



# **In-vitro Antimicrobial Activities and Phytochemical Screening of Selected Plant Extracts against Some Medically and Agriculturally Important Pathogens**

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## **Authors' contributions**

This work was carried out in collaboration among all authors. Author YA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author DZ performed antimicrobial and biochemical test with co-investigator. Author AK managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

## **Article Information**

DOI: 10.9734/EJMP/2020/v31i1030293

### Editor(s):

- (1) Dr. Francesca Aiello, University of Calabria, Italy.
- (2) Prof. Marcello Iriti, University of Milan, Italy.

### Reviewers:

- (1) Maria Bintang, Bogor Agricultural University, Indonesia.
- (2) A. Jayachitra, Madurai Kamaraj University, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55206>

**Original Research Article**

**Received 01 January 2020**

**Accepted 06 March 2020**

**Published 30 July 2020**

## **ABSTRACT**

In Ethiopia, many plants are used for medicinal drive-by old-style naturopaths without any scientific justification for their therapeutic values. The principal aim of this study were to evaluate the *in vitro* antibacterial and antifungal activities of the leaf, root and stem bark extracts of *Rhamnus prinioides* (gesho), *Justicia schimperiana* (sensesel) and *Ruta chalepensis* (Tena adam) against some common pathogenic species of bacteria and fungi. The results of this study shown that the crude extracts of ethanol, methanol, hexane and water crude extracts had antimicrobial activities on most bacterial and fungi species of some solvent extracts. Ethanol and methanol crude extracts had the highest growth inhibitory effects as compared with those of the aqueous and hexane crude extracts. But, the four solvent crude extracts had fewer antimicrobial activities than commercially available drug(chloramphenicol and clotrimazole). *Campylobacter jejuni* and *Staphylococcus aureus* were

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found to be the most vulnerable microbes to the crude ethanol (99.5%). The growth-inhibitory events of the crude extracts were found to be significantly dissimilar for both concentrations (30 and 60 mg/ml) in all plant parts ( $p < 0.05$ ). In general, this study did not only indicate that the antibacterial activities of *R. prinioides* (gesho), *J. schimperiana* (sensel) and *R. chalepensis* (Tena adam). It also accesses a scientific justification for its old-style use against some diseases.

**Keywords:** Antimicrobial activities; MIC; *J. schimperiana*; phytochemical screening; *R. prinioides*; *R. chalepensis*.

## 1. INTRODUCTION

Plants were used as a means of treatment of diseases and injuries from the initial days of civilization on the world and its long journey from ancient time to the current time. It has effectively used plants and plant yields as actual therapeutic tools for struggling against diseases and numerous other health vulnerabilities [1]. 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 [2]. Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs. The native usage of natural plants as main wellbeing medicines due to their pharmacological properties is somewhat common in Asia, Latin America and Africa [3]. Herbal medicine is the oldest form of healthcare known to mankind and over 50% of all modern clinical drugs are of natural products origin, and at present natural products still play important roles in drug development in the pharmaceutical industry [4].

Many research efforts have been directed towards the provision of empirical proofs to back up the use of plant species in trade and medicinal practices in recent [5]. Several researchers have examined the effects of plants used traditionally or by indigenous healers to support treatment of various diseases; scientific validations are being made globally to get evidence for traditionally reputed herbal plants. However, there still exist a large number of plants with tremendous medicinal potentials that have not been investigated. Medicinal plants being an effective source of both traditional and modern medicines are genuinely useful for primary health care. Consequently, in the past few years, a number of studies have been conducted in different countries to prove such applications [6,7,8].

Medicinal plants are an abundant source of antimicrobial molecules. A wide range of

medicinal plants extracts is used to treat several infections as they have potential antimicrobial activity [9]. Some of these bioactive molecules are screened and traded in the market as raw material for many herbal industries. Medical practitioners whether allopath's (medical doctors), homoeopaths, naturopaths, herbalists or shamans had to know the plants in the area and how to use them since many of their drugs were derived from plants [10]. Plants are common clinical phenomena [11,12] and some medicinal plants may produce adverse long-term effects such as hepatotoxicity [13]. Ethiopian medicinal plants are shown to be very effective against some ailments of human and domestic animals [14]. *Rhamnus prinioides* (gesho), *Justicia schimperiana* (sensel) and *Ruta chalepensis* (Tena adam) are widely traditional medicinal plants for the treatment of various ailments.

Different parts of *Ruta chalepensis* has been used for centuries in traditional medicine to treat convulsions, dropsy, fever, mental disorders, menstrual problems, neuralgia, rheumatism, and other bleeding and nervous disorders [15]. The previous phytochemical studies on this plant revealed that the presence of several alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, tannins and terpenoids were reported [16]. The plant also has pharmacological evaluation as analgesic, anthelmintic, anti-acetylcholinesterase, anticancer, anti-inflammatory, antimicrobial, antioxidant and antiparasitic properties [17].

*Rhamnus prinioides* (*Rhamnaceae*) is one of the medicinal plants that have been used traditionally for the treatment of different infectious diseases. *Rhamnus prinioides* has different ethnomedicinal uses in different countries of Africa. In Kenya, traditionally, the different parts of plant are used in the management of Ear, Nose and Throat (ENT) infections, gonorrhoea, malaria and brucellosis [18]. In Ethiopia, the leaves, fruits or roots of *Rhamnus prinioides* is used to treat tonsillitis [19]. In addition, the different parts of

the plant have been used in the management of scabies, hepatitis, tinea capitis, 'chiffa' (Eczema), ringworm and dandruff [20]. Moreover, the leaf of the plant is used for the management of waterborne and related diseases [21].

*Justicia schimperiana* belongs to the family *Acanthaceae* and it is a shrub with branched stems. In Ethiopia, *J. schimperiana* was used for the treatment of stomach complaints, malaria, hepatitis, asthma, jaundice, epilepsy [22].

The therapeutic effectiveness of these medicinal plants, however, varies with the geographical location, altitude and climate type from where the plants are growing. Thus, the principal aim of this study was to evaluate the *in vitro* antibacterial and antifungal activities and phytochemical screening of the leaf, root and stem bark extracts of the selected plants, against some common pathogenic species of bacteria (i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Campylobacter jejuni*) and fungi (i.e. *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces boulardii*).

## 2. MATERIALS AND METHODS

### 2.1 Experimental Design

The research was designed based on the laboratory chemical analysis in a commercial Chemical relational database (CRD). The treatments were including three plants with three parts of each, extracted by four solvents and eight test pathogens in three replications (3x3x4x3x3). DMSO was used as a negative control. Chloramphenicol and clotrimazole were used as a positive control for antibacterial and antifungal strain respectively.

### 2.2 Sample Collection and Preparation of Plant Extract

Healthy leaves, roots and barks of selected plants (*Ruta chalepensis*, *Justicia schimperiana*, *Rhamnus prinoides*) to be used in this study, were randomly collected from Haramaya University main campus. The plants were identified at Haramaya University Herbarium with the help of a plant taxonomist. The collected plant parts were washed thoroughly with tap water followed by sterile distilled water and cut into smaller sizes using a knife. Then the leaves, roots and barks of all plants were dried under shade on a paper towel for one week with

occasional shifting at room temperature. The resulting dry parts were ground into fine powder with the help of suitable sterile grinder and stored in sterile air tight containers. Twenty grams of each dried sample (dried and ground plant leaves/roots/barks) of selected plants were dissolved in 100 ml of four different solvents distilled water, methanol, ethanol and hexane [23] and kept on a rotary shaker rotating at 190-220 rpm for 24h at room temperature. All solutions were filtered through Whatman No.1 sterile filter paper and the resulting filtrates were collected as sources of crude extracts. The solvents in the filtrates were evaporated using a Rota vapor (STERILIN. Ltd., Stone Staffordshire, England) and the crude extracts were weighed and kept in sample vials with stoppers at 4°C until used against the test pathogens [24].

### 2.3 Test for Antibacterial Activities of Crude Plant Extracts

The antimicrobial activities of aqueous (distilled water); methanol, ethanol, and hexane extracts were determined by the filter paper disc diffusion methods as described by Omenka and Osuoha [25].

#### 2.3.1 Paper Disc Technique

Sterile filter paper discs (6.0 mm diameter) were cut and soaked with the test extracts and dried at 40°C for 30 min. The paper discs were then being aseptically placed on Mueller – Hinton agar (MHA) plates inoculated with dense inoculums suspension of each test pathogen. The plates were incubated at 37°C for 48h and examined for zone of inhibitions.

### 2.4 The Test Bacterial and Fungal Species and their Culturing Methods

The selected bacterial and fungal pathogens were obtained from Ethiopian Public Health Institution (EPHI). Four species of pathogenic bacteria (i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Campylobacter jejuni*) and four fungi (i.e. *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces boulardii*) were used in this study. All bacterial and fungal cultures were grown on nutrient agar and potato dextrose agar plate at 37°C/30°C for 24 h respectively. Few colonies (4–5) of similar morphology of the respective fungal and bacterial species were transferred with a sterile inoculating

loop to a liquid medium and incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard were obtained. The inoculate of the respective bacterial species were streaked on to nutrient agar plates using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth obtained following incubation. 6mm in diameter sterile disc were filled with aliquots of the plant extract solution (100 µl each) and the disc plates were then being allowed to stay for 1–2 h at room temperature. Finally, the discs were put on plates and incubated at 37°C for 18–24 h and the resulting diameters of zones of inhibition were measured using a caliper. Chloramphenicol and clotrimazole were used as positive controls at a concentration of 0.1 mg/ml and DMSO was used as a negative control [26]. Disc diffusion method previously described for an antibacterial was used in determining antifungal action and fungal strains are grown on PDA plate.

## 2.5 Determination of Minimum Inhibitory Concentration (MIC)

The agar dilution method was used to determine the MIC for the crude extracts. The ethanol, methanol, hexane and aqueous extracts of the different plant parts (stem bark, root and leaves) of *R. prinioides* (gesho), *J. schimperiana* (sensesl) and *R. chalepensis* (Tena adam) that showed significant antimicrobial activities in the previous test were selected for determination of MIC [27]. The Mueller- Hinton agar media were first prepared as described in the above section and sterilized by autoclaving. The sterilized media were allowed to cool at 50°C and 18 ml of molten agar was added to test tubes, which contained 2 ml of different concentrations of the crude extracts.

The mixture of the media (molten agar and crude extracts) and the test drugs were thoroughly mixed and poured into pre-labelled sterile Petri-dishes on a level surface. Additional Petri-dishes containing only the growth media were prepared in the same way for the comparison of the growth of the respective organisms. The concentrations of the extracts used in this test were 22, 24, 26 and 28 mg/ml. The plates were allowed to dry at room temperature. The suspensions of the respective pathogens whose densities were adjusted to 0.5 McFarland turbidity units ( $1.5 \times 10^8$  CFU/ml) were inoculated onto the series of agar plates using a standard inoculating loop. Three loopfuls of suspension were transferred into each plate. The plates were then incubated

at 37°C and 30°C for 24 h for bacterial and fungi species respectively. The lowest concentration, which inhibited the growth of the respective organisms, was taken as MIC.

