

THE IN-VITRO ANTIMICROBIAL EFFICACY OF FOUR METHANOL AND HEXANE EXTRACTED HERBAL CONCOCTIONS AGAINST *Pseudomonas aeruginosa* AND *Bacillus Subtilis* BACTERIA.

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Abstract

Antimicrobial activities of hexane- and methanol-extracted concoctions of *Kigelia Africana* (root, bark, and fruit), *Carica papaya* leaves, *Zingiber officinale* (ginger) rhizomes and *Jateorhiza palmitate* (Calumba) root (usually used as herbal medicines by many people from different parts of the world) were examined against two species of pathogenic microorganisms: *Pseudomonas Aeruginosa* and *Bacillus Subtilis* using sensitivity testing of streaked cultures on Petri dishes. All concoctions from all four herbal plants showed antimicrobial activities, especially the methanol-extracted concoctions against the tested microorganisms. Phytochemical screening of the samples revealed the presence of some bioactive components like alkaloids, saponins, tannins, anthraquinones, anthocyanins, flavonoids, and terpenes. These phytochemical properties determine the anti-microbial potential of the herbal extracts.

List of abbreviations used in the study

L: liter

D0: Not diluted (crude extract)

D1: First dilution

D2: Second dilution

CIP: Ciprofloxacin

OXA: Oxacillin

SUL: Sulfamethoxazole

Alkn: Alkanol

Terp: Terpenes

Saps: Saponins

Flav: Flavonoids

Tans: Tannins

Anthos: Anthocyanins

Anthras: Anthraquinones

J. palmata: Jateorhiza palmata

K. africana: Kigelia africana

C. papaya: Carica papaya

Z. officinale: Zingiber officinale

Bacillus; Bacillus Subtilis.

Pseudomonas A; Pseudomonas Aeruginosa

K. Africana fruit: Kigelia Africana fruit

K. Africana root bark: Kigelia Africana root bark

K. Africana bark: Kigelia Africana bark

Dia: Diameter

HCl: Hydrochloric acid

aq: Aqueous

g: Grams

v/v: volume by volume

w/v: weight by volume

EBM: Eosin methyl blue

NA: Nutrient agar.

INTRODUCTION

The control of infectious diseases is seriously threatened by the steady increase in the number of micro-organisms that are a resistant wide range of bacterial agents. Resistant infections lead to increased morbidity and prolonged hospital admissions, as well as to prolonged periods during which individuals are infectious and can spread infections to other individuals [1] Although the most potent and recently developed antimicrobial drugs are available throughout the world, their use in developing countries is confined to those who afford them. This has led to widespread and uncontrolled use of antibiotics [2] by patients who quite often do not take a full course of treatment because they cannot afford to buy expensive antibiotics. Another problem in developing countries is the quality and potency of antimicrobial drugs. In some countries, many different antimicrobial drugs are produced locally. In India, for example, there are over 80 different brands of fluoroquinolone ciprofloxacin. In Vietnam, a locally acquired 500 mg capsule of ciprofloxacin costs 400 dong (about 2 pence)[3] Antibiotic resistance is a form of drug resistance whereby some (or, less commonly, all) sub-populations of a microorganism, usually bacterial species, can survive after exposure to one or more antibiotics and such pathogens are known as multidrug-resistant (MDR) or

more colloquially, superbugs [4]. Antibiotic resistance in bacteria is a more serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century. This is particularly so because it pertains to pathogenic organisms that cause diseases in human beings [5].

This phenomenon has led to more investigations into alternative ways such as medicinal herbs [6]. Naturally occurring antibiotics may be modified to give semisynthetic derivatives. These often differ from their parent compound in their antimicrobial activity or their pharmacological properties. Medicinal plants are reservoirs of various metabolites and provide an unlimited source of important chemicals that have diverse biological properties and represent a rich source from which antimicrobial agents can be obtained [7]. Such naturally occurring medicinal plants having antibacterial properties include *Zingiber officinale*, *Carica papaya*, *Jateorhiza palmitate*, and *Kigelia Africana*. They contain a wide range of chemical compounds commonly referred to as phytochemicals. Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a well-known spice used in daily diets in many Asian countries. In China, ginger has been used to aid digestion, and treat stomach upset, diarrhea, and nausea, for more than 2000 years. Ginger is a hot herb and is a useful medicinal agent [8].

