family Asteraceae. The genus *Crassocephalum* is represented approximately 100 species distributed in Asia, Africa, Australia, Malaysia, China, Nepal and Sri Lanka. (Rajesh 2011). *Crassocephalum vitellinum* is an erect little-branched herb to 1 ½ meter tall, smooth or finely hairy. Leaves with lamina elliptic to ovate in outline, lowest leaves lyrate-pinnatifid, up to 20 cm long and 10 cm wide, base often with a pair of stipule-like lobes, margins coarsely toothed, upper leaves smaller, not lobed or with a lobe each side towards base, petiole up to 4 cm long. Heads in cymes, few to many, nodding at first, later erect, heads 4 mm diameter. Flower heads are cylindrical, green, with red florets visible on top. Seeds are floating balls of numerous silky white hairs. This plant is native to tropical Africa.

The genus crassocephalum is ranked high among some of the astonishing herbs for having enormous medicinal potentialities; they are widespread over Asia, Africa and Central & Southern America. . Characterizations of each species in this genus (family Astereceae) are based on the leaves and habitat (Grayer et al., 2002). The color of the leaves is green t and sometimes almost black.

Nature has provided a complete house of remedies to cure all ailments of mankind since the dawn of civilization. In addition to food crops, man cultivated herbs for medicinal needs and knowledge of drugs has accumulated over thousand years. As a result, man's inquisitive nature, there are many effective means of ensuring health care (Kokate 2004).

Many human infectious diseases are known to be treated with herbal remedies throughout the history of humanity. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Prashant et al 2011). Therefore, the study of medicinal plants in different parts of the world is important to medicine sector, in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits. The control of bacterial infections has been remarkably effective since the discovery of antibacterial drugs. However, some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics has led to the search of new antibacterial agents in particular from medicinal plants (Boyan et al 2008). Higher plants have been shown to be a potential source for new anti-microbial agents (Sooad et al

2013).The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases (Moses A.G et al 2013).

A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have appeared in the literatures (D. Olusoga et al 2012).

The present study was to screen the antibacterial activities of *crassocephalum vitellinum* extracts; against common bacteria species, *Staphylococcus aureus*, *Escherichia coli* and Klebsiella pneumoniae. According to Buerton (1990), the most important goal of ethnopharmacology should focus on identification of drugs that treat human illness through analysis of plants claimed to be useful. It is therefore important to investigate *Crassocephalum vitellinum* which is particularly claimed to treat gonorrhea, syphilis and skin infections in the western region of Uganda.

Although synthetic drugs brought a revolution in management of different ailments, plants constitutes a very important place as raw materials for some important drugs used in allopathic medicine and moreover synthetic drugs are not easily accessed by the majority of the population especially those living in the rural settings and entirely depend on herbalists whom they can easily access (Bhatocharjeesk 1998).Therefore the study of the therapeutic properties of *crassocephalum vitellinum*, could lead to discovery of new compounds that can be modified to produce locally available medicinal compounds.

2.2 Traditional Medicinal uses of *Crassocephalum vitellinum*:

According to survey conducted among the traditional herbalists in Western Uganda, *crassocephalum vitellinum* is used as an antibacterial agent in the treatment of Neisseria gonorrhea, syphilis, urinary tract infections and skin infections (Monik 2009).Another study in Rwanda that investigated antioxidant properties of *crassocephalum vitellinu*m revealed that the plant has hepato-protective properties(Marie et al 2011).

Furthermore, Loko.YL et al (2012) carried out a study that investigated how the various communities were using *Crassocephalum vitellinum* as a medicinal agent, which revealed that the plant was used as antibiotic, anti-helminthes, anti-inflammatory, anti-diabetic anti-malarial and blood regulation properties. It also treats indigestion, liver complaints, cold, hepatic insufficiency. Therefore, different studies indicate that the plant has antibacterial properties but it is also necessary to find out which microorganisms does the plant inhibit, which is one of the interest of this present study. When people from the rural communities get an infectious disease, they are mostly treated by traditional healers

because of their expertise in such procedures as making diagnoses, treating wounds, setting bones and making herbal medicines and claim that their medicine is cheaper and more effective than modern medicine (Boyan 2008).

About 80% of developing countries, citizens use traditional medicine based on plant products (Segni et al 2011). In addition, the use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use traditional medicine provided they are proven to be efficacious and safe (WHO 2005). In developing countries, a huge number of people lives in extreme poverty and some are suffering and dying for want of safe water and medicine, they have no alternative for primary health care (R. Senthil et al 2013). There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of compounds which will be added to the potential list of drugs.

CHAPTER THREE: METHODOLOGY

3.1 STUDY DESIGN

This was an experimental study that involved laboratory analysis and use of isolated microorganisms.

3.2 SETTING OF THE STUDY

The study will be carried out in the pharmacognosy and microbiology Laboratories of Kampala International University-Western Campus, Ishaka, Bushenyi District, Uganda.

COLLECTION AND PLANT IDENTIFICATION OF CRASSOCEPHALUM VITELLINUM

The fresh leaves of *crassoceohalum vitellinum* were collectedin the month of July from the local areas of Bushenyi District. The plant was identified and authenticated by a botanist in Mbarara University of science and technology (Voucher number Wangoye khalim 001).

PREPARATION OF CRUDE DRUG FOR EXTRACTION

The leaves of *crassocephallum vitellinum* were air-dried for three weeks. After sufficient air-drying, the leaves were coarsely pulverized using an electrical grinder and passed through a sieve. The powder was weighed and stored in air-tight container for further use.

PREPARATION OF EXTRACTS

(a)Method of extraction:

The pulverized powder was extracted by successive extraction using maceration as explained by Hosamani.P.A (2012).

(b)Materials/equipments:

- Shaker
- Hexane
- Ethyl acetate
- Methanol
- Air-dried powder of *crassocephalum vitellinum* leaves
- Wattman's filter papers
- 3 plastic beakers of 1 litre each
- 3 glass beakers of 250 ml each
- Funnel
- Spatula
- Electrical weighing balance.

EXTRACTION PROCEDURE:

(a)Hexane extract:

150g of *crassocephallum vitellinum* powder were soaked with 500 ml of hexane 95% v/v in a closed plastic container and placed on an electrical shaker for 48 hours.

The liquid was strained off and the solid residue pressed to recover as much as occluded solution. This mixture of strained and expressed liquids was filtered using wattman's filter papers.

The filtrate (extract) was concentrated in an oven at 40^0c for 6 hours and its weight was recorded. After concentration of the extract, a yellowish extract was obtained.

