PHYTOCHEMICAL AND ANTIBIOTIC LIKE ACTIVITY OF (*CRASSULA OVATA)* JADE PLANT ON DIFFERENT STRAINS OF BACTERIA

Declaration

This proposal/thesis is my original work and has not been presented for a degree in any other University.

Mwangi Denis Muiruri

Sign

Date

This work has been submitted for examination with my approval as a University supervisor

Mr. Wambura Mwangi (JKUAT)

Sign

Date

Abstract

The *Crassula ovata* plant has been used for many years as an ornamental plant, and also as a medicinal plant in some communities like the Khoi of South Africa and in Chinese culture. Locally the plant is being used by homeowners who have it in their vicinity as a remedy for diarrhea and disinfecting wounds. However, the major problem of using this plant is its ineffectiveness to heal wounds and diarrhea in most cases where it is being used. It brings so many questions in mind like does the *Crassula ovata* plants inhibit certain specific microorganisms, or is the concentration of the extract to blame, or even the method used to extract the plant. The mode of extraction used in this study involved both aqueous extraction and methanolic extraction, to ensure all plant constituents are extracted for better results. The microorganisms that were tested against the plant extracts are the major day to day sources of diarrhea and wound infection. F explants extracts are used at varying concentrations. The observable results were quantitatively analyzed to see which plant extract and at which concentration causes the most inhibition on the microorganisms. The plant extract with the most inhibition was found to be the water extraction at the concentration of , and it would be recommended that the *Crassula ovata* plant be used to the specifications as observed in the study.

Acknowledgements

The author wishes to express his deepest appreciation to Mr. Wambura Mwangi. His support, guidance, and knowledge throughout this thesis work were invaluable. The author would also like to thank Mr. J. Muthanga and Mr. Kamau for their advice and assistance throughout the laboratory work.

Sincere appreciation is extended to all those graduate students who gave their wise encouragements and support throughout the entire course work.

The author would also like to express his thanks to his parents, siblings, and God; for their constant influence on his continued education. They all provided love and support throughout this entire process, as well.

Table of Contents

CHAPTER ONE

INTRODUCTION

Plants have been used as medicines since time in memorial. Plants having medicinal value are widely and successfully used on every continent. In Asian countries, the practice of herbal medicine is extremely well established and documented. As a result, most of the medicinal plants that are internationally recognized come from this region, particularly from China and India. For Europe and North America, the use of herbal medicine is increasing fast, especially for correcting imbalances such as diabetes caused by modern diets and lifestyles. Many people now take medicinal plant products on a daily basis, to maintain good health as much as to treat illness. In Africa, attitudes towards traditional, herbal medicines vary strongly mostly due to diversity in cultures and traditions. Another reason for this is the confusion between herbal medicine and witchcraft. The use of medicinal plants is commonly associated with superstition, and therefore rejected by some people in favor of western medicine. On the other hand, there are millions of Africans who prefer traditional methods of treatment.

The valuable medicinal properties contained in certain plants are not in doubt. In recent years, for example, the Chinese plant *Artemisia annua*, has become the essential ingredient in a new generation of anti-malaria drugs. East African countries have adopted the plant and growing it to supply pharmaceutical manufacturers in Europe. The bark of the tree *Prunus africana* is used mostly in making treatments for prostate cancer. *Sutherlandia*, a native plant of South Africa, is being increasingly recognized for its value to HIV/AIDS diagnostics and treatment. Other African plants, such as the Devil's Claw and the African Geranium, are gaining popularity as herbal medicines, particularly in Europe.

Medicinal plants hence represent an important opportunity to rural communities in Africa as in Kenya, as a source of affordable medicine and as a source of income for those who grow the plants. Governments too need to be thinking about how to promote the benefits that medicinal plants have to offer, which may involve integrating herbal medicine into conventional healthcare systems, and funding more effective research into possible applications of the plants. This raises important issues, such as regulation of traditional healers and ensuring certain standards are met.

Many plants grown in Kenya have valuable medicinal properties. Paw paws, for instance, can be used to treat asthma, rheumatism and intestinal worms. Lemongrass helps in relieving fever. Sap from the *Aloe vera* is excellent for treating burns. These plants, and many others, are easily grown in home gardens for domestic use. *Moringa oleifera* is another plant with great potential, both in terms of home use and as a source of income. It has high levels of iron, calcium and Vitamin A, and can be used to boost the immune system, as well as treat a range of illnesses. It is normally prepared by drying the leaves and then pounding them into a powder. This can then be mixed with flours, or with other foods such as meat. For HIV/AIDS patients it offers an excellent source of nutrients which can help to support their immune system and slow down the advance of the disease.

Having a selection of different medicinal plant products can broadens the market and the economy of the region as well. For example, the *Mondia whytei* processors in Kenya sell the raw roots of the plant, but also produce a powdered form. This is preferred by hospitals, which use it to increase patient's appetite and to increase milk production in nursing mothers. The powder is also used to make fortified foods for the sick. Other products for sale include Mondia seeds and seedlings. Once products have been formulated for sale, gaining official recognition and approval from the authorities is valuable. In Kenya, the organization Action for Natural Medicine (NAMEDO) is working with the National Drug Authority to have its products such as soaps, creams and oils approved. The organization is also working with the National Bureau of Standards, so that the products are standardized. This makes it much easier to market the products, for example through clinics or supermarkets.