Kigelia Africana commonly known as the Sausage tree naturally occurs in the tropical regions of South Africa. The fruit of *Kigelia Africana* has been used traditionally as dressings for wounds and ulcers while an infusion of the bark and roots has been used to treat pneumonia [9]. Another important medicinal plant, *Carica papaya*, belongs to the family of Caricaceae and has several species of Caricaceae as well with various names papaya, pawpaw, papaya, kebaya, wan shou Kuo, pepol, chich put, Tinti, as well as fan Kua and so on. Papaya plant its part fruit, leaves, seed, roots as well as latex have phytochemical compounds [10]. These plant parts extracts have been used as a remedy against a variety of diseases. The papaya plant (*Carica papaya* L.) is widely found in Indonesia and many parts of Africa. Almost all parts of the plant can be utilized by humans for food or medicinal purposes [11].

Calumba which is the common synonym for *Jateorhiza palmitate* is a dioecious, climbing shrub producing usually perennial woody stems from 2 - 5 meters long from a tuberous rootstock. In some areas, such as Mozambique, the stems can be annual. The tuber is gathered from the wild for local medicinal use [12]. The plant has a long history of traditional medicinal use and became very popular in Europe, where it was especially valued as a treatment for digestive problems for people with weak stomachs. The plant is cultivated in several countries as a medicinal plant [13].

The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies, and academia since many infectious agents are becoming resistant to synthetic drugs. The situation has further been complicated by the rapid development of multidrug resistance by

microorganisms to the antimicrobial agents available. In this paper I will screen the antibacterial activity of *Carica papaya* leaf extract, *Kigelia Africana* bark, root and fruit extracts, *Jateorhiza palmitate* extract, and *Zingiber officinale* extract against two different pathogenic bacteria and the herb's antibacterial efficacy will be compared with three different commercial antibiotics.

RESEARCH MATERIALS AND METHODS

2 Materials and Method of extraction of plant samples

2.1 Processing of *Carica Papaya* leaves

The leaves were washed under a tap running water and then rinsed with sterile distilled water. They were placed under the shed until dry and then pulverized using sterile laboratory mortar and pestle to obtain a powdered material. The powder was stored in airtight glass containers and protected from sunlight until required for analysis.

2.2 Processing of *Kigelia Africana*

The fresh bark, fruit, and root bark were cut from the *Kigelia Africana* tree. They were then cut and pounded into small pieces and pieces were dried under the shed. Dried pieces were ground into smooth powder using a wooden mortar and pestle. The powder was kept in an airtight container for analysis.

2.3 Processing of *Zingiber officinale*

The ginger rhizomes were washed with distilled water, cut into smaller pieces, and allowed to dry (air-day) under the shed. They were then ground to powder using a mortar and pestle and the powder was kept for analysis.

2.4 Processing of *Jateorhiza palmata*

The roots were washed with distilled water, cut into small pieces, allowed to dry under the shed, and then ground to powder for analysis.

Extraction of bioactive compounds from the samples using sequential Soxhlet extraction

Materials

- Powder of papaya leaves, Calumba root, ginger root, sausage tree root, bark, and fruit.
 - Mortar and pestle
 - Glass vials
 - Methanol
 - n-hexane
 - Distilled water
 - Soxhlet extraction apparatus
 - Extraction thimbles
 - Water
 - Rotatory evaporator
 - Airtight glass containers
-
- Analytical balance
 - Stirring glass rod
 - Whitman filter paper no. 1

Extraction procedure

For sequential extraction, each powdered plant material was weighed (25 g) as well as the experiment was done in triplicates and placed in extraction thimbles. The thimble plus the contents were placed in the Soxhlet apparatus which was fitted to a 250 ml round-bottomed flask containing 150 ml of n-hexane. The sample was refluxed for about 72 hours. The solvent (n-hexane) was dried under reduced pressure using a rotary evaporator. To obtain a complete dry extract, each extract was transferred to a beaker and heated at 55°C on a hot plate with continuously stirring until the crystalline powder was obtained. The dry extract was kept at 4°C in a refrigerator until an assessment of its antimicrobial activities was done. After n-hexane extraction, the residues were refluxed again with methanol (150 ml). The drying procedure for the methanol extract was the same as that of n-hexane. The extraction of each herbal plant material was done in three replicated so as to get an average percentage yield and a standard deviation.