(b)Ethyl acetate extract:

The dried residue left after hexane extraction was further extracted with 500 ml of ethyl acetate 95 % v/v in a closed plastic beaker, placed on a shaker for 48 hours. Both the strained and expressed liquid was filtered with wattman's filter papers.

After filtration, the solvent was evaporated in an oven at 40^0c for 6 hours. A dark brown residue extract was obtained.

(c)Methanol extract:

131g of the dried residue left after ethyl acetate extraction was further extracted with 500 ml of methanol 95% v/v in a closed plastic beaker placed on an electrical shaker for 48 hours.

The liquid was strained off and solid residue pressed with a clean piece of cloth to recover as much as occlude solution. This mixture was filtered with wattman's filter papers. The filtrate was concentrated in an oven at 40^0c.A dark brown extract was obtained.

After completion of sequential extraction, the percentage yields of extraction with each solvent were calculated using the following formula;

$$\% \text{ yield} = \frac{\text{weight of extract+ container- weight of container alone}}{\text{Initial weight of the powder/residue}} \times 100.$$

QUALITATIVE PHYTOCHEMICAL TESTS

All the extracts of *crassocephallum vitellinum* were subjected to qualitative chemical tests for the identification active phytoconstituents. These tests were carried out based on the standard methods described by Evans (2002) and Harbone (1998). These experiments were conducted in the pharmacognosy laboratory of Kampala international university-Western campus.

(a)TEST FOR ALKALOIDS

Mayer's tests:

2 ml of each extract were acidified with 3 drops of HCL and stirred on a water bath.3 drops of Mayer's were added to 1 ml of each sample. The formation of a cream precipitate shows the presence of alkaloids.

Wegner's test:

To 2 ml of each extract, 3 drops of 10% HCL were added. The mixtures were stirred on a water bath. Then 3 drops of Wegner's reagent were added to the filtrate. A reddish brown precipitate shows the presence of alkaloids.

(b)TEST FOR REDUCING SUGARS

Benedict's test:

To 1 ml each extract, 4 drops of distilled water were added. The mixture was filtered. 3 drops of Benedict's solution were added to each filtrate and boiled for 5 minutes. A brick red colour precipitate indicates the presence of reducing sugars.

(c)TEST FOR SAPONINS

Frothing test:

2ml of each extract were shaken with 5 ml distilled water and heated to boil. Formation of a layer of froth shows the presence of saponins.

(d) TEST FOR FLAVONOIDS

Shinoda'stest:

To 4 ml of each extract, 1 ml of 50% methanol solution and metal magnesium were added. The mixtures were warmed and 5 drops of concentrated HCL were also added. Presence of a purple colour shows flavonoids.

(e)TEST FOR TANNINS

To 3 ml of each extract, 3 drops of ferric chloride solution were added. A brown or green colour shows the presences of tannins.

(f)TEST FOR STEROIDS

3drops of acetic anhydride were added to 2 ml of each extract. The mixtures were boiled and cooled. 1 ml of concentrated sulphuric acid was added down the side of each test tube containing the sample. Formation of a brown ring at the junction of the two layers, with the upper layer turning green indicates the presence of steroids.

(g)TEST FOR TERPENOIDS

Salkowski test:

2ml of each extract were mixed with 2 ml of chloroform. To the mixture, 3 ml of concentrated sulphuric acid were carefully added to for a layer. A reddish brown colouration at the interface shows the presence of terpenoids in the sample.

(h)TEST FOR CARDIAC GLYCOSIDES

Keller-Killani test:

2 ml of extract were treated with 4 drops of glacial acetic acid and 1 drop of ferric chloride solution plus 2 drops of concentrated sulphuric acid. A brown ring at the interface shows the presence of cardiac glycosides.

(i)TEST FOR AMINO ACIDS

Ninhydrin test:

To 2 ml of each extract, 1 ml of ninhydrin (0.25% w/v) was added and the mixtures boiled for 5 minutes. The formation of a blue colour in the test samples shows the presence of amino acids in the sample.

(j) TEST FOR PHENOLS

2 ml of distilled water were added to 1 ml of each extract. 2 drops of ferric chloride (10%) solution were added to each test sample. Formation of a green colour indicates the presence of phenols.

(k) TEST FOR DITERPENES

Copper acetate test:

To 1 ml of each extract, 4 drops of copper acetate solution were added. Formation of emerald green colour shows the presence of diterpenoids.

(m)TEST FOR PHLOBTANNINS

1 ml of each extract was dissolved in 2 ml of distilled water and filtered. The filtrates were boiled with 1 ml of 2% HCL. A red precipitate shows the presence of phylobtannins in the sample.

(n) TEST FOR ANTHRAQUINONES

1ml of each extract was boiled with 2 drops of 10% HCL for 5 minutes in water bath.

The mixtures were filtered and allowed to cool. 1 ml of chloroform was added to each filtrate and 2 drops of 10% ammonia were also added to each test and heated. A rose-pink colour shows the presence of anthraquinones.

ANTIBACTERIAL ACTIVITY OF THE LEAF EXTRACTS OF *CRASSOCEPHALLUM VITELLINUM.*

METHOD AND MATERIALS USED:

Qualitative evaluation of antibacterial activity of the plant extracts against *Escherichia coli*, *staphylococcus aureus* and *Klebsiella pneumoniae* was done by agar well diffusion (punch hole) technique as described by Sharmavaishale et al (2013).

Materials:

- Muller Hinton A gar (9 grams)
- Sterile Petri dishes
- Conical flask
- De-ionized water
- Autoclave
- Incubator
- Sterile cotton swabs
- Micropipettes and sterile micropipette tips.
- Isolates of *staphylococcus aureus*, *Escherichia coli* and *klebsiella pneumoniae*
- Hot air oven.

PROCEDURE:

Muller Hinton Agar was prepared according to the manufacturer's instructions by dissolving 9 grams of the agar in a conical flask containing 250 ml of de-ionized water. This was sterilized by autoclaving at 120^0c for 15 minutes at 15psi pressure.

20 ml of Muller Hinton Agar were poured aseptically into each of the 6 Petri dishes after sterilizing them in a hot air oven at 170^0c for 2 hours. The plates were allowed to solidify at room temperature in sterile conditions.

These plates were further incubated for an overnight to check for their sterility. All the plates did not have growths after an overnight incubation at 37^0c.

Using sterile micropipette tips, 5 wells bored on the surface of the agar. Beneath the bottom surface of the plates, labels with varying concentrations of 75 mg/ml, 150 mg/ml and 300 mg/ml were placed against each well. The remaining 2 wells were labeled **PC and NC** indicating positive and negative controls respectively.