## 2.6 Phytochemical Screening of the Leaf Stem Bark and Root Extracts of the Selected Plants

Phytochemical screening was done to detect the presence of plant constituents such as tannins, phenolic compounds, resins, amino acids, flavonoids, saponins, reducing sugars, glycosides, steroids, triterpenoids, anthocyanidins, sterols and essential oil using the methods described by Brain and Tuner [28].

### 2.6.1 Test for reducing sugars

Two grams of the extract was weighed and placed into a test tube. This was diluted using 20 ml of de-ionized distilled water. This was followed by the addition of Fehling's solution. The mixture warmed to 40°C in water bath. Development of brick-red precipitate at the bottom of the test tube was indicative of the presence of reducing sugar. The same procedure was repeated using dimethyl sulfoxide (DMSO) as the diluents for the ethanol, methanol and hexane extracts [29].

### 2.6.2 Test for resins

Three grams of the ethanol, methanol and hexane extracts were dissolved in 15 ml of acetic anhydride. A drop of concentrated sulfuric acid was added. The appearance of purple color, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material [30].

### 2.6.3 Test for tannins

Two grams of the aqueous extract was weighed and placed in a test tube. Two drops of 5% ferric chloride solution was then added. The appearance of a dark green color was indicative of the presence of tannins. The same procedure was repeated using the ethanol, methanol and hexane extracts [30].

### 2.6.4 Test for steroid

One gram of the ethanol, methanol and hexane extracts was weighed and placed in a test tube. This was dissolved in 2 ml of acetic anhydride,

followed by the addition of 4 drops of chloroform. Two drops of concentrated sulfuric acid were then added by means of a pipette at the side of the test tube. The development of a brownish ring at the interface of the two liquids and the appearance of violet color in the supernatant layer was indicative of the presence of steroid glycosides. Same procedure was repeated using the aqueous extract [30].

#### 2.6.5 Test for flavonoids

Two grams of the ethanol, methanol and hexane extracts was weighed, placed in a test tube, followed by the addition of 10 ml of DMSO. The mixture was heated, followed by the addition of magnesium metal (0.7 g) and 6 drops of concentrated hydrochloric acid. The appearance of red color was indicative of the presence of flavonoids. Same procedure was repeated using aqueous extract [31].

#### 2.6.6 Test for alkaloids

One gram each of the ethanol, methanol and hexane extracts was weighed and placed into two separate test tubes. To the first test tube, 2-3 drops of Dragendoff's reagent was added while 2-3 drops of Meyer's reagent were added to the second test tube. The development of an orange-red precipitate (turbidity) in the first test tube (with Dragendoff's reagent) or white precipitate (turbidity) in the second test tube (with Meyer's reagent) was indicative of the presence of alkaloids. Same procedure was repeated using aqueous extract [30].

#### 2.6.7 Test for saponins

Five grams of the aqueous extract was weighed and placed in a test tube. This was followed by the addition of 5 ml deionized distilled water. The content was vigorously shaken. The appearance of a persistent froth that lasted for 15 min was indicative of the presence of saponins. Same procedure was repeated using DMSO for the ethanol, methanol and hexane extracts [29].

#### 2.6.8 Test for glycosides

To a volume of 3 ml of the ethanol, methanol, hexane and aqueous extracts, 2 ml of chloroform was added. Tetraoxosulphate VI acid was carefully added to form a lower layer. A reddish brown color at interface indicated the presence of a steroidal ring.

#### 2.6.9 Test for phenolic compounds

Two drops of 5% ferric chloride were added to 5 ml of the ethanol, methanol, hexane and aqueous extracts in a test tube. A greenish precipitate was taken as indication of phenolic compounds.

#### 2.6.10 Test for anthraquinones and test for terpenoids

0.5 g of the both plant crude extracts were shaken with 10 ml of aqueous H<sub>2</sub>SO<sub>4</sub> and then filtered while hot, the filtrate was shake with 5 ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution was then added. The presence of violet or red colouration in the ammoniac (lower) phase was taken as an indication of combined Anthraquinones. About 0.2 g both plant extracts were mixed with 2 ml chloroform and 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added to form a layer. A reddish brown coloration of the interface formed indicating the presence of terpenoids.

### 2.7 Data Analysis

All experiments were conducted in randomized complete block design (CRD) with arrangements of three replications of the treatments; and data were analyzed statistically using ANOVA provided by the statistical software SPSS version 20.

## 3. RESULTS AND DISCUSSION

### 3.1 Percentage Yield of Crude Extracts

Methanol, hexane, ethanol, and aqueous crude extracts of all three plants (*R. prinioides* (gesho), *J. schimperiana* (sensesel) and *R. chalepensis* (Tena adam) leaves, stem barks and roots were obtained from the extraction of 20 g powders of the plant parts using ethanol, methanol, hexane and aqueous extracting solvents, as shown in Tables 1, 2 and,3.

As indicated in the Table 1, the yield (amount) of the crude extracts ranged from 1.20% to 16.30% for *R. prinioides*. ECE of the leaf of *R. prinioides* gave maximum yield (16.30%). The lowest yield was obtained from aqueous extract of the roots of *R. prinioides* (1.20%).

**Table 1. The percentage yields of the crude extracts of the leaves, roots, and stem barks of *Rhamnus prinioides* (gesho)**

Plant parts	Weight and percentage yield of crude extracts by extraction solvents							
	Ethanol		Methanol		Hexane		Water	
	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight(g)	Yield (%)	Weight (g)	Yield (%)
Leaf	3.26	16.30dB	2.84	14.20cC	0.24	1.20aA	1.79	8.95bB
Stem bark	1.15	5.75aA	1.47	7.35bA	1.03	5.15aC	1.53	7.65bA
Root	2.00	10.00dC	1.88	9.40cB	0.78	3.90aB	1.24	6.20bA

Values with different superscripts in the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different in extraction potential

**Table 2. The percentage yields of the crude extracts of the leaves, roots, and stem barks of *Justicia schimperiana* (sensel)**

Plant parts	Weight and percentage yield of crude extracts by extraction solvents							
	Ethanol		Methanol		Hexane		Water	
	Weight(g)	Yield (%)	Weight (g)	Yield (%)	Weight(g)	Yield (%)	Weight (g)	Yield (%)
Leaf	4.13	20.65dC	3.14	15.70cC	0.41	2.05aB	2.18	10.90bB
Stem bark	3.01	15.05dB	2.06	10.30cB	1.89	9.45bC	1.31	6.55aA
Root	1.08	5.40bA	1.29	6.45cA	0.06	0.30aA	2.7	13.5dC

Values with different superscripts in the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different in extraction potential

**Table 3. The percentage yields of the crude extracts of the leaves, roots, and stem barks of *Ruta chalepensis* (Tena adam)**

Plant parts	Weight and percentage yield of crude extracts by extraction solvents							
	Ethanol		Methanol		Hexane		Water	
	Weight(g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
Leaf	2.31	11.55cA	2.41	12.05dC	0.58	2.90aA	1.10	5.50bB
Stem bark	2.37	11.85dA	1.57	7.85cB	0.58	2.90aA	0.92	4.60bA
Root	2.30	11.50dA	1.36	6.80bA	0.91	4.55aB	1.89	9.45cC

Values with different superscripts in the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different in extraction potential

Table 2 shows, the yield (amount) of the crude extracts ranged from 0.30% to 20.65% for *J. schimperiana*. ECE of the leaf of *J. schimperiana* gave maximum yield (20.65%). The lowest yield was obtained from hexane extract of the roots of *J. schimperiana* (0.30%).

Table 3 shows, the yield (amount) of the crude extracts ranged from 2.90% to 11.58% for *R. chalepensis*. ECE of the stem bark of *R. chalepensis* gave maximum yield (11.58%). The lowest yield was obtained from hexane extract of the leaf and stem of *R. chalepensis* (2.90%). The results clearly showed that the percentage yield of the crude extracts of the different plant parts of all plants varied from solvent to solvent. This could be attributed to the difference in polarity and extracting potential of methanol, ethanol, hexane and water. The highest percentage yield was observed in ECE and MCE of (leaf and stem) of *J. schimperiana* (sensel). This finding is

in agreement with the results of [31]. As [32] reported, most antimicrobial agents that have been identified from plants are soluble in organic solvents and this reveals the better efficiency of methanol, ethanol and hexane as extracting solvent than water.

### 3.2 Antimicrobial Activities of Crude Extracts as Measured by the Paper Disc Method

In this study, the antimicrobial activities of the ethanol, methanol, hexane and aqueous crude extracts of the stems, roots and leaves of *R. prinioides* (gesho), *J. schimperiana* (sensel) and *R. chalepensis* (Tena adam) were evaluated using paper disc method. The inhibition zone formed following incubation was measured and the mean diameters were achieved. A total of 36 crude extracts (ethanol, methanol, hexane and aqueous) were prepared from all plants and

antibiotics where tested for antimicrobial activities against the test organisms. The antimicrobial activities of the different extracts of all plants against the bacterial and fungal species are presented in Tables 4- 15.

### 3.2.1 Antibacterial and antifungal activities of crude extract of the stem of *R. chalepensis* against the test organisms

The *in vitro* assays of antibacterial and antifungal activities obtained from stem of *R. chalepensis* using four different solvents are presented in Table 4. As can be seen from the table below, the diameters of the zone of inhibition of the ethanol, methanol, hexane and aqueous root extracts were in the range of 5.67 – 19.67 mm, 4.33- 14.00 mm, 3.33 – 13.33 mm and 3.33-14.00 mm, respectively. Most of crude extracts of *R. chalepensis* stem showed a significant growth inhibition against eight tested organisms (Table 4).

### 3.2.2 Antibacterial and antifungal activities of crude extract of the leaf of *R. chalepensis* against the test organisms

The *in vitro* assays of antibacterial and antifungal activities obtained from leaf of *R. chalepensis* using four different solvents are presented in Table 5. As can be seen from the table, the diameters of the zone of inhibition of the ethanol, methanol, hexane and aqueous leaf extracts were in the range of 6.00 – 14.00 mm, 4.00-16.00 mm, 2.00 – 8.67 mm and 4.00- 12.00 mm respectively. This finding is compatible with the finding of [33]. Most of crude extracts of *R. chalepensis* leaf showed a significant growth inhibition against eight tested organisms (Table 5).

The highest inhibition zone (16.00±0.00 mm) was recorded by methanol leaf extract on *S. aureus* at the concentration (60 mg/ml) whereas; the lowest inhibition zone (2.00±0.00 mm) was recorded from hexane leaf extract at the concentration (30 mg/ml) on *S. bouhardi*. Antimicrobial activities of leaf extract of *R. chalepensis* against different pathogens were also reported by Zeichende et al. [34].