3.1 Research study location

The research was conducted in 104 and 105 Laboratories of Sharda University in Great Noida

3.2 Data collection instruments

In order to collect data for this study, experiments were conducted in the 105 laboratory where *Pseudomonas Aeruginosa* and *Bacillus Subtilis* were cultured in a culture media, and paper disks impregnated with antibiotics and herbal concoctions were placed in a culture medium. The zone of inhibition (in millimeters) was measured and used as a reference of the effectiveness of either synthetic drugs or herbs in the medium and later compared to find which one of the two types of medicines was the best treatment for which bacteria.

3.3 Study design

In order for this study to reflect on what was on the ground, a quantitative type of research was used. The quantitative research focused on how effective the herbal concoctions were compared with commercial synthetic antibiotics. The experiment used a completely randomized design with three replicates.

3.4 Data collection

All the data was collected from the findings of the experiments that were conducted by the researcher. Phytochemical screening of the four herbs for the presence of saponins, terpenes, alkaloids, tannins, flavonoids, anthraquinones, and anthocyanins was done in 104 laboratories. In the Life science department, the efficacy of each extract from each concoction was compared against the commercial antibiotics by comparing their zones of inhibition in each petri dish. The categorization of the zones of inhibition was in accordance with Benson, (2012) and was done in laboratory 105.

3.5 Data Analysis

Data analysis was based on how big the zone of inhibition measured in millimeters was on different bacteria from the point of the antibiotic disk which indicated the effectiveness of the medicine. T-test and analysis of variance (ANOVA) were used to test the efficacy differences between the concoction extracts and commercial antibiotics.

3.5.2 Phytochemical analysis of *J. palmitate* roots, *C. papaya* leaves, *Z. officinale* tuber, and *K. Africana* root, bark, and fruit

Jateorhiza palmitate root (Njoka), *Carica papaya* leaves, *Zingiber Officinale* tuber, and *Kigelia Africana* (Sausage tree; Mvunguti) fruit, bark, and root were screened for the presence of alkaloids, saponins, tannins, terpenoids, flavonoids, anthraquinones and anthocyanins using standard methods

described by Harbone (1998), Sofowora (1993) and other researchers. All the phytochemical screening was done at Sharda University 104 Laboratory

3.5.2.1 Alkaloids screening (Wanger test)

Apparatus

- Erlenmeyer flask
- Stirring rod
- Conical flask (100ml)
- Whitman filter paper No. 1
- Test tubes
- Droppers (Pasteur pipettes)

Reagent

1. **1 ml of Potassium iodide**
2. **5% (v/v) Hydrochloric acid (HCl)**

Preparation

12.50 ml of HCl was measured and added to 237.50 ml of distilled water in a 250ml volumetric flask. The solution was shaken gently to ensure homogeneity.

Procedure

Each plant material was weighed (5g) and placed in a separate 100 ml conical flask. To each conical flask, 50ml of 5% (v/v) HCl (aq) solution was added. The mixtures were stirred using a glass stirring rod for 5 minutes. The mixtures were left to stand for 24 hours. After 24 hours, the mixtures were filtered using filter paper. To 1ml of each of the sample filtrates, in a test tube, 1 ml of potassium Iodide was added and shaken. The test was proven to be positive if reddish-brown precipitates were formed.

2. Terpenes / Terpenoids

Apparatus

- Conical flask
- Stirring rod
- Stopper
- Dropper
- Whitman filter paper No. 1

Reagents

- Diethyl ether
- Concentrated Sulphuric acid

Procedure

1g of each powder sample was weighed and put in a well-labeled separate conical flask of Diethyl ether was poured into each conical flask and the mixtures were stirred for at least 5 minutes. The conical flasks were stoppered tightly to avoid volatilizing the solvent. The volatilizing was left to stand for 48 hours. The mixtures were filtered and 1 ml of each filtrate was put into a separate test tube to it conc. Sulphuric acid (0.5 ml) was added to the filtrate. The appearance of orange-yellow colors developed upon dilution with water was taken as an indication of the presence of Terpanenes and or terpenoids in the plant sample.

3.5.2.3 Saponins**Apparatus**

- Conical flasks
- Measuring cylinder
- Stirring rod
- Whitman filter paper no. 1
- Droppers
- Test tubes
- Watch
- Ruler

Reagent

- Distilled water

Procedure

1g of each sample was weighed and placed in separate conical flasks. 20ml of distilled water was measured and poured into the conical flasks. The mixture was stirred for 5 minutes and then let to stand for 24 hours. The extracts were filtered using Whitman filter paper No. 1 and each filtrate (10ml) was transferred into separate test tubes and shaken vigorously for 10 seconds. The foam was

measured at zero minutes (immediately after shaking) and after 10 minutes. The foam that persisted for 10 minutes was measured in each test tube and taken as evidence of the presence of saponins.