The plant extracts were prepared at varying concentrations of 75mg/ml, 150mg/ml and 300mg/ml by dissolving the weighed semisolid extract with tween 80 and normal saline.

The positive control (ceftriaxone) was prepared at a concentration of 2mg/ml and normal saline was used as the negative control.

The 3 wells were filled with 250uL of each extract at 3 varying concentrations labeled against each well using sterile micropipette and the remaining 2 wells were filled with 250uL ceftriaxone and normal saline respectively.

Using sterile cotton swabs, isolates of *Escherichia coli*, *staphylococcus aureus* and *Klebsiella pneumoniae* were streaked on the surface of Agar with wells filled with extracts.

The plates were incubated at 37^0c for 24 hours. The antibacterial activity was determined by measuring the diameter of the zones of inhibition in millimeters (mm) ±SD.

The experiment was done in duplicate for each extract and the mean zone of inhibition was calculated.

CHAPTER IV: RESULTS

Result 01: Percentage yields of extraction

Serial no.	Successive extracts	Nature of extracts	Color	Weight of powder residue(grams)	Weight of extract (grams)	Percentage yield (w/w)
1	Hexane	Semisolid	Yellowish	150	2.2	1.47
2	Ethyl acetate		Dark brown	140	1.8	1.29
3	Methanol		Dark brown	131	4.0	3.05

RESULT 02: Summary of data showing the preliminary phytochemical screening of the various leaf extracts of *Crassocephalum Vitellinum.*

Serial no.	Constituents	Hexane extract	Ethyl acetate extract	Methanolic extract
01	Alkaloids			
	(a) Mayer's test	+	+	+
	(b)Wegner's test	+	+	+
02	Test for reducing sugars			
	Benedict test	+	+	+
03	Test for saponins			
	Frothing test	-	-	+
04	Test for flavonoids			
	Shinoda's test	-	+	-
05	Test for tannins	+	+	+
06	Test for steroids	+	-	+
07	Test for Terpenoids			
	Salkowski test	+	-	+
08	Test for cardiac glycoside	+	+	+
09	Test for amino acids			
	Ninhydrin test	-	-	-
10	Test for phenols	-	-	+
11	Test for quinones	+	-	-
12	Test for diterpenoids			
	Copper acetate test	+	-	+
13	Test for phylobotannins	-	-	-
14	Test for anthraquinones	-	-	-

(+) = Present, (-) = Absent

Result 03: Mean Zones of inhibition for plant extracts against Escherichia coli and Staphylococcus aureus and Klebsiella pneumonia

Sample	concentration(mg/ml)	Mean zones of inhibition in millimeters±SD		
		Staphylococcus areus	Escherichia coli	Klebsiella pnemoniae
Hexane extract				
	75	0	0	0
	150	0	0	0
	300	13.5±2.12	18.5±4.95	14.2±1.06
Ceftriaxone	2	44.5±0.71	39.5±0.71	44±1.41
Normal saline	-	0	0	0
Ethyl acetate extract				
	75	0	0	0
	150	0	0	0
	300	0	0	0
Ceftriaxone	2	41±1.41	39±1.41	38±2.83
Normal saline	-	0	0	0
Methanolic Extract				
	75	0	0	0
	150	0	0	0
	300	18±2.83	0	0
Ceftriaxone	2	40.5±0.71	39.5.5±0.71	44±1.41
Normal saline	-	0	0	0