Herbal and conventional doctors are enemies of each other. Each having little respect for the skills and knowledge of the other. For example, hospitals and clinics can be swamped by people with relatively minor ailments, some of which might be treated with herbal remedies. This would allow hospitals to devote more of their resources to deal with serious diseases and operations. However, for a government health ministry to promote or encourage people to use herbal medicines normally requires a radical change in thinking of its people and policy implementation. In The Gambia, this process is already underway, with the government working to have traditional healers registered in union and/or associations. This makes it easier for their practice to be monitored, and to ensure that it is in accordance with the national traditional medicine policy. The policy aims to protect the patient's rights, and to introduce standards for

traditional medicine, and to protect the intellectual property rights of traditional healers. Integrating plant medicine into national policy involves the health, agriculture, environment and trade ministries. In order to ensure farmers are supported in growing the plants. Harvesting from the wild can be controlled and quality standards introduced for those trading in medicinal plants and their products.

CHAPTER TWO

LITERATURE REVIEW

Brief description of *Crassula ovata*

Scientific classification and morphological description

Crassula ovata, commonly known as the jade plant or the money tree, belongs to the *Crassulaceae* or the Orpine family. They are a family of dicotyledonous plants, succulent with small white or pink flowers. Their main water reservoir is in their succulent leaves. (Springer 2003)

The jade plant is an evergreen plant up to 1 - 3 m tall, with thick branches and smooth, rounded, fleshy leaves that grow in opposite sides along the branches which are also short, stubby and well-proportioned. The leaves are a rich jade green color, 30 -90 mm long and 18 - 40 mm wide, egg-shaped to elliptic, often with a red margin and a somewhat pointed end. They are in opposite pairs, the one pair arranged at right angles to the next, and they are clustered towards the ends of the branches. New stem growth is the same color and texture as the leaves, but becomes brown and woody with age. Under the right conditions, they may produce small white or pink star-like flowers in early spring. The flowers later develop into small capsules, each of which hold many tiny seeds (Gary 2004).

Ecology and Distribution

The *Crassula ovata* plant is able to maintain minimum water loss while photosynthesizing efficiently through Crassulacean Acid Metabolism (CAM). Its stomata are closed during the day but open at night where Co□is taken in and stored in the form of organic crassulacean acids. In daytime, these acids are broken down and the Co□ released is recycled in the photosynthetic process. This way the plants lose much less water yet can photosynthesize normally during the daytime hours. However, during extremely dry periods they will not even open their stomata at night, and will re-cycle the Co□ within their cells. This causes slow metabolism hence little growth but at the same time keeping the cells healthy. This is called CAM-idling (Walter *et al*., 2012)

The plants succulent water-storing leaves, stems and roots give it the ability to survive droughts, being grazed on, trampled on or knocked over, as it is able to root from any piece of its stem, and even a single leaf. Any discarded leaves left around the foot of the plant send down roots and grow into new plants.

The flowers of *Crassula ovata* attract wasps, flies, bees, butterflies, and beetles. Wind helps disperse the fine dust-like seeds. The stems also make good bases for wasps to build their nests (Eggli 2002).

Crassula ovata is a native plant to South Africa. It is also a common houseplant all over the world, but it is mostly located in the Northern Hemisphere particularly in cold and/or dry areas where water is scarce. *Crassula ovata* is a vibrant part of the Eastern Cape and KwaZulu-Natal valley thicket vegetation, together with a variety of euphorbias, aloes, *Portulacaria afra* and other succulent plants. It strives from Willowmore to East London then northwards to Queenstown and KwaZulu-Natal where it grows on rocky hillsides (Leistner 2000).

In Kenya the *Crassula ovata* is found growing in areas with adequate rainfall which is well distributed throughout the year. These are areas within the Central, Rift Valley, Nyanza, and few areas in the Eastern region of Kenya. The *Crassula ovata* rarely grows in the North Eastern part of the country due to the scarce availability of precipitation. Also in the coastal region it's very rare to find this plant. There is no variation in the *Crassula ovata*'s phytochemical composition regardless of where they are from. The only difference might occur in their succulence depending on the geographical location which will affect water availability in the area where the plant is found (Gary 2004).

Mode of Propagation

Crassula ovata is famously propagated either by leaf cuttings or stem cuttings. Both of these types of cuttings require high humidity. In the wild, stems and leaves will often break off and fall to the ground, and after a few weeks, they may grow roots and form a new plant. They can also be cut and placed in a water container until roots grow usually in about two weeks, then planted in soil.