3.5.2.4 Flavonoids

Apparatus

- Conical flasks
- Whitman filter paper No. 1
- Test tubes

Reagents

- 1. Few drops of Sodium hydroxide**
- 2. Dilute acetic acid**

Procedure

Infusions of the dry powdered plant materials of 5g each were immersed in 50ml distilled water stirred and left to stand for 24 hours. The extract was filtered using Whitman filter paper no.1. 1 ml of the extract was taken and placed in a test. A few drops of Sodium hydroxide solution were added to each filtrate in test tubes and shaken. Emergence of intense yellow color that turns colorless after adding dilute acetic acid was taken as evidence for the presence of flavonoids.

3.5.2.5 Tannins

Apparatus

- Conical flasks
- Stirring rod
- Droppers
- Test tubes
- Erlenmeyer flask
- Buchner funnel
- Whitman filter paper no. 1

Reagents

- 1. Aqueous ferric chloride solution**

Preparation

27.5g of Ferric chloride was weighed and dissolved in a 50ml conical flask with distilled water and then diluted with distilled water to make 100 ml of 0.5M aqueous ferric chloride solution.

Procedure

1g of each of the powdered plant materials was weighed and macerated in 20ml of distilled water stirred for about 5 minutes and left to stand for 24 hours. The mixtures were filtered after 24 hours with filter paper. 1ml of each filtrate was poured into separate test tubes and 10 drops of 0.5M Ferric chloride aqueous solution were added. Blue, black, or green precipitates were taken as evidence for the presence of tannins.

3.5.2.6 Anthraquinones**Apparatus**

- Conical flasks
- Test tubes
- Droppers
- Measuring cylinders

Reagents**1. 0.5 N Potassium hydroxide****1. Preparation**

2. 1.4g of Potassium hydroxide was measured and poured into a 100 ml volumetric flask. Then 50 ml of distilled water was added. The flask was swirled to mix the solution.

2. 5% Hydrogen peroxide**1. Preparation**

2. 8.3ml of Hydrogen peroxide was measured and poured into a 100ml volumetric flask. 50 ml of distilled water was added and shaken to mix the solution.

3. Acetic acid 0.1 ml

4. Toluene 5 ml

Procedure

2g of each of the powdered plant materials were weighed and placed in separate conical flasks. Each sample was moistened with 20 ml of distilled water and left to stand for 24 hours. After 24 hours the mixtures were filtered using filter paper and 1ml of the filtrate of each sample was treated with 5 ml of 0.5 N Potassium hydroxide and 0.5ml of 5 % Hydrogen peroxide. After cooling the suspension was filtered. and the filtrate was treated with 0.5 ml of Acetic acid and then mixed with 5ml of Toluene. The upper layer was separated with the help of a pipette and transferred to a test

tube. Then 2ml of 0.5N Potassium hydroxide was added to it. The development of cherry red color and violet after the mixtures were shaken was an indication of the presence of anthraquinones.

3.5.2.7 Anthocyanins

Apparatus

- Conical flasks
- Water bath
- Measuring cylinder
- Dropper
- Test tubes
- Volumetric flask
- Pipette filler

Reagents

1. 2M HCl (aq) solution

1. Preparation

2. 22ml of 32% HCl was measured and poured into a 250 ml volumetric flask that already contained some distilled water (100 ml). The solution was diluted with distilled water. The flask was shaken gently to obtain a homogeneous solution.

2. Distilled water

Procedure

1g of each of the powdered plant materials was weighed and placed in separate conical flasks. 20 ml of distilled water was measured and poured into each conical flask, stirred for 5 minutes, and left to stand for 24 hours. After 24 hours, the mixtures were filtered using filter paper and 1ml of each filtrate was poured into separate test tubes. To each test tube, 5ml of the prepared 2 M HCl (aq) solution was added and heated in a water bath for 30 minutes. Red or purple color indicated the presence of anthraquinones.