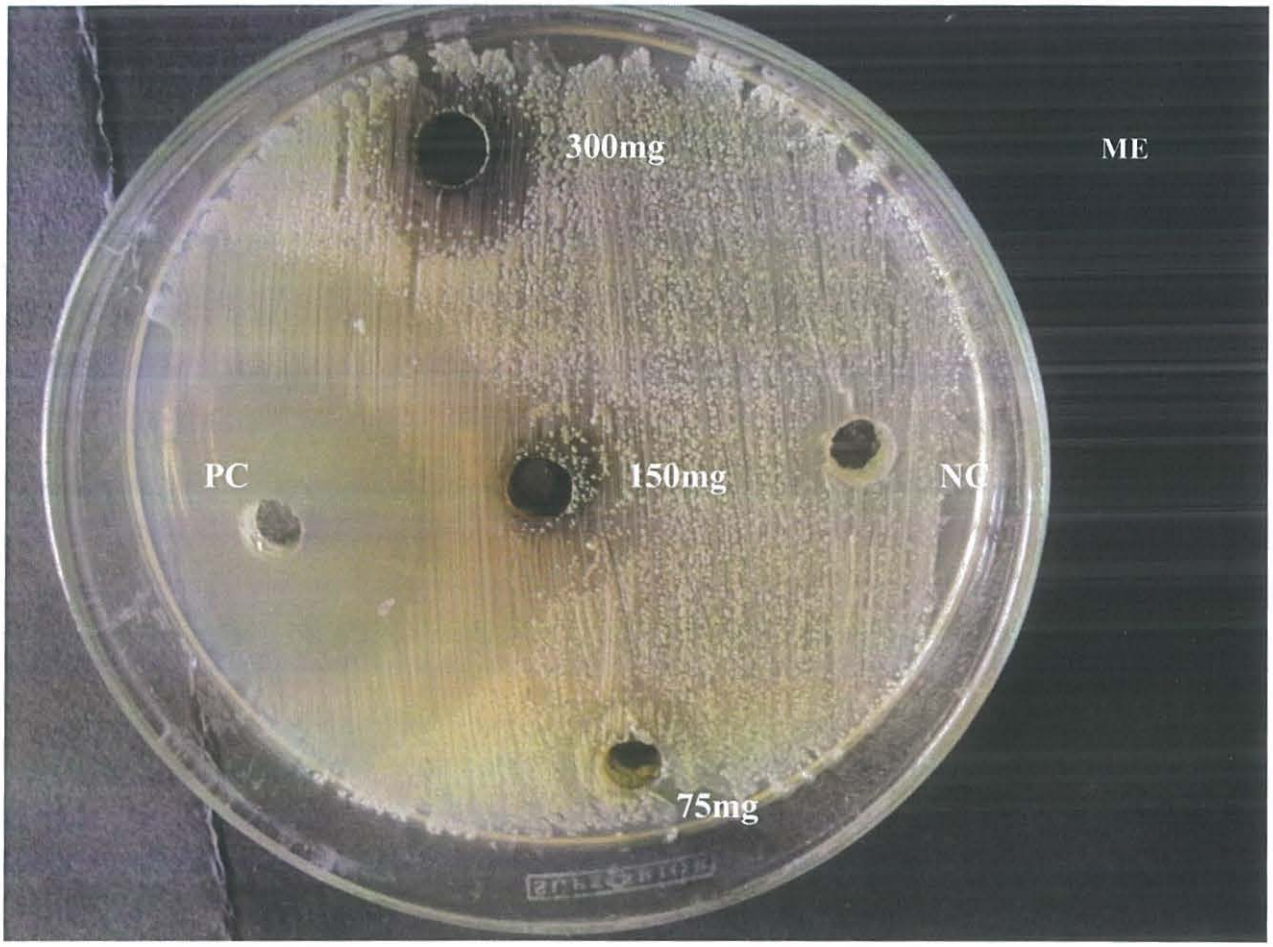

Figure A: *Staphylococcus aureus* / methanolic extract, MI=18±2.83

Abbreviations: ME; Methanol extract, PC; Positive control. NC; Negative control

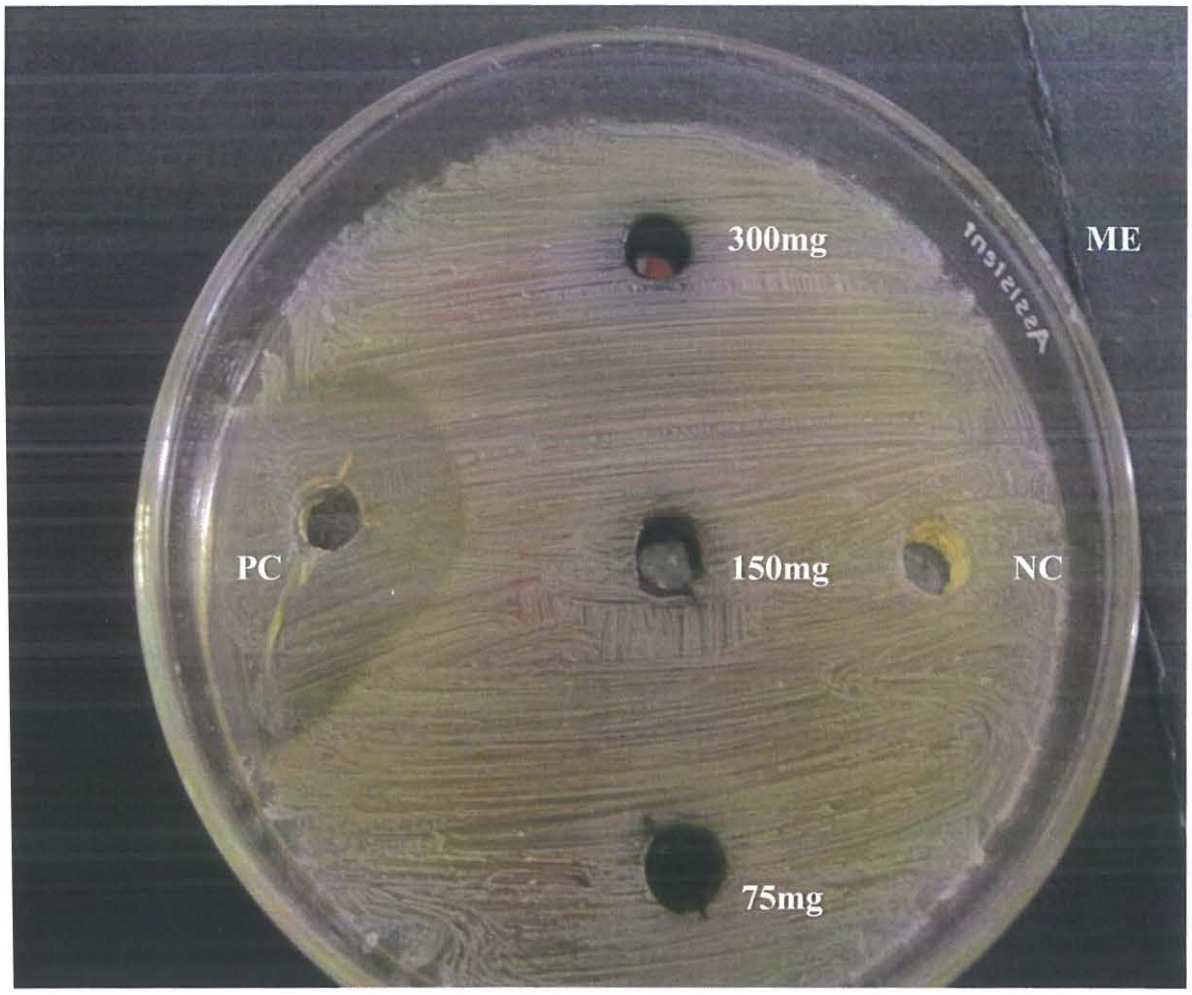

Figure C: *Klebsiella pneumoniae*/Methanolic extract, MI=0

Abbreviations: ME; Methanol extract, PC; Positive control, NC; Negative control.