In cultivation, new plants are made by cutting new growth (stems or leaves) and letting them dry. Roots will develop in or out of soil, though inserting the stem into moist soil will increase rooting. (Hudson *et al*., 2002)

Traditional Uses and Cultural Aspects

Traditionally many communities have developed a habit of using the fluid extract from the leaves to treat warts which are small circumscribed tumor of the outer layer of the skin. Warts are flat or elevated from the surrounding skin and are firm. They are caused by forms of the contagious human papilloma virus (HPV); warts vary in size and may be accompanied by pain, particularly if they occur on the feet (plantar warts). The leaf of *Crassula ovata* was sliced in half and attached the moist inside to the wart for a few hours, or overnight. The unsightly growth would fall off with just three applications (Springer 2003).

In Asian cultures particularly in China (700AD), jade plant is a popular element. Medicine-men prescribed a tea of the jade plant to treat symptoms of diabetes. Because of its abundance and its softness in ancient times, it could easily be shaped into various forms thus it was used in the art of Bonsai. The plant was spread around as luxurious gift to royalties all over the Chinese empire.
The jade plant is used in the Chinese ritual practice of Feng Shui to attract the flow of money. Feng Shui creates balance and harmony of energies within a space. Practitioners believe that the money tree brings about balance to the southeastern corner of a home. The jade plant is one of the plants used in this ritual practice. In many businesses, a jade plant is often placed near a cash register as in Chinese tradition as a way to attract prosperity (Springer 2003).
In Africa, jade leaves are boiled in milk and consumed to stop diarrhea. The Khoi and other African tribes ate the roots and stems of the jade plant. The plant was grated and cooked after which they were eaten with thick milk. The leaves were also boiled in milk as a remedy for diarrhea, treating epilepsy, corns and as a purgative.
The Jade plant has attracted more common names including the Penny Plant, Money Tree, Dollar Plant, and Tree of Happiness in the Far East, United States and Germany. The plant is traditionally grown in square porcelain tubs with lion feet to bring good financial luck, and (Doreen *et al*., 2000).
The *Crassula ovata* plant is in Kenya mostly grown in local homesteads for its ornamental value. However some people keep this plant also for its medical values. The Kamba community believe that the juice extracted from this plant help heal burn wounds on the skin. Other communities like the Maasai use it as a relief for stomach upsets.

Diseases Controlled by *Crassula ovata* plant

Microorganisms are a common human skin, gut flora, soil, water, and gastrointestinal tract inhabitant. However, these microorganisms can also be major causes of abnormalities in the human body system. Bacteria such as some *Staphylococcus* species live on normal skin and on mucous membranes and cause no harm. Some bacteria; however, invade normal skin, broken skin or wounds causing wound infection. The most common causative organisms associated with wound infections include Staphylococcus aureus and *Pseudomonas aeruginosa.*

Staphylococcus aureus is a Gram-positive bacteria and a potential pathogen. It is a leading cause of bacterial disease in humans. It can be transmitted from the nasal membranes of an asymptomatic carrier to a susceptible host. This bacterium causes Furunculosis, a medical condition in which large areas of the skin are covered in persistent boils. Folliculitis is also caused by the *Staphylococcus aureus* bacterium. It's an inflammation of one or more follicles, especially of the hair, producing small boils. These infections are commonly found in young children aged 5-12, or any immuno-suppressed person. *Staphylococcus aureus* can cause impetigo (skin infection), scalded skin syndrome and food poisoning. (Gibbons *et al.,* 1978)

Pseudomonas aeruginosa is an opportunistic pathogen of humans that can invade virtually any tissue. It is a leading cause of hospital-acquired (nosocomial) gram-negative infections, but its source is often exogenous (from outside the host). *Pseudomonas aeruginosa* causes wound infections, athlete's foot, gram negative folliculitis, chronic paronychia, and pneumonia.
(Balcht *et al.,* 1994)

Escherichia coli are a Gram-negative, rod-shaped bacterium, a consistent resident of the small intestine. Some strains of *Escherichia coli* are pathogens that cause intestinal infections, urinary tract infections and neonatal meningitis. Some serotypes can be a source of severe food poisoning in humans. But there are harmless strains that are part of the normal flora of the gut, which benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine. *Escherichia coli* and related bacteria constitute about 0.1% of the gut flora, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease.
(Vogt and Dippold 2005)

Candidiasis is an overgrowth of a fungus hence causing irritation and swelling. Pathogenicity among yeast-like fungus is extremely variable; however, the most virulent and harmful is

Candida albicans. Involvement of the *Candida albicans* may be localized to the mouth, throat, skin, toes, scalp, fingers, vagina, nails, bronchi, lungs or gastrointestinal tract. It may also be systemic as in septicemia (circulating in the blood and causing damage to blood vessels and blood cells), endocarditis and meningitis. Factors predisposing people to candidiasis include AIDS, burn wounds, young individuals and/or infants, pregnancy, oral birth control, high fruit diets, antibiotic therapy, immunosuppressants, cancer treatments, steroids, heart surgery, genetic deficiency, endocrine deficiency diabetes, use of catheters, and use of dirty needles.

Bacillus subtilis cells are rod-shaped, Gram-positive bacteria that are naturally found in soil and vegetation. *Bacillus subtilis* bacteria are non-pathogenic. They can contaminate food; however, they seldom result in food poisoning (Balcht *et al.*, 1994).