### 3.2.3 Antibacterial and antifungal activities of crude extract of the root of *R. chalepensis* against the test organisms

The ethanol, methanol, hexane and aqueous crude extracts of the root of *R. chalepensis* at

concentrations of 30 and 60 mg/ml were evaluated for in-vitro antimicrobial activities against the eight test organisms.

Most crude root extracts of *R. chalepensis* showed a significant growth inhibition against some test organisms. As shown from Table 6, the inhibition zones of the ethanol, methanol, hexane and aqueous crude extracts of root were in the range of 6.00 – 14.00 mm, 4.00 – 12.67 mm, 4.33-7.33 mm and 2.00- 8.33 mm respectively. The highest inhibition zone (14.00±0.00 mm) was recorded by ethanol root extract on *E. coli* at the concentration (60 mg/ml) whereas; the lowest inhibition zone (2.00±0.00 mm) was recorded from water root extract at the concentration (30 mg/ml) on *E. coli*.

Antimicrobial activities of different parts extract of *R. chalepensis* against different pathogens were also reported by Rojas et al. [35,36,37,38]. In general, most of the tested microorganisms were inhibited by several plants extracts of different solvents used in this study. For example, while ethanol extracts of *R. chalepensis* inhibited all eight microorganisms, methanol extracts inhibited all most all, hexane and water extracts inhibited few of them. Thus, the efficacy of plant extracts evaluated as antimicrobial agents was dependent on the solvent of extraction. Alzoreky and Nakahara [39] reported that, both methanol and ethanol were proved to be good solvents in extracting inhibitory substances from medicinal plants. In contrast, [40] and [32] found that methanol was more efficient than ethanol/acetone in extracting phytochemicals from plant materials. In accordance with these dissimilar findings, the results of the current study revealed that the solvent type is not the only factor that should be taken in consideration during extraction of plant constituents but also the plant species as well as the test microorganism and concentration played an important role in the antimicrobial efficacy.

### 3.2.4 Antibacterial and antifungal activities of crude extract of the stem bark of *J. schimperiana* against the test organisms

The *in vitro* assays of antibacterial and antifungal activities obtained from stem of *J. schimperiana* using four different solvents are presented in Table 7. As can be seen from the table, the diameters of the zone of inhibition of the ethanol, methanol, hexane and aqueous leaf extracts were in the range of 4.00 – 18.67 mm, 8.00-

18.00 mm, 4.00 – 60.67 mm and 2.67-12.00 mm, respectively. Most of crude extracts of *J. schimperiana* stem showed a significant growth inhibition against all eight tested organisms (Table 7).

The highest inhibition zone (18.67±1.15mm) was recorded by ethanol stem extract on *J. schimperiana* at the concentration (60 mg/ml) whereas; the lowest inhibition zone (2.67±0.11 mm) was recorded from water root extract at the concentration (30 mg/ml) on *S. boulardi*. Antimicrobial activities of chewing its stick of *J. schimperiana* against *C. albicans* and other pathogenic microorganisms also reported by Abebe et al. [11].

### 3.2.5 Antibacterial and antifungal activities of crude extract of the leaf of *J. schimperiana* against the test organisms

The *in vitro* assays of antibacterial and antifungal activities obtained from leaf of *J. schimperiana* using four different solvents are presented in Table 16. As can be seen from the table, the diameters of the zone of inhibition of the ethanol, methanol, hexane and aqueous root extracts were in the range of 6.00 – 24.00 mm, 6.00-20.00 mm, 3.33– 12.00 mm and 2.67- 12.00 mm, respectively. All crude extracts of *J. schimperiana* leaf showed a significant growth inhibition against most tested organisms (Table 8).

The highest inhibition zone (24.00±0.00 mm) was recorded by ethanol leaf extract on *C. jejuni* at the concentration (60 mg/ml) whereas; the lowest inhibition zone (2.67±0.11 mm) was recorded from water root extract at the concentration (30 mg/ml) on *P. auruginosa*.

### 3.2.6 Antibacterial and antifungal activities of crude extract of the root of *J. schimperiana* against the test organisms

The ethanol, methanol, hexane and aqueous crude extracts of the root of *J. schimperiana* at concentrations of 30 and 60 mg/ml were evaluated for *in vitro* antimicrobial activities against the eight test organisms. The inhibition zones that resulted from these extracts are shown in Table 9. Most crude root extracts of *J. schimperiana* showed a significant growth inhibition against some taste organisms. As shown from Table 9, the inhibition zones of the ethanol, methanol, hexane and aqueous crude

extracts of root were in the range of 5.00 – 16.00 mm, 4.67 – 15.33mm, 5.00 -12.67 mm and 4.33-10.33 mm respectively. The highest inhibition zone (16.00±0.00 mm) was recorded by ethanol root extract on *S. aureus* and *A. flavius* at the concentration (60 mg/ml) whereas; the lowest inhibition zone (4.33±0.51 mm) was recorded from water root extract at the concentration (30 mg/ml) on *P. auruginosa*.

### 3.2.7 Antibacterial and antifungal activities of crude extract of the stem bark of *R. prinioides* against the test organisms

The *in vitro* assays of antibacterial and antifungal activities obtained from Stem bark of *R. prinioides* using four different solvents are presented in Table 10. As can be seen from the table, the diameters of the zone of inhibition of the ethanol, methanol, hexane and aqueous root extracts were in the range of 4.00 – 10.67 mm, 3.67- 10.00 mm, 8.00 – 10.00 mm and 4.33-8.33 mm, respectively. Most crude extracts of *R. prinioides* stem showed a significant growth inhibition against most tested organisms (Table 18). The highest inhibition zone (10.67±0.00 mm) was recorded by ethanol stem extract on *S. aureus* at the concentration (60 mg/ml) whereas; the lowest inhibition zone (3.67±0.00 mm) was recorded from methanol stem extract at the concentration (30 mg/ml) on *A. flavius*.

### 3.2.8 Antibacterial and antifungal activities of crude extract of the leaf of *R. prinioides* against the test organisms

The ethanol, methanol, hexane and aqueous crude extracts of the leaf of *R. prinioides* at concentrations of 30 and 60 mg/ml were evaluated for *in vitro* antimicrobial activities against the eight test organisms. The inhibition zones that resulted from these extracts are shown in Table 19. Most crude leaf extracts of *R. prinioides* showed a significant growth inhibition against some taste organisms. As shown from Table 19, the inhibition zones of the ethanol, methanol, hexane and aqueous crude extracts of leaf were in the range of 4.00 – 13.60 mm, 3.00 – 10.30 mm, 3.67 – 8.33 mm and 2.00 – 8.33 mm respectively. The highest inhibition zone (13.67±0.05 mm) was recorded from ethanol leaf extract on *C. jejuni* at the concentration (60 mg/ml) whereas; the lowest inhibition zone (2.00±0.00 mm) was recorded from water leaf extract at the concentration (30 mg/ml) on *C. albicus*.



Table 4. Antibacterial and antifungal activities of crude extracts of the stem of *R. chalepensis* against the test organisms (mean  $\pm$  SD, n=3)

Test organism	Conc. In mg/ml	Zone of Inhibition (mm) in different extract solvents					
		EsE	MsE	HsE	WsE	Cont.	+Con.0.1 mg/ml
<i>E. coli</i>	30	5.67 $\pm$ 0.57 <sup>CA</sup>	4.33 $\pm$ 0.57 <sup>BA</sup>	4.33 $\pm$ 1.52 <sup>DB</sup>	3.33 $\pm$ 1.15 <sup>AA</sup>	-	12.33 $\pm$ 0.57 <sup>DB</sup>
	60	9.33 $\pm$ 1.52 <sup>DE</sup>	7.00 $\pm$ 1.00 <sup>BD</sup>	7.67 $\pm$ 1.52 <sup>CE</sup>	4.33 $\pm$ 1.52 <sup>AB</sup>	-	14.67 $\pm$ 1.15 <sup>CD</sup>
<i>P. auruginosa</i>	30	5.67 $\pm$ 0.57 <sup>AA</sup>	6.67 $\pm$ 0.57 <sup>BD</sup>	5.67 $\pm$ 0.57 <sup>AC</sup>	5.67 $\pm$ 0.57 <sup>AC</sup>	-	15.33 $\pm$ 0.57 <sup>CE</sup>
	60	9.00 $\pm$ 1.00 <sup>AE</sup>	9 $\pm$ 1.00 <sup>AE</sup>	11.00 $\pm$ 4.33 <sup>bH</sup>	8.33 $\pm$ 0.57 <sup>AE</sup>	-	17.67 $\pm$ 0.57 <sup>CF</sup>
<i>S. aureus</i>	30	19.0 $\pm$ 100 <sup>EH</sup>	11.67 $\pm$ 0.57 <sup>CH</sup>	8.33 $\pm$ 0.57 <sup>AF</sup>	9.67 $\pm$ 0.57 <sup>BF</sup>	-	12.00 $\pm$ 00 <sup>DB</sup>
	60	19.67 $\pm$ 0.5 <sup>DI</sup>	14.00 $\pm$ 0.00 <sup>BI</sup>	9.67 $\pm$ 0.57 <sup>AG</sup>	14.00 $\pm$ 0.00 <sup>bG</sup>	-	15.33 $\pm$ 1.15 <sup>CE</sup>
<i>C. jejuni</i>	30	6.33 $\pm$ 0.57 <sup>CB</sup>	5.00 $\pm$ 00 <sup>bB</sup>	3.33 $\pm$ 1.15 <sup>AA</sup>	-	-	15.33 $\pm$ 1.15 <sup>dE</sup>
	60	8.67 $\pm$ 0.57 <sup>BE</sup>	6.00 $\pm$ 0.57 <sup>AC</sup>	13.33 $\pm$ 1.15 <sup>CI</sup>	-	-	18.00 $\pm$ 00 <sup>dF</sup>
<i>A. niger</i>	30	6.33 $\pm$ 0.57 <sup>CB</sup>	6.67 $\pm$ 0.57 <sup>CD</sup>	6.00 $\pm$ 0.00 <sup>BD</sup>	4.00 $\pm$ 0.00 <sup>AB</sup>	-	10.67 $\pm$ 1.15 <sup>dA</sup>
	60	7.33 $\pm$ 0.57 <sup>AC</sup>	9.67 $\pm$ 0.57 <sup>CF</sup>	8.00 $\pm$ 0.00 <sup>BF</sup>	7.33 $\pm$ 1.15 <sup>AD</sup>	-	12.33 $\pm$ 0.57 <sup>dB</sup>
<i>A. flavius</i>	30	9.0 $\pm$ 1.00 <sup>CE</sup>	7.00 $\pm$ 0.00 <sup>BD</sup>	6.33 $\pm$ 0.57 <sup>AD</sup>	-	-	15.67 $\pm$ 0.57 <sup>dE</sup>
	60	14 $\pm$ 0.00 <sup>CG</sup>	9.33 $\pm$ 0.57 <sup>BF</sup>	7.67 $\pm$ 1.15 <sup>AE</sup>	-	-	20.00 $\pm$ 00 <sup>dE</sup>
<i>C. albicus</i>	30	7.67 $\pm$ 0.57 <sup>AC</sup>	9.00 $\pm$ 00 <sup>BE</sup>	-	-	-	12.00 $\pm$ 00 <sup>CB</sup>
	60	10 $\pm$ 0.00 <sup>AF</sup>	10.67 $\pm$ 1.15 <sup>bG</sup>	-	-	-	15.33 $\pm$ 1.15 <sup>CE</sup>
<i>S. boulardi</i>	30	8.00 $\pm$ 0.00 <sup>BD</sup>	6.67 $\pm$ 0.57 <sup>AD</sup>	-	-	-	12.00 $\pm$ 00 <sup>CB</sup>
	60	10 $\pm$ 0.00 <sup>BF</sup>	9.00 $\pm$ 0.00 <sup>AE</sup>	-	-	-	14.00 $\pm$ 0.00 <sup>CC</sup>