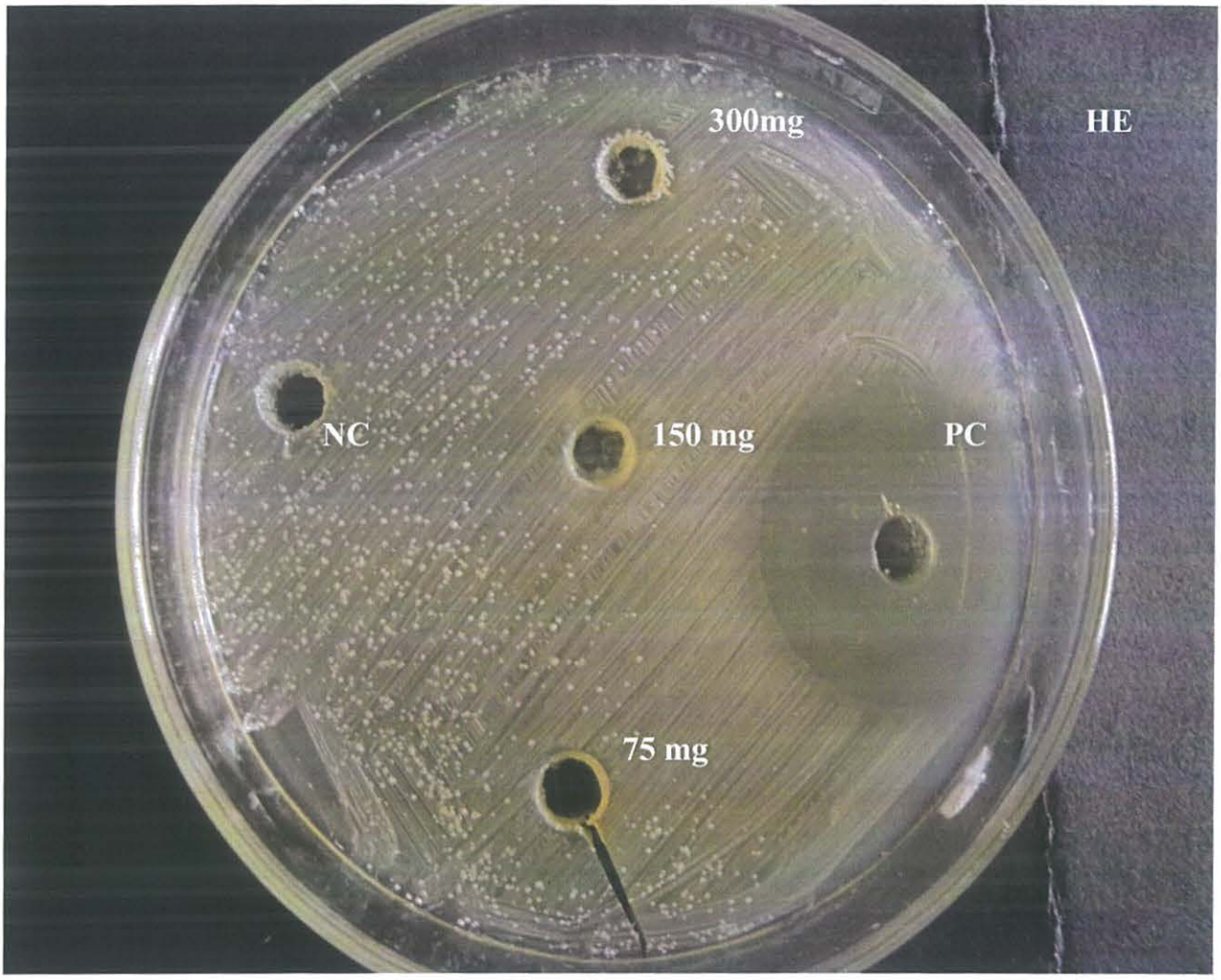

Figure D: *Staphylococcus aureus*/Hexane extract, MI=13.5±2.12

Abbreviations: HE; Hexane extract, PC; Positive control, NC; Negative control.

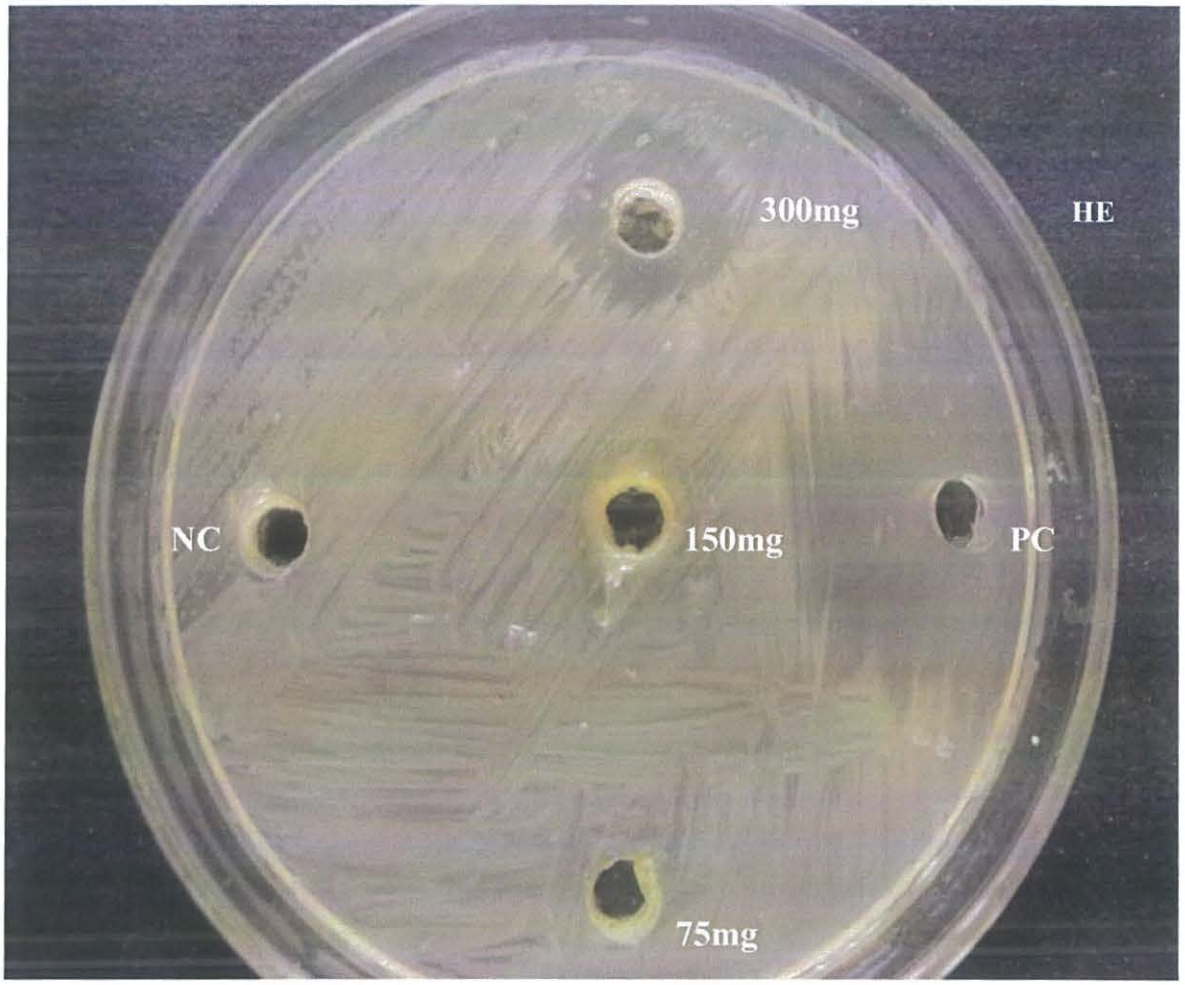

Figure E*: Escherichia coli*/Hexane extract, MI=18.5±4.95

Abbreviations: HE; Hexane extract, PC; Positive control, NC; Negative control.