Statement of the problem

The main problem or general question pertaining the *Crassula ovata* is whether it has relevantly effective antibiotic or antifungal traits. *Crassula ovata* plants are mostly used as house plants and do not have many commercial uses other than for ornamental value. However it's usually integrated into most homesteads also because of its healing properties or medicinal values. In many occurrences, the plant extracts do not always treat some stomach upsets or even fresh wounds, despite its prominent successful use in other past communities from different nations. Even after continuous application of the plant extracts, the stomach upset or wound still continues to persist.

The question left unanswered is whether the extraction procedure is efficient, or maybe the concentration of the extract is too high or too low to be effective. Or perhaps which part of the *Crassula ovata* plant is most effective to use.

The *Crassula ovata* has had successful ratings in its past ancient uses, but very much limited success in present time. There is need to research further why this has come to be. Among the many reasons for these changes might be the fact that since these plants were originally from what we now know as highly productive nations (China and South Africa), due to the industrial advancements and resulting increased environmental pollution, the plant genome has been altered. This could to a great deal alter the overall efficiency of the plants antibiotic traits.

Justification

This research was conducted so as to test the medicinal value of Crassula *ovata* plant, and whether it has any phytochemical components which inhibit growth of microorganisms. There is also the need for more research on the evolution of the specific microorganisms *Crassula ovata* is said to inhibit. This is because it is a known fact that these microorganisms and others are mutating almost every day, thereby increasing their survival and reducing the effects of any antibiotic stimuli. Unfortunately, no much research has been put in effect to solve this riddle.

Objectives

Broad objectives

- To determine the phytochemical components and antibiotic traits of the *Crassula ovata* plant.

Specific objectives

1. To determine the phytochemical components of the *Crassula ovata* plant.
2. To test the antibiotic activity of the *Crassula ovata* plant extracts against a range of selected microorganisms.

Hypothesis

Null hypothesis

There is no difference in the phytochemical components of the *Crassula ovata* plant and the proliferation of the selected microorganisms.

CHAPTER THREE

MATERIALS AND METHODS

Sample and Sampling Technique

The samples for experimentation include the fresh leaves and stem of the *Crassula ovata* plant. The *Crassula ovata* plant species were acquired randomly from a local homestead in Ruiru, Kenya. The plant samples were stored not more than two hours before extraction.

Extraction of crude extracts from the plant

This was carried out according to Walter *et al*., (2012).

Methanolic extraction

2 grams of the plants leaves and stem were cut into smaller pieces and put in 100ml of 90% methanol to dissolve the crude extracts and left overnight. The plant material was then separated from the methanol by washing with 100ml of sterile distilled water. Serial dilution of the extract was then done four to five times starting with a dilution of ×10° to × .

Aqueous extraction

2 grams of the plants leaves and stem were crushed using a pestle and mortar while adding 100ml sterile distilled water to dissolve the crude extracts. The extracts were then put into sterile conical flasks and stored at room temperature. Serial dilution of the extract was then done four to five times starting with a dilution of ×10° to × .

Small circular paper discs were put into the containers containing the different plant extracts from the aqueous extraction and the methanolic extraction. The discs with the methanolic extracts were then put in an oven at 40ºC for 30minutes to dry.

Phytochemical Tests

The phytochemical tests were carried out as per procedures by Seidel (2012). Fresh plant samples were obtained, weighed and divided for the various assays. Plant extracts required in some assays were obtained from prior extractions.

Alkaloid Test

0.05g of the sample was added to 1%HCL and filtered. The filtrate is tested carefully with various alkaloid reagents as follows;

Mayer's test

To 1ml of the filtrate, a drop or two of Mayer's reagent was added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

Dragendorff's test

To 1ml of the filtrate 1 or 2mls of Dragendorff's reagent was added. A prominent yellow precipitate confirms the test as positive.

Carbohydrate Test

Barfoed's test

To 1ml of filtrate, 1ml of Barfoed's regent was added and heated in a boiling water bath for 2 minutes. A red precipitate confirms sugar presence.

Benedict's test

To 0.5ml of filtrate, 0.5ml Benedict's reagent was added and the mixture heated in a boiling water bath for 2 minutes. A characteristic colored precipitate confirms the presence of sugar.

Detection of Saponins

1ml of plant extracts were dissolved in anhydride-tetrachloride to which 4 drops of concentrated sulfuric acid was added to the mixture. A blue, green or red color accompanied by a pink ring shows presence of Saponins.

Flavanoids Test

1ml of the extract was put into a test tube followed by addition of Hydrochloric acid (4 drops) and Magnesium turnings. Development of a pink or magenta red indicates the presense of Flavanoids.

Tannins Test

1ml of the crude extract was dissolved in water which contains 1% gelatin and 10% NaCl. The presence of tannins is indicated by the presence of a blackish blue color. Catecol tannins are indicated by a greenish black coloration.

Sterols and Steroids Test

1ml of the extract was put in a test tube in which 0.5ml sulfuric acid, acetic anhydride and chloroform in similar amounts were added. A red coloration would indicate presence of sterols. A green color indicates presence of steroids.