Key: EsE = Ethanol stem extract, MsE =Methanol stem extract, HsE= Hexane stem extract, WsE= Water stem extract chloramphenicol/ clotrimaxole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different ( $p < 0.05$ ) 0 = no inhibition zone

Table 5. Antibacterial and antifungal activities of crude extracts of the leaf of *R. chalepensis* against the test organisms (mean  $\pm$  SD, n=3)

Test organism	Conc. In Mg/ml	Zone of Inhibition (mm) in different extract solvents					
		EIE	MIE	HIE	WIE	Cont.	+Con.0.1mg/
<i>E. coli</i>	30	10.67 $\pm$ 1.15 <sup>bE</sup>	7.00 $\pm$ 0.00 <sup>aC</sup>	-	10.00 $\pm$ 0.00 <sup>bC</sup>	-	14.33 $\pm$ 0.57 <sup>cE</sup>
	60	13.33 $\pm$ 1.52 <sup>cG</sup>	10.33 $\pm$ 0.57 <sup>aF</sup>	-	12.00 $\pm$ 0.00 <sup>bD</sup>	-	16.00 $\pm$ 0.00 <sup>dF</sup>
<i>P. auruginosa</i>	30	6.00 $\pm$ 0.00 <sup>aA</sup>	8.00 $\pm$ 0.00 <sup>bD</sup>	-	-	-	12.00 $\pm$ 0.00 <sup>cC</sup>
	60	8.00 $\pm$ 0.00 <sup>aB</sup>	10 $\pm$ 0.00 <sup>bF</sup>	-	-	-	14.67 $\pm$ 1.15 <sup>cE</sup>
<i>S. aureus</i>	30	12 $\pm$ 0.00 <sup>cF</sup>	14.67 $\pm$ 0.57 <sup>dH</sup>	8.00 $\pm$ 0.00 <sup>aC</sup>	10.00 $\pm$ 0.00 <sup>bC</sup>	-	16.00 $\pm$ 0.00 <sup>eF</sup>
	60	14.00 $\pm$ 0.00 <sup>cH</sup>	16.00 $\pm$ 0.00 <sup>dI</sup>	8.67 $\pm$ 0.57 <sup>aD</sup>	12.00 $\pm$ 0.00 <sup>bD</sup>	-	18.00 $\pm$ 0.00 <sup>eG</sup>
<i>C. jejuni</i>	30	8.00 $\pm$ 0.00 <sup>aB</sup>	9.00 $\pm$ 0.00 <sup>bE</sup>	-	-	-	10.00 $\pm$ 0.00 <sup>cB</sup>
	60	9.67 $\pm$ 0.57 <sup>aC</sup>	12.00 $\pm$ 0.00 <sup>bG</sup>	-	-	-	12.67 $\pm$ 1.15 <sup>cD</sup>
<i>A. niger</i>	30	6.00 $\pm$ 0.00 <sup>bA</sup>	6.67 $\pm$ 1.15 <sup>cC</sup>	-	4.00 $\pm$ 0.00 <sup>aA</sup>	-	16.00 $\pm$ 1.15 <sup>dF</sup>
	60	9.33 $\pm$ 1.15 <sup>bC</sup>	9.33 $\pm$ 0.57 <sup>bE</sup>	-	8.00 $\pm$ 0.00 <sup>aB</sup>	-	20.00 $\pm$ 0.00 <sup>cH</sup>
<i>A. flavius</i>	30	6.00 $\pm$ 0.00 <sup>aA</sup>	8.00 $\pm$ 0.00 <sup>bD</sup>	-	-	-	10.00 $\pm$ 0.00 <sup>cB</sup>
	60	8.00 $\pm$ 0.00 <sup>aB</sup>	10.33 $\pm$ 0.00 <sup>bF</sup>	-	-	-	12.00 $\pm$ 0.00 <sup>cC</sup>
<i>C. albicus</i>	30	8.00 $\pm$ 0.00 <sup>bB</sup>	6.00 $\pm$ 0.00 <sup>aB</sup>	-	-	-	10.00 $\pm$ 0.00 <sup>cB</sup>
	60	10 $\pm$ 0.00 <sup>bD</sup>	8.33 $\pm$ 0.57 <sup>aD</sup>	-	-	-	12.00 $\pm$ 0.00 <sup>cC</sup>
<i>S. boulardi</i>	30	8.00 $\pm$ 0.00 <sup>cB</sup>	4.00 $\pm$ 0.00 <sup>bA</sup>	2.00 $\pm$ 0.00 <sup>aA</sup>	4.00 $\pm$ 0.00 <sup>bA</sup>	-	9.33 $\pm$ 1.15 <sup>dA</sup>
	60	10.00 $\pm$ 0.00 <sup>dD</sup>	8.00 $\pm$ 0.00 <sup>bD</sup>	4.00 $\pm$ 0.00 <sup>aB</sup>	8.00 $\pm$ 0.00 <sup>cB</sup>	-	12.67 $\pm$ 1.15 <sup>eD</sup>

Key: EIE = ethanol stem extract, MIE = methanol stem extract, HIE= Hexane stem extract, WIE= Water stem extract chloramphenicol/ clotrimazole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column(upper case) are significantly different ( $p < 0.05$ , - = no inhibition zone)

Table 6. Antibacterial and antifungal activities of crude extracts of the root of *R. chalepensis* against the test organisms (mean  $\pm$  SD, n=3)

Test organism	Conc. In Mg/ml	Zone of Inhibition (mm) in different extract solvents					
		ErE	MrE	HrE	WrE	_Cont.	+Con.0.1mg/ml
<i>E. coli</i>	30	8.00 $\pm$ 0.00bC	-	-	2.00 $\pm$ 0.00aA	-	10.00 $\pm$ 0.00cA
	60	14.00 $\pm$ 0.00cF	-	-	4.67 $\pm$ 1.15aB	-	13.33 $\pm$ 1.15bC
<i>P. auruginosa</i>	30	6.00 $\pm$ 0.00bA	4.00 $\pm$ 0.00aA	-	-	-	10.67 $\pm$ 1.15cA
	60	8.00 $\pm$ 0.00bC	7.33 $\pm$ 1.15aB	-	-	-	14.00 $\pm$ 0.00cD
<i>S. aureus</i>	30	10 $\pm$ 0.00dD	8.00 $\pm$ 0.00cC	4.33 $\pm$ 0.57aA	6.00 $\pm$ 0.00bC	-	12.67 $\pm$ 1.15eB
	60	12.00 $\pm$ 0.00dE	10.00 $\pm$ 0.00cD	7.33 $\pm$ 1.15aB	8.33 $\pm$ 0.57bD	-	17.33 $\pm$ 1.15eF
<i>C. jejuni</i>	30	6.00 $\pm$ 0.00aA	-	-	-	-	12.00 $\pm$ 0.00bB
	60	7.33 $\pm$ 1.15aB	-	-	-	-	14.00 $\pm$ 0.00bD
<i>A. niger</i>	30	8.00 $\pm$ 0.00aC	10.00 $\pm$ 0.00bD	-	-	-	12.00 $\pm$ 0.00cB
	60	12.00 $\pm$ 0.00aE	12.67 $\pm$ 0.57bE	-	-	-	14.67 $\pm$ 1.15cD
<i>A. flavius</i>	30	7.33 $\pm$ 1.15aB	8.00 $\pm$ 0.00bC	-	-	-	10.00 $\pm$ 0.00cA
	60	10.00 $\pm$ 0.00aD	12.00 $\pm$ 0.00bE	-	-	-	14.00 $\pm$ 0.00cD
<i>C. albicus</i>	30	8.00 $\pm$ 0.00aC	-	-	-	-	14.00 $\pm$ 0.00bD
	60	10.00 $\pm$ 0.00aD	-	-	-	-	15.33 $\pm$ 1.15bE
<i>S. boulardi</i>	30	10.00 $\pm$ 0.00cD	8.00 $\pm$ 0.00bC	-	4.00 $\pm$ 0.00aB	-	14.00 $\pm$ 0.00dD
	60	12.00 $\pm$ 0.00cE	10.00 $\pm$ 0.00bD	-	8.00 $\pm$ 0.00aD	-	16.00 $\pm$ 0.00dE

Key: ErE = ethanol stem extract, MrE = methanol stem extract, HrE= Hexane stem extract, WrE= Water stem extract chloramphenicol/clotrimazole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different ( $p < 0.05$ , - = no inhibition zone)

**Table 7. Antibacterial and antifungal activities of crude extracts of the stem bark of *J. schimperiana* against the test organisms (mean  $\pm$  SD, n=3)**