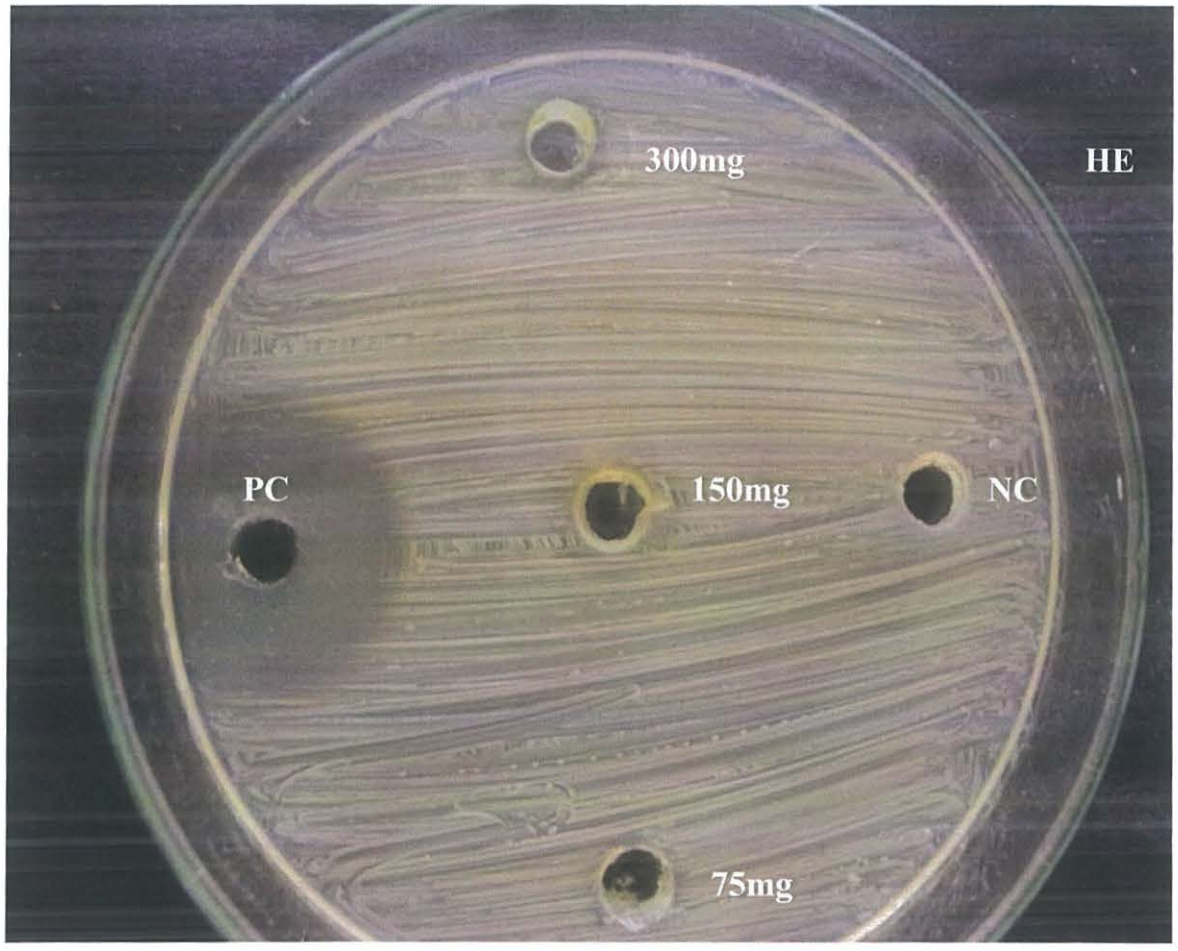

Figure F: *Klebsiella pneumonia*/Hexane extract, MI=14.2±1.06

Abbreviations: HE; Hexane extract, PC; Positive control, NC; Negative control.