3.5.4 Culturing of inoculated bacteria with a drop of antibiotic and a drop of herb

Materials

- Petri dishes
- Nutrient agar (NA)
- Incubators
- Antibiotic solutions of Oxacillin, Sulfamethoxazole, and Ciprofloxacin)

- Herbal extracts: *Kigelia Africana*, *Carica papaya* *Zingiber officinale*, *Jateorhiza palmate*
- Pipette filler
- Beakers
- Wire streaking loop
- Bunsen burner
- Ethanol
- Two different bacteria (*Bacillus subtilis* and *Pseudomonas aeruginosa*.)
- Forceps
- Foil paper
- Autoclave
- Cotton wool
- Distilled water
- Hexane
- Methanol

Procedure

Preparation of Nutrient Agar Broth (NA)

7.0g of NA was weighed as well as 2g agar powder and poured into a 500 ml conical flask. Was diluted with 250ml of distilled water, stoppered the conical flasks, and wrapped their tops with foil paper. The diluted broths were sterilized in an autoclave at 15 P S I of pressure for 15 minutes at 121 degrees Celsius. After the pressure turned to zero, the flasks with broth were removed from the autoclave container and waited to cool a little. Using ethanol and a towel working table was wiped to reduce the number of other microbes. The Petri dishes were laid on the wiped table and the sterilized NA was poured into each petri dish with about 8-10ml of the agar. The broth in the plates was allowed to solidify. The Petri dishes were placed in a freezer at 4⁰C.

3.5.5 Inoculating inoculum on media

The working table was wiped using cotton wool immersed in alcohol. Then the Bunsen burner was left lit throughout. The streaking method described by Kirby-Bauer in 1966 was used. Inoculum of clinically isolated bacteria was obtained from lab 1o6 (*Pseudomonas. a*) and (*Bacillus.s*) 106 laboratory was used by growing each strain on NA for 24 hours at 37 ⁰C.

The susceptibility assay was carried out with a 1 mg/ml concentration of each of the extracts. 7mm in diameter punched paper was immersed in extracts for one minute and then placed on the bacteria-

streaked surface of NA Petri dishes. The extracts served as negative controls. Commercial antibiotic solutions .0.1ml contain 5mcg of Ciprofloxacin, 0.1 ml contain 1mcg of Oxacillin and 0.1 ml contained 23.75 mcg of Sulfamethazine served as the positive controls for the bactericidal activity. The aseptically inoculated Petri dishes were incubated in the incubator at 37 °C and removed after 24 hours. The Petri dishes were checked for zones of inhibition in both the antibiotic and herbal disk and measurements of diameters using a ruler were taken in millimeters on the zones of inhibition and recorded.

The aliquots of extract and their dilutions of 50% and 25% were reconstituted by 1:1 dilution using methanol and n-hexane organic solvent for the extracts and were put in each disk made on the culture plates previously seeded with the test organisms. The antibacterial potential of the test compound was determined on the basis of the diameter of the zone of inhibition around the disk. To ensure the precision and accuracy of the results, the experiments were done in replicates.

The efficacy of the herbal extract was done using the average of the replicates compared with the standardized rating of the zone of inhibition in the Kirby-Bauer method of antimicrobial sensitivity testing.

3.5.6. Calculations for making standard solutions for tablet antibiotics

Cipro

- 0,1ml -5mcg
- 5 ml – 250mcg change to mg divided by 1000
- 100 multiplied by 5ml -0.25mg multiplied by 100
- 500ml -25mg divide by 2

250ml -12.5mg standard stock solution

First solution

250ml -12.5mg (divide by 250 both sides) **sol 1**

Extract or take 1ml

1ml -0.5mg multiplied by 1000(50mcg)

Take 1ml

1ml plus 9ml solvent -50mcg

Add to make

10 ml – 50mcg **sol 2**

Take 1ml or 100microliter

1ml – 5mcg (this is the one I will put in the disk)

OXACILLIN

- 0.1ml-1mcg
- 5ml-50mcg
- 1500ml -**1500mcg** (change to mg and liters divide by 1000)
- **1.5ml- 15mg (standard stock solution)**

First solution**1.5ml -15mg(sol1)****Take 1ml which will have 10mcg**1ml plus 9ml of solvent (10ml) -10mcg **sol2****Take 1ml****1ml -1mcg (this is what I will put in the disk)****SULF**

- 0.1ml -23.75mcg
- 0.1ml multiplied by 1000-23.75mcg multiplied by 1000
- 100ml -23750 mcg divide by 1000 to get (23.75mg)
- **100ml -23.75mg standard stock solutions' sol 1**