Test Organism	Conc. In Mg/ml	Zone of Inhibition (mm) in different extract solvents					
		EsE	MsE	HsE	WsE	_Cont.	+Con.0.1mg/ml
<i>E. coli</i>	30	10.67 $\pm$ 1.15 <sup>cD</sup>	10.00 $\pm$ 2.00 <sup>bC</sup>	6.67 $\pm$ 1.15 <sup>aC</sup>	-	-	10.00 $\pm$ 0.00 <sup>bA</sup>
	60	13.33 $\pm$ 0.1.55 <sup>cG</sup>	12.67 $\pm$ 1.15 <sup>bE</sup>	10.67 $\pm$ 1.15 <sup>aH</sup>	-	-	14.00 $\pm$ 0.00 <sup>dE</sup>
<i>P. auruginosa</i>	30	11.33 $\pm$ 1.15 <sup>bE</sup>	11.33 $\pm$ 1.15 <sup>bD</sup>	8.67 $\pm$ 1.15 <sup>aF</sup>	-	-	14.00 $\pm$ 0.00 <sup>cE</sup>
	60	14.00 $\pm$ 0.00 <sup>cH</sup>	13.33 $\pm$ 1.15 <sup>bF</sup>	10.00 $\pm$ 0.00 <sup>aG</sup>	-	-	16.00 $\pm$ 0.00 <sup>dF</sup>
<i>S. aureus</i>	30	15.33 $\pm$ 0.23 <sup>cJ</sup>	16.00 $\pm$ 0.00 <sup>dH</sup>	8.00 $\pm$ 0.57 <sup>aE</sup>	10.00 $\pm$ 0.00 <sup>bG</sup>	-	19.33 $\pm$ 1.15 <sup>aH</sup>
	60	18.00 $\pm$ 0.00 <sup>cK</sup>	18.00 $\pm$ 0.00 <sup>dI</sup>	10.67 $\pm$ 1.15 <sup>aH</sup>	12.00 $\pm$ 0.57 <sup>bH</sup>	-	24.33 $\pm$ 0.57 <sup>dJ</sup>
<i>C. jejuni</i>	30	14.67 $\pm$ 1.15 <sup>cI</sup>	8.67 $\pm$ 1.15 <sup>bB</sup>	5.33 $\pm$ 1.15 <sup>aB</sup>	-	-	20.00 $\pm$ 0.00 <sup>dI</sup>
	60	18.67 $\pm$ 1.15 <sup>cK</sup>	14.67 $\pm$ 1.15 <sup>bG</sup>	7.33 $\pm$ 1.15 <sup>aD</sup>	-	-	24.00 $\pm$ 0.00 <sup>dJ</sup>
<i>A. niger</i>	30	4.00 $\pm$ 0.00 <sup>bA</sup>	-	-	3.00 $\pm$ 1.00 <sup>aB</sup>	-	11.33 $\pm$ 1.15 <sup>cB</sup>
	60	5.33 $\pm$ 1.15 <sup>bB</sup>	-	-	4.67 $\pm$ 1.51 <sup>aD</sup>	-	12.00 $\pm$ 0.00 <sup>cC</sup>
<i>A. Flavivus</i>	30	9.33 $\pm$ 1.15 <sup>bC</sup>	-	-	4.00 $\pm$ 0.00 <sup>aC</sup>	-	16.00 $\pm$ 0.00 <sup>cF</sup>
	60	11.33 $\pm$ 1.15 <sup>bE</sup>	-	-	5.33 $\pm$ 0.00 <sup>aE</sup>	-	18.00 $\pm$ 0.00 <sup>cG</sup>
<i>C. albicus</i>	30	12.00 $\pm$ 0.00 <sup>dF</sup>	8.00 $\pm$ 0.00 <sup>cA</sup>	4.00 $\pm$ 0.00 <sup>bA</sup>	3.33 $\pm$ 1.50 <sup>aB</sup>	-	12.00 $\pm$ 0.00 <sup>dC</sup>
	60	14 $\pm$ 0.00 <sup>dH</sup>	8.67 $\pm$ 8.86 <sup>cB</sup>	5.33 $\pm$ 0.15 <sup>aB</sup>	8.00 $\pm$ 0.00 <sup>bF</sup>	-	16.00 $\pm$ 0.00 <sup>cF</sup>
<i>S. boulardi</i>	30	-	-	-	2.67 $\pm$ 0.11 <sup>aA</sup>	-	12.00 $\pm$ 0.00 <sup>bC</sup>
	60	-	-	-	5.33 $\pm$ 0.15 <sup>aE</sup>	-	13.33 $\pm$ 1.15 <sup>bD</sup>

Key: EsE = ethanol stem extract, MsE = methanol stem extract, HsE = Hexane stem extract, WsE = Water stem extract chloramphenicol/ clotrimazole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column(upper case) are significantly different ( $p < 0.05$ , - = no inhibition zone)

**Table 8. Antibacterial and antifungal activities of crude extracts of the leaf of *J. schimperiana* against the test organisms (mean  $\pm$  SD, n=3)**

Test Organism	Conc. In Mg/ml	Zone of Inhibition (mm) in different extract solvents					
		EIE	MIE	HIE	WIE	_Cont.	+Con.0.1mg/ml
<i>E. coli</i>	30	13.33 $\pm$ 1.11 <sup>bF</sup>	11.33 $\pm$ 1.15 <sup>aC</sup>	-	-	-	14.00 $\pm$ 0.00 <sup>cC</sup>
	60	19.33 $\pm$ 1.55 <sup>bI</sup>	13.33 $\pm$ 1.15 <sup>aE</sup>	-	-	-	16.00 $\pm$ 0.00 <sup>cD</sup>
<i>P. auruginosa</i>	30	-	-	-	2.67 $\pm$ 1.15 <sup>aA</sup>	-	10.00 $\pm$ 0.00 <sup>bA</sup>
	60	-	-	-	4.00 $\pm$ 0.00 <sup>aC</sup>	-	12.00 $\pm$ 0.00 <sup>bB</sup>
<i>S. aureus</i>	30	12.00 $\pm$ 0.00 <sup>cD</sup>	10.00 $\pm$ 0.00 <sup>bB</sup>	4.67 $\pm$ 1.15 <sup>aB</sup>	10.00 $\pm$ 0.00 <sup>bG</sup>	-	18.00 $\pm$ 0.00 <sup>dE</sup>
	60	16.00 $\pm$ 0.00 <sup>dH</sup>	15.33 $\pm$ 0.05 <sup>bF</sup>	8.00 $\pm$ 0.00 <sup>aD</sup>	12.00 $\pm$ 0.00 <sup>cH</sup>	-	20.00 $\pm$ 0.00 <sup>eG</sup>
<i>C. jejuni</i>	30	20.00 $\pm$ 0.00 <sup>cJ</sup>	18.00 $\pm$ 0.00 <sup>bH</sup>	10.00 $\pm$ 0.00 <sup>aE</sup>	-	-	18.00 $\pm$ 0.00 <sup>bE</sup>
	60	24.00 $\pm$ 0.00 <sup>dK</sup>	20.00 $\pm$ 0.00 <sup>bI</sup>	11.33 $\pm$ 1.15 <sup>aF</sup>	-	-	22.00 $\pm$ 0.00 <sup>cI</sup>
<i>A. niger</i>	30	-	6.00 $\pm$ 0.00 <sup>bA</sup>	3.33 $\pm$ 1.12 <sup>aA</sup>	-	-	18.00 $\pm$ 0.00 <sup>cE</sup>
	60	-	14.00 $\pm$ 0.00 <sup>bF</sup>	6.00 $\pm$ 0.00 <sup>aC</sup>	-	-	20.67 $\pm$ 0.51 <sup>cH</sup>
<i>A. flavivus</i>	30	10.67 $\pm$ 1.15 <sup>cC</sup>	12.00 $\pm$ 0.00 <sup>dD</sup>	10.00 $\pm$ 0.00 <sup>bE</sup>	3.33 $\pm$ 0.15 <sup>aB</sup>	-	18.00 $\pm$ 0.00 <sup>dE</sup>
	60	14.67 $\pm$ 1.15 <sup>cG</sup>	15.33 $\pm$ 0.12 <sup>aG</sup>	11.00 $\pm$ 0.00 <sup>bF</sup>	8.00 $\pm$ 0.00 <sup>aE</sup>	-	20.00 $\pm$ 0.00 <sup>eG</sup>
<i>C. albicus</i>	30	6.00 $\pm$ 0.00 <sup>aA</sup>	-	-	6.00 $\pm$ 0.00 <sup>aD</sup>	-	18.67 $\pm$ 1.10 <sup>bF</sup>
	60	10.00 $\pm$ 0.00 <sup>bB</sup>	-	-	8.67 $\pm$ 0.05 <sup>aF</sup>	-	20.65 $\pm$ 0.15 <sup>cH</sup>
<i>S. boulardi</i>	30	-	-	10.00 $\pm$ 0.00 <sup>aE</sup>	-	-	18.00 $\pm$ 0.00 <sup>bE</sup>
	60	-	-	12.00 $\pm$ 0.00 <sup>aG</sup>	-	-	20.00 $\pm$ 140 <sup>bG</sup>

Key: EIE = ethanol leaf extract, MIE = methanol leaf extract, HIE = Hexane leaf extract, WIE = Water leaf extract chloramphenicol/ clotrimazole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column(upper case) are significantly different ( $p < 0.05$ , - = no inhibition zone)

**Table 9. Antibacterial and antifungal activities of crude extracts of the root of *J. schimperiana* against the test organisms (mean ± SD, n=3)**

Test Organism	Conc. In Mg/ml	Zone of Inhibition (mm) in different extract solvents					
		ErE	MrE	HrE	WrE	_Cont.	+Con.0.1mg/ml
<i>E. coli</i>	30	9.00±1.00 <sup>dC</sup>	7.00±0.00 <sup>cD</sup>	5.00±0.00 <sup>aA</sup>	6.00±0.00 <sup>bC</sup>	-	12.67±1.15 <sup>eC</sup>
	60	12.33±0.57 <sup>cF</sup>	10.33±0.57 <sup>bF</sup>	9.33±0.57 <sup>aF</sup>	9.00±0.00 <sup>aF</sup>	-	18.00±0.00 <sup>dF</sup>
<i>P. auruginosa</i>	30	7.67±0.57 <sup>aB</sup>	7.00±0.00 <sup>cD</sup>	5.33±0.51 <sup>bB</sup>	4.33±0.51 <sup>aA</sup>	-	9.00±0.00 <sup>eA</sup>
	60	11.67±0.51 <sup>cE</sup>	10.00±0.00 <sup>aF</sup>	10.67±0.21 <sup>bG</sup>	10.33±0.11 <sup>aB</sup>	-	17.67±0.01 <sup>dE</sup>
<i>S. aureus</i>	30	11.67±0.57 <sup>dE</sup>	10.33±0.57 <sup>cF</sup>	8.67±0.57 <sup>bE</sup>	8.00±1.00 <sup>aE</sup>	-	18.00±0.00 <sup>eF</sup>
	60	16.00±0.00 <sup>dG</sup>	15.33±0.00 <sup>cH</sup>	12.67±0.57 <sup>bH</sup>	8.00±0.01 <sup>aE</sup>	-	20.00±0.00 <sup>eH</sup>
<i>C. jejumi</i>	30	5.00 ±1.00 <sup>aA</sup>	5.00±0.00 <sup>aB</sup>	-	-	-	16.00±0.00 <sup>bD</sup>
	60	10.00±0.00 <sup>aD</sup>	10.00±0.00 <sup>aD</sup>	-	-	-	18.00±0.00 <sup>bF</sup>
<i>A. niger</i>	30	8.00±0.00 <sup>bB</sup>	7.67±0.57 <sup>aE</sup>	-	-	-	16.00±0.00 <sup>cD</sup>
	60	15.33±1.15 <sup>bG</sup>	13.33±0.15 <sup>aB</sup>	-	-	-	18.00±0.51 <sup>cF</sup>
<i>A. flavus</i>	30	8.00 ±0.00 <sup>bB</sup>	6.00±0.00 <sup>aC</sup>	-	-	-	16.00±0.00 <sup>cD</sup>
	60	16.00±0.00 <sup>bH</sup>	10.33±0.12 <sup>aF</sup>	-	-	-	19.00±0.33 <sup>cG</sup>
<i>C. albicus</i>	30	8.00±0.00 <sup>bB</sup>	9.67±0.57 <sup>aF</sup>	6.00±0.00 <sup>bC</sup>	5.00±0.00 <sup>aB</sup>	-	10.00±1.10 <sup>eB</sup>
	60	10.33±0.00 <sup>cD</sup>	14.00±0.00 <sup>eG</sup>	8.00±0.00 <sup>bD</sup>	7.00±0.05 <sup>aD</sup>	-	12.00±0.15 <sup>dC</sup>
<i>S. boulardi</i>	30	7.00±0.00 <sup>bA</sup>	4.67±0.00 <sup>aA</sup>	-	-	-	10.00±0.00 <sup>cB</sup>
	60	10.33±0.57 <sup>bD</sup>	7.67±0.57 <sup>aE</sup>	-	-	-	12.00±0.00 <sup>cC</sup>