Media preparation and incorporation with bacteria

Based on Baker et al., (2001), Mueller Hinton agar was prepared by measuring 28.5g and dissolving it in 750ml distilled water. Nutrient broth was also prepared and put into glass bottles. The prepared media, nutrient broth, pipette tips, paper discs, distilled water, the pestle and mortar were autoclaved at 121°C for 15 minutes. The agar was left to cool to about 40-37°C then aseptically poured into sterile Petri dishes. This was done on the bench, using flame to keep media bottle sterile.

Sterile nutrient broth was inoculated with fresh bacteria strains. Bacteria was picked from a frozen culture by scratching the sterile loop across the surface of the culture or they were picked from a liquid culture by immersing loop in it. The bacteria were evenly spread across the surface of the plate using a glass spreader.

Disc diffusion method

Several circular sterile paper discs, were each infused with the different dilutions of the crude extracts, then evenly spaced over the surface of the plate. The discs were gently pushed down into the agar to make contact with the bacteria. The plates were left to grow overnight in an incubator at 37°C. Colonies would be visible after 12-16 hours growth at 37°C. Plates should be inverted in the incubator to prevent condensation from dripping on the colonies.

The colonies that form would be then counted as colonies per unit after incubation. The zones of inhibition were also measured on each plate. The minimal inhibitory concentration (MIC) of the crude extract to specific bacteria can then be determined.

CHAPTER FOUR

RESULTS AND DISCUSSION

PHYTOCHEMICAL TESTS RESULTS

The biologically active compounds of the *Crassula ovata* plant are tested so as to draw valuable conclusions from the observed results.

Table 1: Phytochemical test results of the *Crassula ovata* plant crude extracts

TEST	OBSERVATIONS Aqueous extracts	OBSERVATIONS Methanolic extracts
Alkaloid test i. Mayer's test ii. Dragendorff's test	 + -	 - -
Flavanoids test	-	-
Sterols and steroids Test	+	+
Saponins Test	+	+
Tannins Test	-	-
Carbohydrate Test i. Barfoed's Test ii. Benedict's Test	 + +	 + +

The phytochemical screening of *Crassula ovata* stem and root extracts showed the presence of Carbohydrates, saponins, steroids, and alkaloids.

ANTIMICROBIAL ACTIVITY RESULTS

Table 2: Observations for the aqueous plant leaf extracts at various concentrations

BACTERIA	Inhibition in mm per dilution $\times 10^{0}$ $\times 10^{-1}$ $\times 10^{-2}$ $\times 10^{-3}$ $\times 10^{-4}$

Bacillus subtilis	0	0	0	0	0
Escherichia coli	6.4	6.2	6.1	6.1	6.1
Candida albicans	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0

The zones of inhibition of the different microorganisms after exposure to aqueous plant leaf extracts. The *E coli* bacteria showed the only inhibition at the normal concentration of $\times 10^0$.

Table 3: Observations for the aqueous plant stem extracts at various concentrations

BACTERIA	Inhibition in mm per dilution $\times 10^0$ $\times 10^{-1}$ $\times 10^{-2}$ $\times 10^{-3}$ $\times 10^{-4}$				
Bacillus subtilis	0	0	0	0	0
Escherichia coli	6.1	0	0	0	0
Candida albicans	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0

The zones of inhibition of the different microorganisms after exposure to aqueous plant stem extracts. The *E coli* bacteria showed the only inhibition at the normal concentration of $\times 10^0$.

Table 4: Observations for the methanolic plant leaf extracts at various concentrations

BACTERIA	Inhibition in mm per dilution $\times 10^{0}$	$\times 10^{-1}$	$\times 10^{-2}$	$\times 10^{-3}$	$\times 10^{-4}$
Bacillus subtilis	0	0	0	0	0
Escherichia coli	6.5	6.2	0	0	0
Candida albicans	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0

The zones of inhibition of the different microorganisms after exposure to methanolic plant leaf extracts. The *E coli* bacteria showed the only inhibition at the normal concentration of $\times 10^{0}$

Table 5: Observations for the methanolic plant stem extracts at various concentrations

BACTERIA	Inhibition in mm per dilution $\times 10^{0}$	$\times 10^{-1}$	$\times 10^{-2}$	$\times 10^{-3}$	$\times 10^{-4}$
Bacillus subtilis	0	0	0	0	0
Escherichia coli	6.1	0	0	0	0
Candida albicans	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0

The zones of inhibition of the different microorganisms after exposure to methanolic plant stem extracts. The *E coli* bacteria showed the only inhibition at the normal concentration of $\times 10^0$.

Table 6: Anova (Single Factor) analysis of methanolic and aqueous extracts of *Crassula ovata* plant

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Escherichia coli	2	12.3	6.15	0.005
Bacillus subtilis	2	0	0	0
Candida albicans	2	0	0	0
Staphylococcus aureus	2	0	0	0
Pseudomonas aeruginosa	2	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	60.516	4	15.129	15129	2.17E-10	5.192168
Within Groups	0.005	5	0.001			
Total	60.521	9				

The anova analysis of the effects of the plant extract on the various microorganisms. These shows a difference in the calculated F value and tabulated F value.