Take 1ml and add 9ml of solvent to make 10ml10 ml -237.5 mcg **sol 2****Take 1ml****1ml -23.75mcg (this is what I will put in the disk)****PLANT EXTRACT**

1mg -1ml

15 mg – 15ml (Standard stock solution)**Take 1ml****1ml -1mg (this is what I will put in the disk)****PERCENTAGES OF PLANT EXTRACT AND ANTIBIOTICS**

- **Take 1ml of the aliquot**
- **Take 500 micro liter-100 percentage**
- **Take 500 microliter + 500micro litre -50 percent**
- **500micro litre+ 500micro litre – 25 percent**

Preparation of the inoculum**Weigh 0.85g of NaCl and dissolve in 100ml distilled water.****Take 1ml of Nacl and put in one spinoff**

In one of them put a scratch of bacteria. Mix, and keep for 6 to 5 minutes, if the solution is turbid it means the bacteria is growing.

Micropipette 10 microliter, put in the plates and spread over the media using the spreader.

Put the ant microbial agent on the plates and incubate.



The arrow on the map above shows calumba root collected from Mwabvi Wild Reserve as indicated on the map



The arrow on the map shows sausage balk and fruit collected from Karonga district hospital as indicated on the map



The arrow on the map shows sausage root, ginger root, and papaya leaves collected from Area 25 as indicated on the map.

RESULTS AND DISCUSSION

Different parts of a total of four selected medicinal plants [(*K. Africana* (root, bark, and fruit), *J. palmitate* (root), *C. papaya* (leaf), and *Z. officinale* (root)] extracts were tested for the presence of alkaloids, terpenes, tannins, saponins, anthocyanin's, flavonoids and anthraquinones (Table 1).

Table 1: Phytochemical results

SAMPLE	ALKN	TERP	SAPS	FLAV	TANS	ANTHOS	ANTHROS
<i>Z.officinale</i>	-	+	++	+	+	-	-
<i>K. root</i>	+	-	-	++	++	-	-
<i>J. palmate</i>	-	+++	+	++	-	-	-
<i>K. bark</i>	+++	+	-	++	++	-	-
<i>K. fruit</i>	++	+	-	++	++	-	-
<i>C. papaya</i>		++	-	+	+++	-	-

Key: +++ large quantities, ++ moderate quantity, + mild and - absent.

Photochemistry and Evaluation of the antibacterial potential of *C. papaya*, *Z. officinale*, *J. palmitate*, and *K. Africana* root, bark, and fruit.

Phytochemicals and antimicrobial activity were determined in selected medicinal plants namely *Z. officinale*, *C. papaya*, *J. palmate*, and *K. Africana*. The tested herbal concoctions were found to have tannins, saponins, anthraquinones, anthocyanins, alkaloids, and flavonoids. These results were consistent with results by [1], who tested that the antimicrobial potency of the assay was due to phytochemicals. From the phytochemical analysis results (Table 1), it was observed that *Zingiber officinale*, and *Kigelia* (bark, and fruit); had a lot more chemicals than the other two herbs.

After the laboratory analysis of *Kigelia* (bark, root and fruit); it was found that bark contained terpenes, alkaloids, flavonoids, and tannins. *K. Africana* fruit contained alkaloids, flavonoid terpenes, and tannins only whereas *K. Africana* root contained alkaloids, flavonoids, and tannins. These results are in line with the findings of [2]

J. palmate contained terpenes, which was consistent with what [3] found in his study. Saponins and flavonoids were also present in *J. palmate* which is in contrast with what [4] found in his study for he found tannins.

Z. officinale contained tannins, terpenes, saponins, and flavonoids which were also reported in [1] study findings. Lastly, *C. papaya* contained terpenes, flavonoids, and tannins and this result was in line with what [5][6] reported in their study.

The bioactive compounds were extracted using n-hexane and methanol in sequence using a Soxhlet extractor for 72 hours for a sample of 25g with 150ml of each solvent were done in triplicates. The presence of bioactive substances has been reported to confer resistance to plants against bacteria, fungi, and pests. [7], Therefore, this explains the demonstration of in-vitro antibacterial activity by the plant extracts used in this study meaning that the tested herbs could be used as alternative antimicrobials for some antibiotics [8].



Alkanol results in K Balk

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