Key: ErE = ethanol root extract, MrE = methanol root extract, HrE= Hexane root extract, WrE= Water root extract chloramphenicol /clotrimaxole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different ( $p < 0.05$ , - = no inhibition zone)

**Table 10. Antibacterial and antifungal activities of crude extracts of the stem bark of *R. prinioides* against the test organisms (mean ± SD, n=3)**

Test Organism	Conc. In Mg/ml	Zone of Inhibition (mm) in different extract solvents					
		EsE	MsE	HsE	WsE	_Cont.	+Con.0.1mg/ml
<i>E. coli</i>	30	5.67±0.57 <sup>cB</sup>	4.00±0.00 <sup>aB</sup>	-	-	-	5.00±0.00 <sup>bA</sup>
	60	8.00±0.00 <sup>bD</sup>	6.00±0.00 <sup>aD</sup>	-	-	-	10.00±0.00 <sup>cC</sup>
<i>P. auruginosa</i>	30	7.33±0.27 <sup>bC</sup>	5.33±0.05 <sup>aC</sup>	-	-	-	10.00±0.00 <sup>cC</sup>
	60	7.33±0.00 <sup>aC</sup>	10.00±0.00 <sup>bG</sup>	-	-	-	12.00±0.00 <sup>cE</sup>
<i>S. aureus</i>	30	7.67±0.57 <sup>bC</sup>	7.33±0.67 <sup>bE</sup>	8.00±0.00 <sup>cA</sup>	4.33±1.00 <sup>aA</sup>	-	12.00±0.00 <sup>dE</sup>
	60	10.67±0.00 <sup>cE</sup>	10.00±0.05 <sup>bG</sup>	10.00±0.00 <sup>bB</sup>	4.33±0.01 <sup>aA</sup>	-	13.00±0.00 <sup>dF</sup>
<i>C. jejumi</i>	30	4.00 ±0.00 <sup>aA</sup>	6.00±0.00 <sup>bD</sup>	-	-	-	12.00±0.00 <sup>cE</sup>
	60	7.67±0.05 <sup>aC</sup>	8.00±0.00 <sup>aF</sup>	-	-	-	14.00±0.00 <sup>bG</sup>
<i>A. niger</i>	30	-	-	-	4.33±0.55 <sup>aA</sup>	-	12.00±0.00 <sup>bE</sup>
	60	-	-	-	8.33±0.33 <sup>aB</sup>	-	14.00±0.00 <sup>bB</sup>
<i>A. flavus</i>	30	4.00 ±0.00 <sup>aA</sup>	3.67±0.00 <sup>aA</sup>	-	-	-	10.00±0.00 <sup>bC</sup>
	60	5.67±0.55 <sup>aB</sup>	7.67±0.11 <sup>bE</sup>	-	-	-	11.00±0.33 <sup>cD</sup>

Key: EsE = ethanol stem extract, MsE = methanol stem extract, HsE= Hexane stem extract, WsE= Water stem extract chloramphenicol/ clotrimaxole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different ( $p < 0.05$ , - = no inhibition zone)

### 3.2.9 Antibacterial and antifungal activities of crude extract of the root of *R. prinioides* against the test organisms

The ethanol, methanol, hexane and aqueous crude extracts of the root of *R. prinioides* at concentrations of 30 and 60 mg/ml were evaluated for *in vitro* antimicrobial activities against the eight test organisms. The inhibition zones that resulted from these extracts are shown in Table 20. Most crude root extracts of *R. prinioides* showed a significant growth inhibition against some test organisms. As shown from Table 20, the inhibition zones of the ethanol, methanol, hexane and aqueous crude extracts of root were in the range of 2.67 – 17.00 mm, 4.00 – 15.67 mm, 2.67-10.00 mm and 3.00 -10.67 mm respectively. The highest inhibition zone (17.00±1.00 mm) was recorded from ethanol leaf extract on *A. flavius* at the concentration

(60mg/ml) whereas; the lowest inhibition zone (2.67±0.35 mm) was recorded from ethanol and hexane extract at the concentration (30 mg/ml) on *S. boulandi* and *P. auruginosa*. Antibacterial and antifungal activities of crude extract of *R. prinioides* against the test organisms also reported by [41].

Several investigators had also reported that plants contain antibacterial substances [42,43]. The present study also showed that there was variation in the degree of antibacterial activities of the extracts due to that high level of phytochemicals present in organic solvent extracts than in aqueous extracts [44]. Similarly, a number of studies have also reported the antimicrobial efficacy of ethanol, methanol and hexane extracts of other plants [45,46,47,48, 33,49,50].

**Table 11. Antibacterial and antifungal activities of crude extracts of the leaf of *R. prinioides* against the test organisms (mean ± SD, n=3)**

Test Organism	Conc. In Mg/ml	Zone of Inhibition (mm) in different extract solvents					
		EIE	MIE	HIE	WIE	_Cont.	+Con.0.1mg/ml
<i>E. coli</i>	30	6.00±1.00 <sup>bC</sup>	4.67±0.57 <sup>aB</sup>	6.00±0.00 <sup>bC</sup>	6.00±0.00 <sup>bE</sup>	-	10.00±1.15 <sup>cB</sup>
	60	9.33±0.15 <sup>bF</sup>	6.00±0.57 <sup>aC</sup>	8.00±0.00 <sup>bE</sup>	8.00±0.00 <sup>cG</sup>	-	10.00±0.00 <sup>cB</sup>
<i>P. auruginosa</i>	30	-	-	-	4.00±0.00 <sup>aB</sup>	-	9.33±0.57 <sup>bB</sup>
	60	-	-	-	5.00±0.00 <sup>aD</sup>	-	11.00±0.00 <sup>bD</sup>
<i>S. aureus</i>	30	6.00±0.00 <sup>aC</sup>	8.00±0.00 <sup>bD</sup>	6.00±0.00 <sup>aC</sup>	6.00±0.00 <sup>aE</sup>	-	10.67±0.57 <sup>aC</sup>
	60	10.00±2.00 <sup>cG</sup>	9.00±0.05 <sup>bE</sup>	7.00±0.00 <sup>aD</sup>	8.33±1.11 <sup>aA</sup>	-	13.00±0.00 <sup>dF</sup>
<i>C. jejuni</i>	30	11.00±1.00 <sup>dH</sup>	8.67±0.57 <sup>cE</sup>	3.67±0.57 <sup>aA</sup>	6.00±0.00 <sup>bE</sup>	-	14.00±0.00 <sup>cG</sup>
	60	13.60±0.05 <sup>cI</sup>	10.67±0.57 <sup>bF</sup>	8.33±0.55 <sup>aE</sup>	8.33±0.57 <sup>aG</sup>	-	15.00±0.00 <sup>dH</sup>
<i>A. niger</i>	30	5.00±0.00 <sup>aB</sup>	8.00±0.00 <sup>bD</sup>	-	-	-	12.00±0.00 <sup>cE</sup>
	60	7.00±1.15 <sup>aD</sup>	10.30±0.15 <sup>bF</sup>	-	-	-	14.00±0.51 <sup>cG</sup>
<i>A. flavius</i>	30	-	-	-	-	-	8.00±0.00 <sup>aA</sup>
	60	-	-	-	-	-	10.00±1.00 <sup>aC</sup>
<i>C. albicus</i>	30	8.00±0.00 <sup>bE</sup>	-	-	2.00±0.00 <sup>aA</sup>	-	10.00±1.10 <sup>cB</sup>
	60	10.67±0.00 <sup>bG</sup>	-	-	6.00±0.05 <sup>aE</sup>	-	11.00±0.15 <sup>cD</sup>
<i>S. boulandi</i>	30	4.00±0.00 <sup>bA</sup>	3.00±0.00 <sup>aA</sup>	4.00±0.00 <sup>bB</sup>	4.67±0.57 <sup>cC</sup>	-	16.00±0.00 <sup>aI</sup>
	60	8.00±0.00 <sup>aE</sup>	6.00±0.00 <sup>aC</sup>	8.67±0.57 <sup>dF</sup>	7.33±0.57 <sup>bF</sup>	-	18.00±0.00 <sup>eJ</sup>

Key: EIE = ethanol leaf extract, MIE = methanol leaf extract, HIE= Hexane leaf extract, WIE= Water leaf extract chloramphenicol/clotrimazole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different (p<0.05, - = no inhibition zone)

**Table 12. Antibacterial and antifungal activities of crude extracts of the root of *R. prinioides* against the test organisms (mean ± SD, n=3)**