DISCUSSION

Based on the observations made during the study, it was observed that the *Crassula ovata* plant had active components of carbohydrates, alkaloids, sterols, steroids and saponins. These are active chemical components that are involved in inhibition of microbial activity. However the degree of the effect of these active components depends on the plant species and the overall concentration used.

The antimicrobial activity of the *Crassula ovata* leaf and stem extracts were studied at different concentrations against four pathogenic bacterial strains and one fungal strain. The antimicrobial activities of the extracts increased linearly with increase in concentration of the extracts. The *Escherichia coli* bacteria were fairly sensitive than any other microbes to the plants extracts especially the leaf aqueous extracts. The growth inhibition zones measured an average of 6.2 mm for all the sensitive microbes, although not a strong value to show activity. The results show that the aqueous leaf extracts of *Crassula ovata* were found to be fairly effective against *Escherichia coli,* but no effectiveness on the other microbes tested.

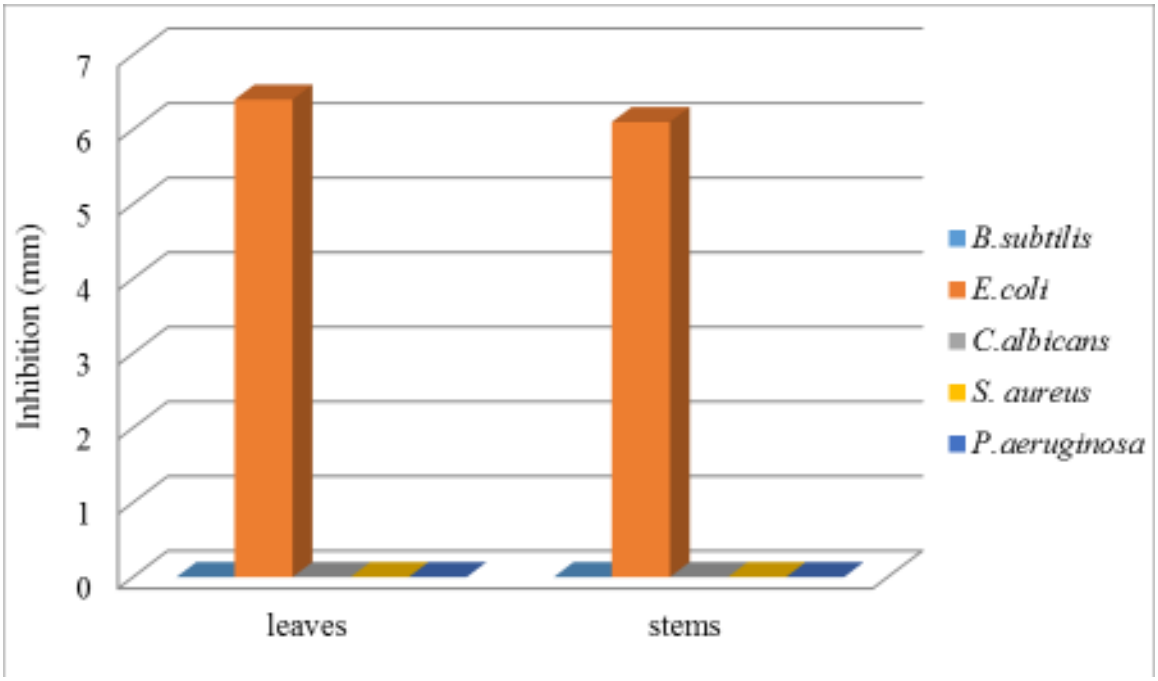

Figure 1: Antimicrobial activity against different microbes exposed to methanolic extracts

The methanolic extracts only showed inhibition of *E. coli* bacteria at concentration only. No inhibition was observed for the other microorganisms.