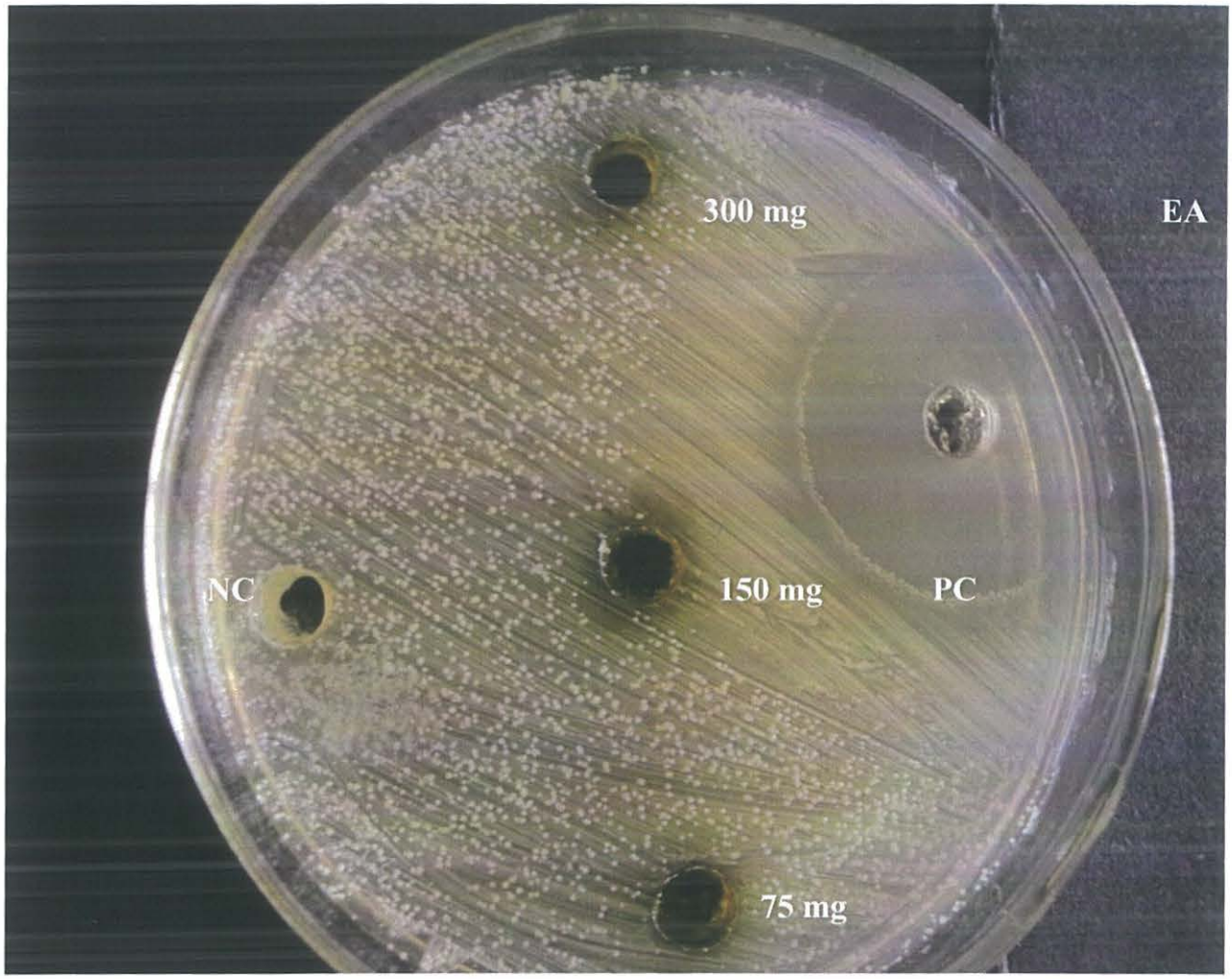

Figure G: *Staphylococcus aureus*/ethyl acetate extract, MI=0

Abbreviations: EA; Ethyl acetate extract NC; Negative control, PC; Positive control

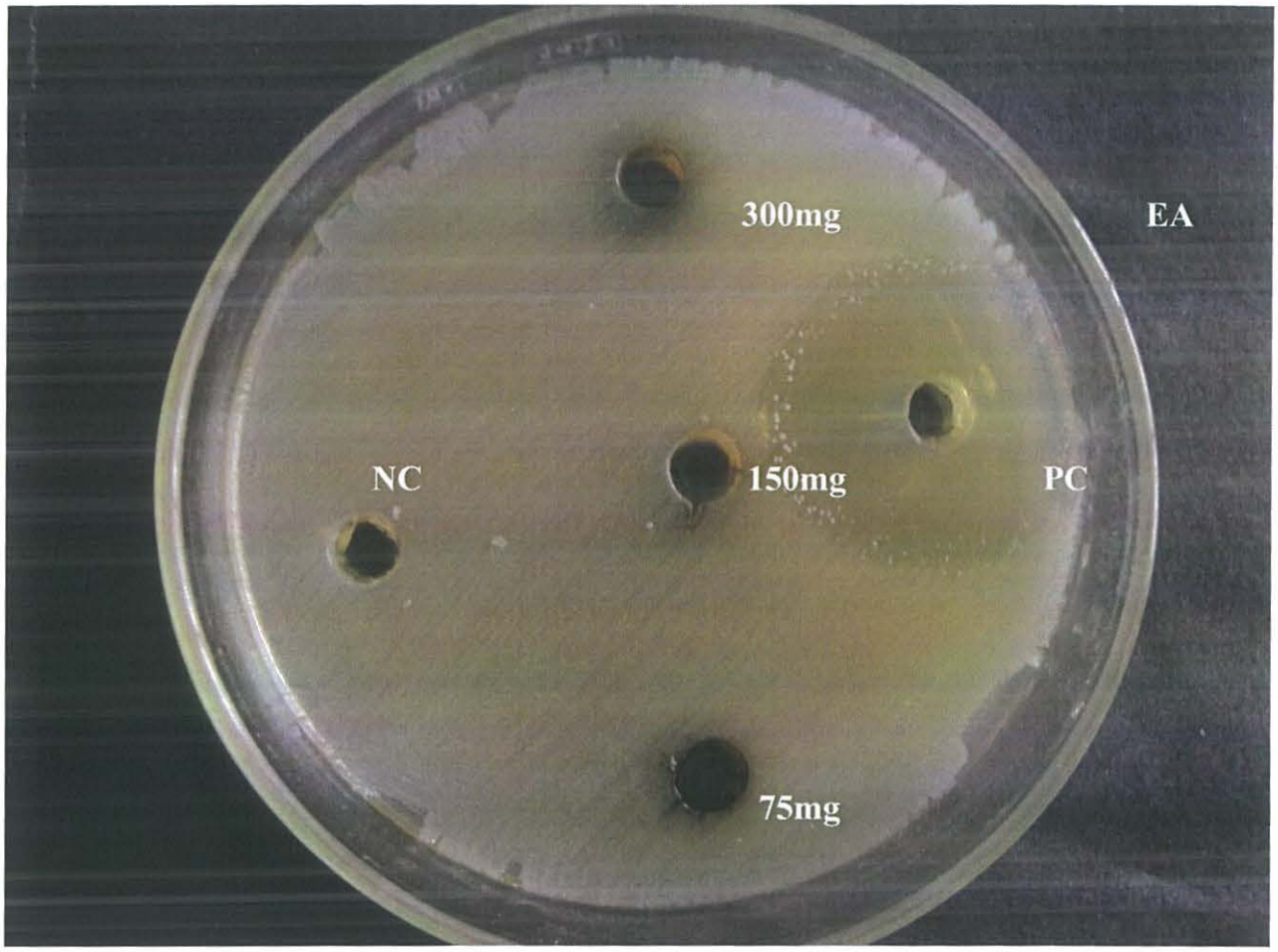

Figure H: *Escherichia coli*/ethyl acetate extract, MI=0

Abbreviations: EA; Ethyl acetate extract NC; Negative control, PC; Positive control