Test Organism	Conc. In Mg/ml	Zone of Inhibition (mm) in different extract solvents					
		ErE	MrE	HrE	WrE	-Co nt	+ Con.
<i>E. coli</i>	30	6.00±0.00 <sup>CD</sup>	4.00±0.00 <sup>BA</sup>	-	3.0±0.00 <sup>AA</sup>	-	10.00±0.00 <sup>DC</sup>
	60	7.33±1.15 <sup>BE</sup>	6.00±1.00 <sup>AD</sup>	-	6.00±0.00 <sup>AC</sup>	-	12.00±0.00 <sup>CE</sup>
<i>P. auruginosa</i>	30	5.00±0.00 <sup>BC</sup>	6.00±0.00 <sup>CD</sup>	2.67±0.35 <sup>AA</sup>	-	-	12.00±0.00 <sup>DE</sup>
	60	6.00±0.00 <sup>AD</sup>	10.00±0.00 <sup>BH</sup>	10.0±0.00 <sup>BD</sup>	-	-	14.00±0.00 <sup>FE</sup>
<i>S. aureus</i>	30	12.67±0.57 <sup>dG</sup>	7.67±0.57 <sup>cE</sup>	4.00±0.00 <sup>aB</sup>	5.33±0.57 <sup>bB</sup>	-	12.33±0.57 <sup>eE</sup>
	60	15.33±0.15 <sup>dH</sup>	9.00±1.00 <sup>bG</sup>	7.00±0.00 <sup>aC</sup>	10.67±0.5 <sup>cD</sup>	-	15.33±0.15 <sup>dC</sup>
<i>C. jejuni</i>	30	5.33 ±0.57 <sup>bC</sup>	4.67±0.57 <sup>aB</sup>	-	-	-	8.67±0.00 <sup>cB</sup>
	60	8.00±1.00 <sup>aF</sup>	10.00±0.00 <sup>bH</sup>	-	-	-	12.33±0.00 <sup>cE</sup>
<i>A. niger</i>	30	4.00±0.00 <sup>aB</sup>	-	-	-	-	10.00±0.00 <sup>bC</sup>
	60	8.00±0.00 <sup>aF</sup>	-	-	-	-	12.00±0.00 <sup>bE</sup>
<i>A. flavius</i>	30	12.33±0.57 <sup>cG</sup>	13.67±0.57 <sup>dI</sup>	4.33±0.57 <sup>aB</sup>	-	-	11.00±1.00 <sup>bD</sup>
	60	17.00±1.00 <sup>dI</sup>	15.67±0.15 <sup>cJ</sup>	6.67±1.15 <sup>aC</sup>	-	-	14.00±0.57 <sup>bF</sup>
<i>C. albicus</i>	30	4.33±0.57 <sup>aB</sup>	5.00±0.00 <sup>bC</sup>	-	-	-	10.00±0.00 <sup>cC</sup>
	60	7.33±1.15 <sup>aE</sup>	8.00±0.00 <sup>bF</sup>	-	-	-	12.00±0.00 <sup>dE</sup>
<i>S. boulardi</i>	wq30	2.67±0.57 <sup>aA</sup>	-	-	-	-	8.00±0.00 <sup>bA</sup>
	60	6.33±0.54 <sup>aD</sup>	-	-	-	-	12.33±0.57 <sup>bE</sup>

Key: ErE = ethanol root extract, MIE = methanol root extract, HIE= Hexane root extract, WIE= Water root extract chloramphenicol/ clotrimazole = positive control, DMSO = negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row (lower case) and values with different superscripts on the same column(upper case) are significantly different ( $p < 0.05$ , - = no inhibition zone)

### 3.3 Minimum Inhibitory Concentration (MIC) of the Crude Extracts

The minimum inhibitory concentration (MIC) assay was employed to evaluate the effectiveness of the extracts that showed significant antimicrobial activities in the previous tests. MIC was determined for extracts that showed significant growth inhibition zone at 30 mg/ml. The test was performed using the Agar dilution method. In agar dilution, the extract solution at 30 mg/ml was serially diluted to get 28 mg/ml, 26 mg/ml, 24 mg/ml and 22 mg/ml concentrations. Then, each of the eight test pathogens were added to the dilute ethanol, methanol, hexane and water extracts of concentrations ranging from 22 mg/ml up to 28 mg/ml. The sterilized media were allowed to cool at 50°C and 18 ml of molten agar was added to test tubes, which contained 2 ml of different concentrations of the crude extracts, were mixed and poured into pre-labeled sterile Petri-dishes

on a level surface. The concentrations of the extracts used in this test ranged from 22 – 28 mg/ml. The suspensions of the respective pathogens densities were adjusted to 0.5 McFarland turbidity units ( $1.5 \times 10^8$  CFU/ml). Three loopful of suspension were transferred into each plate using a standard inoculating loop. The plates were then incubated at 37°C for 24 h. The lowest concentration, which inhibited the growth of the respective organisms, was taken as MIC. The plates were allowed to dry at room temperature and the results are shown in table from 13-15.

#### 3.3.1 The minimum inhibitory concentration (MIC) of the crude extracts of leaves, stem barks and roots of *R. chalepensi*

The MIC obtained from *R. chalepensi* using Agar dilution method for different part extracts are shown in Table 13 as follows. The MIC value of the different extracts of *R. chalepensi* indicated

that the highest activity was recorded against *E. coli*, *A. flavus* and *P. auroginosa* (22 mg/ml) in ethanol extracts of the leaf and stem bark (Table 13). The lowest activities were obtained against all other solvent extracts against some pathogens (28 ml/gm).

Generally, the MIC values were recorded for the extracts, which confirm that, the high activity of the extract at low concentrations. Extracts with lower MIC scores are very effective antimicrobial agents. MIC is important because populations of bacteria and fungi, exposed to an insufficient concentration of the extract can develop resistance to antimicrobial agents. The high activity of antimicrobial agents at low concentrations is very essential for

chemotherapeutic purposes because of their low toxicity to patients administered with such agents [27].

### 3.3.2 The minimum inhibitory concentration (MIC) of the crude extracts of leaves, stem barks and roots of *J. schimperiana*

The data revealed that the MIC values of the different extracts of *J. schimperiana* showed that the highest activity was recorded against *E. coli*, *P. auroginosa*, *C. jejuni* and *C. albicans* (22 mg/ml) in ethanol extract of the stem bark and leaf (Table 14). The lowest activity was obtained against all test organisms (28 mg/ml) in some extracts of all parts (root, leaf and stem bark) of *J. schimperiana*.

**Table 13. The minimum inhibitory concentration (MIC) of the crude extracts of leaves, stem barks and roots of *R. chalepensis* against the selected bacterial test organisms in mg/ml**

Test organism	Plant parts	MIC of the four crude extracts (mg/ml)			
		Ethanol	Methanol	Hexane	Water
<i>E. coli</i>	Leaf	22 <sup>aA</sup>	24 <sup>bA</sup>	-	-
	Stem bark	26 <sup>aC</sup>	26 <sup>aB</sup>	26 <sup>aB</sup>	28 <sup>bB</sup>
	Root	-	-	-	-
<i>P. auroginosa</i>	Leaf	-	-	-	28 <sup>aB</sup>
	Stem bark	22 <sup>aA</sup>	24 <sup>bA</sup>	24 <sup>bA</sup>	28 <sup>cB</sup>
	Root	28 <sup>aD</sup>	28 <sup>aC</sup>	-	-
<i>S. aureus</i>	Leaf	26 <sup>aC</sup>	26 <sup>aB</sup>	26 <sup>aB</sup>	28 <sup>bB</sup>
	Stem bark	24 <sup>aB</sup>	26 <sup>bB</sup>	26 <sup>bB</sup>	28 <sup>cB</sup>
	Root	28 <sup>aD</sup>	-	-	-
<i>C. jejuni</i>	Leaf	22 <sup>aA</sup>	24 <sup>bA</sup>	24 <sup>bA</sup>	-
	Stem bark	22 <sup>aA</sup>	24 <sup>bA</sup>	24 <sup>bA</sup>	28 <sup>cB</sup>
	Root	28 <sup>aD</sup>	28 <sup>dC</sup>	-	-
<i>A. niger</i>	Leaf	-	26 <sup>aB</sup>	26 <sup>aB</sup>	-
	Stem bark	24 <sup>aB</sup>	-	-	28 <sup>bB</sup>
	Root	28 <sup>aD</sup>	-	-	-
<i>A. flavus</i>	Leaf	26 <sup>aC</sup>	26 <sup>aB</sup>	26 <sup>aB</sup>	28 <sup>bB</sup>
	Stem bark	24 <sup>aB</sup>	-	-	28 <sup>bB</sup>
	Root	28 <sup>aD</sup>	28 <sup>aC</sup>	-	-
<i>C. albicans</i>	Leaf	22 <sup>aA</sup>	-	-	28 <sup>bB</sup>
	Stem bark	22 <sup>aA</sup>	24 <sup>bA</sup>	24 <sup>bA</sup>	28 <sup>cB</sup>
	Root	28 <sup>bD</sup>	28 <sup>bC</sup>	28 <sup>bC</sup>	26 <sup>aA</sup>
<i>S. boulardi</i>	Leaf	-	-	26 <sup>aB</sup>	28 <sup>bB</sup>
	Stem bark	-	-	-	28 <sup>Ab</sup>
	Root	28 <sup>aD</sup>	28 <sup>aC</sup>	-	-

Values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different at  $p < 0.05$ , - = no growth



**Table 14. The minimum inhibitory concentration (MIC) of the crude extracts of leaves, stem barks and roots of *J. schimperiana* against the selected bacterial test organisms in mg/m**

Test organism	Plant parts	MIC of the four crude extracts (mg/ml)			
		Ethanol	Methanol	Hexane	Water
<i>E.coli</i>	Leaf	22 <sup>aA</sup>	26 <sup>bB</sup>	-	28 <sup>cB</sup>
	Stem bark	22 <sup>aA</sup>	26 <sup>bB</sup>	26 <sup>bB</sup>	28 <sup>cB</sup>
	Root	26 <sup>aC</sup>	-	-	28 <sup>bB</sup>
<i>P.auroginosa</i>	Leaf	24 <sup>aB</sup>	24 <sup>aA</sup>	-	-
	Stem bark	22 <sup>aA</sup>	24 <sup>bA</sup>	24 <sup>bA</sup>	-
	Root	28 <sup>aD</sup>	28 <sup>aC</sup>	-	-
<i>S. aureus</i>	Leaf	24 <sup>aB</sup>	26 <sup>bB</sup>	26 <sup>bB</sup>	28 <sup>cB</sup>
	Stem bark	24 <sup>aB</sup>	26 <sup>bB</sup>	26 <sup>bB</sup>	28 <sup>cB</sup>
	Root	-	-	-	-
<i>C. jejuni</i>	Leaf	26 <sup>bC</sup>	24 <sup>aA</sup>	-	-
	Stem bark	26 <sup>bC</sup>	24 <sup>aA</sup>	24 <sup>aA</sup>	-
	Root	26 <sup>bC</sup>	-	-	-
<i>C.niger</i>	Leaf	24 <sup>aB</sup>	26 <sup>bB</sup>	-	28 <sup>cB</sup>
	Stem bark	24 <sup>aB</sup>	26 <sup>bB</sup>	26 <sup>bB</sup>	28 <sup>cB</sup>
	Root	28 <sup>aD</sup>	28 <sup>aC</sup>	-	-
<i>A.flavius</i>	Leaf	24 <sup>aB</sup>	26 <sup>bB</sup>	-	-
	Stem bark	22 <sup>aA</sup>	26 <sup>bB</sup>	26 <sup>bB</sup>	-
	Root	26 <sup>aC</sup>	26 <sup>aB</sup>	-	-
<i>C.albicus</i>	Leaf	24 <sup>aB</sup>	24 <sup>aA</sup>	-	-
	Stem bark	24 <sup>aB</sup>	24 <sup>aA</sup>	-	-
	Root	28 <sup>aD</sup>	-	-	-
<i>S.boulardi</i>	Leaf	24 <sup>aB</sup>	26 <sup>bB</sup>	26 <sup>bB</sup>	28 <sup>cB</sup>
	Stem bark	26 <sup>aC</sup>	26 <sup>aB</sup>	-	-
	Root	26 <sup>aC</sup>	28 <sup>bC</sup>	26 <sup>aB</sup>	26 <sup>aA</sup>

Values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different at  $p < 0.05$ , - = no growth

### 3.3.3 The minimum inhibitory concentration (MIC) of the crude extracts of leaves, stem barks and roots of *R. prinioides*

The data revealed that the MIC values of the different extracts of *R. prinioides* showed that the highest activity was recorded against *E. coli*, *P. auroginosa* and *A. niger* (22 mg/ml) in ethanol and methanol extract of the stem bark and leaf (Table 15). The lowest activity was obtained against all test organisms (28 mg/ml) in some extracts of all parts (root, leaf and stem bark) of *R. prinioides*.