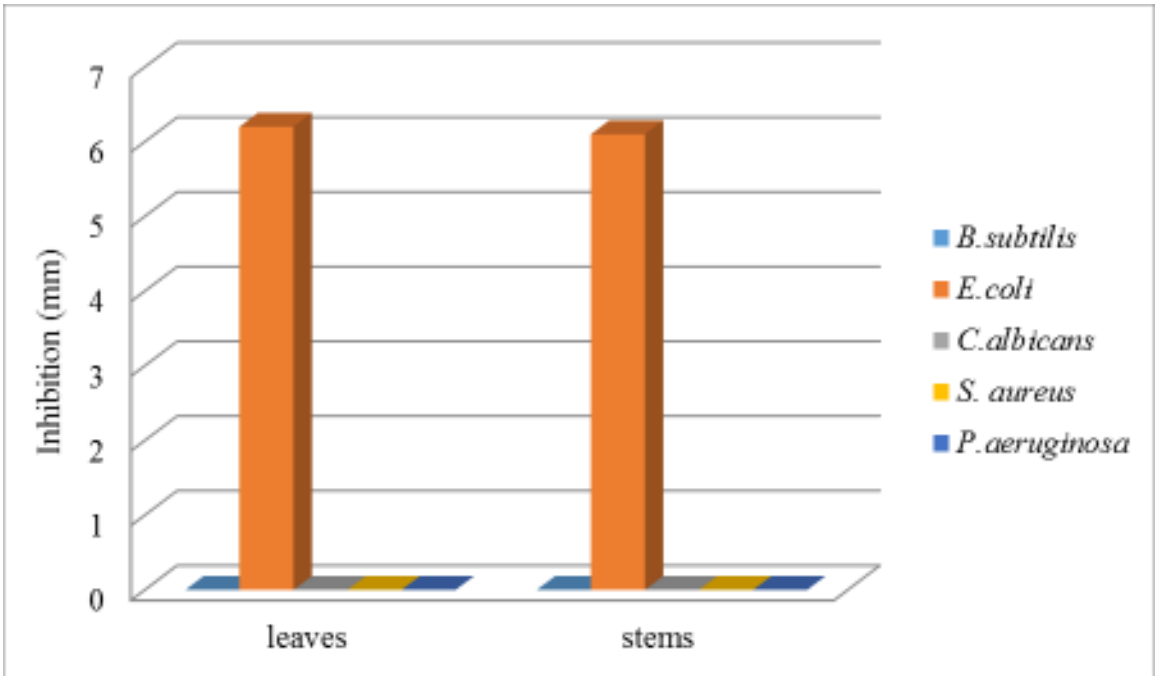

Figure 2: Antimicrobial activity against different microbes exposed to aqueous extracts

The aqueous extracts showed an average inhibition on only *E. coli* bacteria alone.

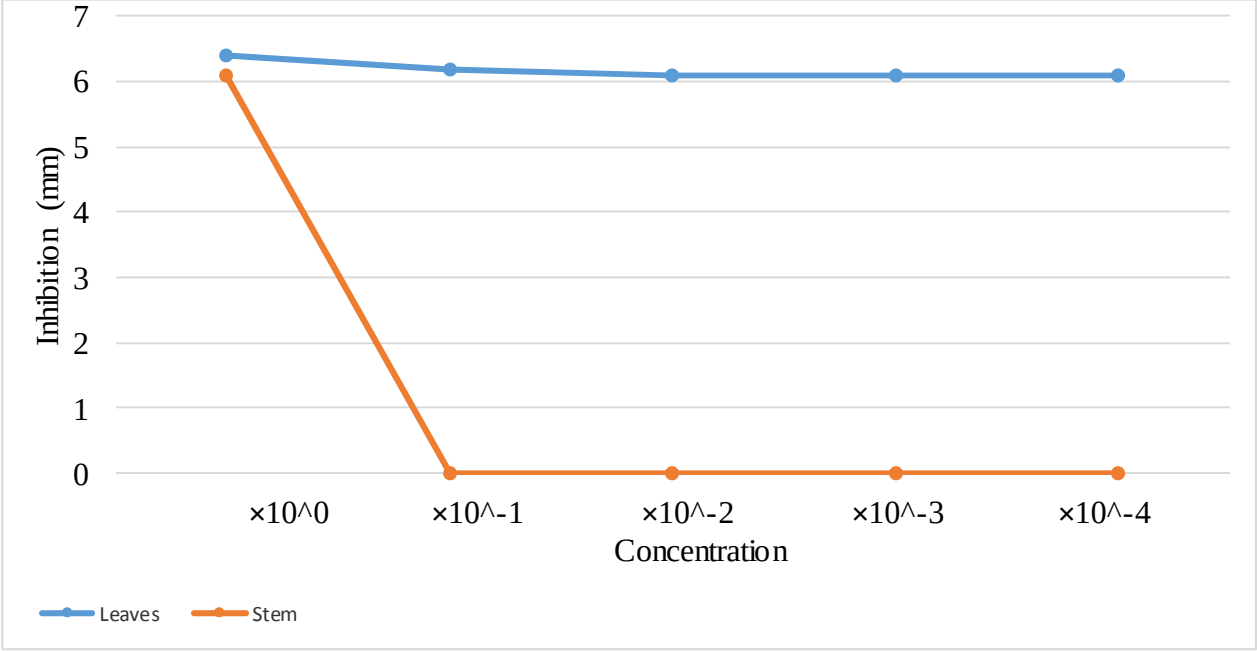

Figure 3: A Chart of Inhibition against Concentration of *E. coli* by aqueous plant extracts

The bacteria E. coli was greatly inhibited at concentration, with leaf extract being at 6.4 mm and stem extracts at 6.2 mm. Inhibition declined for the stem extracts instantaneously while for the leaf extracts it was gradual up to 6.1 mm.

The aqueous extracts of *Crassula ovata* showed strong activity against *Escherichia coli*. The results also revealed the presence of different phytochemical compounds with biological activity that can be of valuable therapeutic index. It has been shown from earlier experiments that plants rich in phenolic compounds have been shown to have antimicrobial activities.