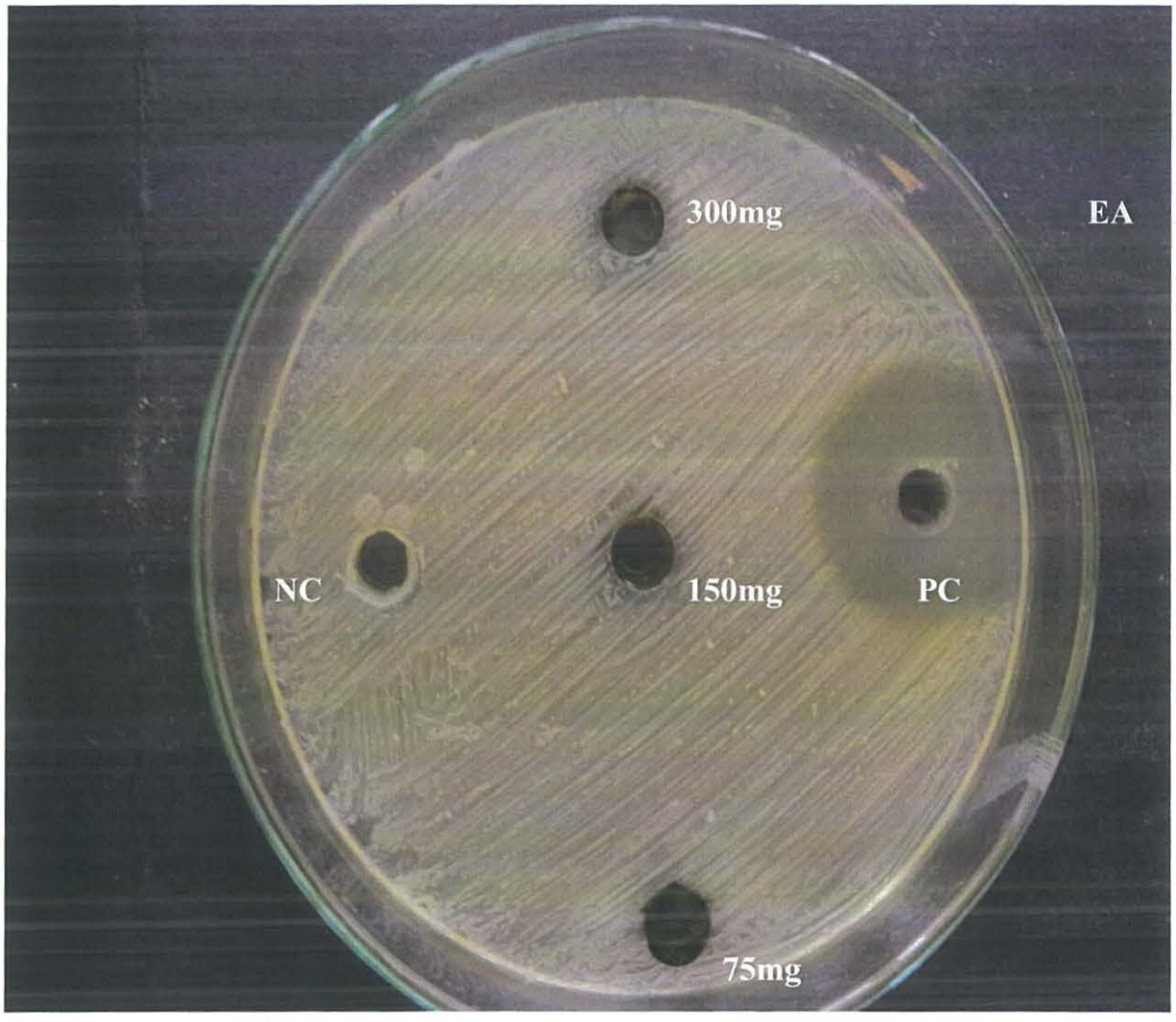

Figure I: *Klebsiella pneumoniae*/ Ethyl acetate extract, MI=0

Abbreviations: EA; Ethyl acetate extract NC; Negative control, PC; Positive control

CHARPTER FIVE: DISCUSSION

The medicinal plants have been the main source of information for drugs over many centuries, in both developed and developing world. It is estimated that 25% of all modern medicines are derived either directly or indirectly from medicinal plants (Essawi 2000).

The medicinal property of plants is due to the presence of different complex chemical substances as secondary metabolites, which are exclusively accumulated in different parts of the plant. *Crassocephalum vitellinum* leaf extracts contained a wide range of metabolites such as alkaloids, steroids, tannins, cardiac glycosides, phenols, terpenoids, and flavonoids among others.Similar compounds were obtained in a study that investigated the phyto-constituents and hepato-protective properties of *Crassocphallum vitellinum in Rwanda* (Marie et al 2011).

These natural metabolites are important as potential antimicrobial crude drug and source for natural compounds as new anti infection agents (Dwivedi et al 2011). The growing rise of resistant microorganisms against the formally potent antibiotics is threatening public health. Therefore there is an urgent need to scale up new antibiotic search because of the concern that an increasing number of bacterial species are becoming resistant to more than one antibiotic (D. Olusoga et al 2012).

This present investigation for the newer antibacterial bioactive compounds is targeted on the unexplored folk medicinal plants being used for centuries in treating local population in Bushenyi District.

In this study, three different polarity extracts of *Crassocephallum vitelinum* at three varying concentrations of 300mg/ml, 150mg/ml and 75mg/ml were tested for antibacterial activity on 3 different human pathogens viz *Staphylococcus areus, Klebsiella pneumonia* and *Escherichia coli*.

Hexane extract showed antibacterial activity against *staphylococcus areus, klebsiella pneumonia* and *Escherichia coli* at the highest concentration. At lower concentrations of 150mg/ml and 75mg/ml, hexane extract was inactive against all the test microorganisms.

In addition, methanolic extract had no activity against *Escherichia coli* and *Klebsiella pneumonia* at all the three concentrations of the extract. However, growth of *staphylococcus aureus* was inhibited at a higher concentration of 300mg/ml with a mean zone of inhibition of 18±2.83mm.

CHARPTER SIX: RECOMMENDATIONS

Further investigations should be performed on *crassocephallum vitellinum* leaf extract to determine the individual metabolites responsible for the exhibited antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia.*

In addition, the toxicological profile of the leaf extract of *crassocephallum vitellinum* should also be performed to determine their safety for human use.

The minimum inhibitory concentration and minimum bactericidal concentration of these extracts need to be determined to assess the effectiveness of these extract.

CONCLUSION:

Based on the local uses and literature review of past investigations, the plant was selected. The preliminary phytochemical studies on *crassocephallum vitellinum* were performed by successive solvent extraction and identified by chemical tests. These tests showed the presence of alkaloids, flavonoids, cardiac glycosides, steroids, tannins, pseudo tannins, reducing sugars and saponins.

Finally, antibacterial activity of the leaf extract of *crassocephallum vitellinum* against *staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* was assessed by agar well diffusion method. Each extract was prepared at three varying concentrations of 300mg/ml, 150mg/ml and 75mg/ml. Hexane extract was active against all the three pathogens at a higher concentration of 300mg/ml. Similarly, methanolic extract was only active against *staphylococcus aureus* at the highest concentration and ethyl acetate extract remained inactive against all the test micro-organisms at all the three varying concentrations of ethyl acetate extract. Therefore, for susceptible microorganisms, antibacterial activity of leaf extracts of *Crassocephallum vitellinum* were increased with increasing concentration of crude extract.

Similarly, ethyl acetate extract was inactive against all the test micro-organisms at all the concentrations of the extract.

For sensitive micro-organisms, the results obtained showed that antibacterial activity both hexane and methanolic extracts of *Crassocephallum vitellinum* were increased with increasing concentration of crude extract and this plant may be a new source for novel antibacterial compound discovery for treating drugs resistant clinical human pathogens. Related results were obtained with ethyl acetate extract of *Crassocephallum bauchiense against staphylococcus aureus* and *Escherichia coli* (Raymond et al 2011)

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