From the anova analysis, the tabulated F value was lesser than the calculated F values did not match, and hence the null hypothesis had to be rejected. The aqueous leaf extracts of the *Crassula ovata* plant gave a promising effectiveness of antimicrobial growth on *Escherichia coli* bacteria alone. This is attributed to the presence of alkaloids like Berberine and Sanguinarine, and saponins as observed during the phytochemical testing of the plant. This showed that the *Crassula ovata* plant is effective only to the *Escherichia coli* bacteria. In this light, more bioprospecting questions arise on whether the plant extracts can be manipulated further to completely inhibit *Escherichia coli* growth and development.

The *Crassula ovata* plant is a common home plant in most parts of Kenya. However, the claims that it can heal wounds are most likely not true. Scientific experimentation carried out in this study helps prove that point. However, due to the plants effect on *Escherichia coli*, the *Crassula ovata* plant's potential to control stomach upset is yet to be further looked into.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

It is evident from the results that the plant extracts of the *Crassula ovata* plant, both from the leaves and stems, methanolic and aqueous, are only able to inhibit the *Escherichia coli* bacteria. Also being a gram negative bacteria, *Pseudomonas aeruginosa* was not affected by the plant extracts. Meaning that there was an active compound in the plants extracts that acted specifically against *E. coli* bacteria. This results also led to the rejection of the null hypothesis since a significant difference was observed in the microbial proliferation and the active compounds in the *Crassula ovata* plant. Other than *Escherichia coli*, the *Crassula ovata* plant is none effective to the other microbes that were tested against.

The objectives of this study were met, both the broad and specific objectives. There were active phytochemical compounds in the *Crassula ovata* plant. This included the Carbohydrates, saponins, steroids, and alkaloids. The plant was found to have an antimicrobial effect on *Escherichia coli* bacteria.

This study proved that there are active phytochemical compounds in the *Crassula ovata* plant, and that these compounds have a relatively minimal effect on microbial activity.

RECOMMENDATIONS

Since the *Crassula ovata* plant is fairly effective to inhibit growth of only *Escherichia coli* bacteria. It would be highly recommended that further research is done to ascertain to which degree the *Crassula ovata* plant extracts can inhibit the *Escherichia coli* bacteria. And also how the different growth stages of the plant could influence this activity.

Other recommendations that the author would suggest include;

- Research on different *Crassula* plant species varieties from different locations and how effective they inhibit different microorganisms.
- Use of more gram negative bacteria against the *Crassula ovata* extracts.

- Isolating the *Crassula ovata's* active compound inhibiting the *E.coli* bacteria and molecularly engineer it using bioinformatics tools to test its potential as a possible drug.

References

1. Baker F.J, R.E Silverstone, and C.J Pallister(2001), Introduction to Medical Laboratory Techniques, 7th Ed, Hodder Headline Group, London, pg 252,239

2. Cheesbrough M (2000), District Laboratory Practice in Tropical Countries-Part 2, Cambridge University Press, UK, pg 322, 337

3. CTA Technical Centre for Agricultural and Rural Co-operation (2007), Medicinal Plants, A J Wageningen, The Netherland, pg. 15-30

4. Eggli U.R, (2002) Illustrated Handbook of Succulent Plants, *Crassulaceae* Illustrated Handbook of Succulent Plants. Springer, Berlin

5. Fett-Neto, A. G., Aoyagi, H., Tanaka, H., and DiCosmo, F. (2004) *Encyclopedia of molecular cell biology and molecular medicine, Volume: 1, pg. 415–438.*

6. Gregory M.M, Bills G.F, Foster M.S (2004), Biodiversity of fungi: inventory and monitoring methods, Elsevier Academic Press , Carlifonia,pp37–47

7. Hajnos W. M, Petruczynik A, Dragan A, Wianwska D, Dawidowicz A, Sowa I(2004), Influence of the extraction mode on the yield of some furanocoumarins from *Pastinaca sativa* fruits, *A journal of Phytochemistry, Volume: 71,pg 181–187*

8. Hammonds S J. (1995), Diagnostic Bacteriology Protocols, Nottingham Trent University, Nottingham, UK, pg 1-4

9. Leistner O.A, (2000) Seed plants of southern Africa: families and genera, National Botanical Institute, Pretoria

10. Morandini, P., and Salamini, F. (2003) Plant biotechnology and breeding: allied for years to come. *Trends in Plant Science. Volume: 8, 70–75.*

11. Rao, A.Q, Bakhsh A., Kiani S, Shahzad K, Shahid A. A, Husnain T, and Riazuddin.S (2009), The myth of plant transformation. *Biotechnology Advancements, Volume: 27, pg 753–763.*

12. Seidel.V,(2012), Initial and Bulk Extraction of Natural Products Isolation, *Phytochemestry Volume: 10, pg 1-4*
13. Walter M. G, Andre B. Canelas, Hilal Taymaz-Nikerel, Rutger D. Douma, Lodewijk P. Jonge, Joseph J. Heijnen (2012), Fast Sampling of the Cellular Metabolite, Wiley Publishers, Weinheim, pg. 23-27