### 3.4 Phytochemical Characteristics of the Leaf Stem Bark and Root Extracts of Selected Plants

Phytochemical screening was done in order to detect the presence of plant constituents such as Tannins, Phenolic compounds, Resins, Amino acids, Flavonoids, Saponins, Reducing sugar, Glycosides, Steroids, Triterpenoids, Anthocyanidins, Sterol and essential Oil using the methods described by [28]. The result, phytochemical screening of leaf, stem and root of *R. chalepensis*, *J. schimperiana*, and *R. prinioides* are shown from Tables 16-18.

**Table 15. The minimum inhibitory concentration (MIC) of the crude extracts of leaves, stem barks and roots of *R. prinioides* against the selected bacterial test organisms in mg/ml**

Test organism	Plant parts	MIC of the four crude extracts (mg/ml)			
		Ethanol	Methanol	Hexane	Water
<i>E.coli</i>	Leaf	22 <sup>2A</sup>	22 <sup>2A</sup>	24 <sup>2B</sup>	24 <sup>2A</sup>
	Stem bark	24 <sup>2B</sup>	24 <sup>2B</sup>	-	-
	Root	28 <sup>2D</sup>	26 <sup>2C</sup>	-	28 <sup>2C</sup>
<i>P.auroginosa</i>	Leaf	-	-	-	26 <sup>2B</sup>
	Stem bark	22 <sup>2A</sup>	26 <sup>2C</sup>	-	-
	Root	28 <sup>2D</sup>	28 <sup>2D</sup>	28 <sup>2D</sup>	-
<i>S. aureus</i>	Leaf	26 <sup>2C</sup>	28 <sup>2D</sup>	26 <sup>2C</sup>	26 <sup>2B</sup>
	Stem bark	26 <sup>2C</sup>	26 <sup>2C</sup>	26 <sup>2C</sup>	26 <sup>2B</sup>
	Root	28 <sup>2D</sup>	24 <sup>2B</sup>	28 <sup>2D</sup>	28 <sup>2C</sup>
<i>C. jejuni</i>	Leaf	26 <sup>2A</sup>	26 <sup>2C</sup>	22 <sup>2A</sup>	26 <sup>2B</sup>
	Stem bark	26 <sup>2C</sup>	24 <sup>2B</sup>	-	-
	Root	28 <sup>2D</sup>	24 <sup>2B</sup>	-	-
<i>A.niger</i>	Leaf	22 <sup>2A</sup>	26 <sup>2C</sup>	-	-
	Stem bark	-	-	-	26 <sup>2B</sup>
	Root	28 <sup>2D</sup>	-	-	-
<i>A.flavius</i>	Leaf	-	-	-	-
	Stem bark	24 <sup>2B</sup>	24 <sup>2B</sup>	26 <sup>2C</sup>	-
	Root	28 <sup>2D</sup>	28 <sup>2D</sup>	28 <sup>2D</sup>	-
<i>C.albicus</i>	Leaf	22 <sup>2A</sup>	-	-	28 <sup>2C</sup>
	Stem bark	-	-	-	-
	Root	28 <sup>2D</sup>	28 <sup>2D</sup>	-	-
<i>S.boulardi</i>	Leaf	26 <sup>2C</sup>	26 <sup>2C</sup>	26 <sup>2C</sup>	-
	Stem bark	24 <sup>2B</sup>	26 <sup>2C</sup>	26 <sup>2C</sup>	28 <sup>2C</sup>
	Root	28 <sup>2D</sup>	-	-	-

Values with different superscripts on the same row (lower case) and values with different superscripts on the same column (upper case) are significantly different at  $p < 0.05$ , - = no growth

**Table 16. Phytochemical characteristics of the leaf stem bark and root extracts of *R. chalepensis***

Phytochemicals	Ethanol extraction			Methanol extraction			Hexane extraction			Water extraction		
	Leaf	Stem	Root	Leaf	stem	Root	Leaf	stem	Root	Leaf	stem	Root
Alkaloids	+	-	-	++	+	-	+	-	-	+	-	+
Tannins	-	+	+	+	-	+	-	-	-	-	+	-
Volatile Oil	-	-	-	+	+	-	+	++	-	+	-	-
Resins	++	+	+	++	-	-	-	+	-	+	-	-
Phenolics	+	++	++	+	+	+	+	+	+	+	+	-
Sterol	+	+	+	-	-	-	-	+	-	+	-	-
Anthocyanidi	+	+	+	-	-	-	-	+	-	-	+	-
Triterpenoids	+	+	+	+	+	-	+	+	+	+	-	-
Steroids	+	+	+	+	+	-	+	+	-	-	-	-
Glycosides	+	-	-	-	-	-	-	+	-	-	-	-
Reducing sugar	+	+	+	+	+	+	+	-	+	+	+	-
Saponins	-	+	+	++	++	+	++	+	+	+	-	-
Flavonoids	+	+	+	++	+	-	+	-	+	+	+	-
Amino acids	-	-	-	-	+	-	+	-	+	+	-	-

Keys: ++ = abundantly present, += present in low concentration, - = absent (not detected)

Table 16 shows Phytochemical compounds found in the leaf stem bark and root different solvent extracts of *R. chalepensis*. Many researchers were reported that tannins bind the cell wall of bacteria, preventing growth and protease activity and can be toxic to filamentous fungi, yeasts and ruminal bacteria [50]. Cardiac glycosides, which have been reported to have antimicrobial properties were found in all the extracts [51]. Saponins were detected in all the extracts. They are effective in the treatment of syphilis and certain skin diseases [50]. Flavonoids are known for their anti-allergic effect as well as a wide variety of activity against Gram-positive and Gram-negative bacteria. The properties of the phytochemical ingredients (Table 16) could have attributed to the results of the antibacterial activities observed in the present study.

As indicated on the above table (Table 17) the preliminary phytochemical screening revealed the presence of these compounds in the extracts of *J. schimperiana* by using different extraction solvents. Ethanol and Methanol Leaf and stem

bark showed the highest compound extracts compared with hexane and water root extract. One of the factor that affect microbial susceptibility is the concentration of the active component; the more the concentration, the higher the activity of the chemical substance [52]. It is reported that, some compounds as an indication of the potential medicinal value of the plants in which they appear. Flavonoids constituent exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, analgesic, anti-allergic, cystostatic and antioxidant properties, anticancer activities reported that tannins are known to react with protein to provide the typical tannins effect which is important for the treatment of ulcers have been found to form irreversible complex with proline-rich protein resulting in the inhibition of cell protein synthesis. Herbs that have tannins as their component are stringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery [53]. This observation therefore supports the use of *J. schimperiana* in herbal cure remedies.

**Table 17. Phytochemical characteristics of the leaf stem bark and root extracts of *J. schimperiana***

Phytochemicals	Ethanol extraction			Methanol extraction			Hexane extraction			Water extraction		
	Leaf	Stem	Root	Leaf	stem	Root	Leaf	stem	Root	Leaf	stem	Root
Alkaloids	+	+	-	++	++	-	++	+	+	-	-	-
Tannins	+	+	+	+	+	-	+	++	-	-	-	+
Volatile Oil	++	++	+	++	+	+	+	-	-	-	+	-
Resins	++	+	-	-	+	+	-	-	+	+	-	+
Phenolics	+	+	+	-	+	-	-	+	+	+	+	+
Sterol	-	+	+	-	-	+	+	-	+	-	-	-
Anthocyanidi	+	-	+	+	-	+	+	+	-	+	+	-
Triterpenoids	+	+	+	-	+	+	-	-	+	-	-	-
Steroids	-	+	-	-	-	-	+	+	-	-	+	-
Glycosides	+	+	+	-	-	+	-	-	-	+	+	-
Reducing sugar	+	+	-	-	-	-	-	+	+	-	+	-
Saponins	-	+	+	-	+	-	+	+	+	-	+	+
Flavonoids	+	+	-	-	+	+	-	+	+	+	-	-
Amino acids	+	+	+	+	-	+	-	+	-	+	-	+

Keys: ++ = abundantly present, += present in low concentration, - = absent (not detected)

**Table 18. Phytochemical characteristics of the leaf stem bark and root extracts of *R. prinioides***

Phytochemicals	Ethanol extraction			Methanol extraction			Hexane extraction			Water extraction		
	Leave	Stem	Root	Leave	stem	Root	Leave	stem	Root	Leave	stem	Root
Alkaloids	++	+	+	++	+	-	+	-	-	+	-	-
Tannins	+	+	+	+	+	+	-	-	-	-	+	-
Volatile Oil	+	-	-	+	+	-	+	+	-	+	-	-
Resins	++	++	++	++	++	-	-	+	-	++	-	-
Phenolics	++	++	++	+	+	+	+	+	+	+	+	+
Sterol	-	-	-	-	+	-	-	-	-	+	-	-
Anthocyanidi	+	+	-	-	+	-	-	+	-	-	+	+
Triterpenoids	++	++	+	+	++	-	+	+	+	+	-	-
Steroids	-	-	-	+	-	-	+	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Reducing sugar	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	++	+	++	+	+	++	+	+	+	-	-
Flavonoids	++	++	-	++	+	-	+	+	+	+	+	-
Amino acids	-	+	+	-	+	-	+	-	+	+	-	-

Keys: ++ = abundantly present, += present in low concentration, - = absent (not detected)

#### 4. CONCLUSION

The findings of this study revealed that (*R. prinioides* (gesho), *J. schimperiana* (sensel) and *R. chalepensis* (Tena adam) exhibited significant antimicrobial effect by the crude extracts against the four bacterial strains (*E. coli*, *S. aureus*, *C. jejuni* and *P. auruginosa*) and four fungi strains (*Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Saccharomyces boulardii*) which is an indication for the presence of antimicrobial agents in it. The antimicrobial effect of crude extract of each solvent was concentration dependent against the tested pathogens. The four solvents employed for the extraction process i.e., water, and organic solvents showed different extraction efficiency, which might be due to their difference in polarity. This work indicated that ethanol, hexane and methanol are better solvents in compared to water for the extraction of the active ingredients of these plants.

Based on the results, it could say that the plant extracts contain chemical constituents of pharmacological significance. The presence of these chemical constituents is an indication that the plant, if properly screened using additional solvents, could yield drugs of pharmaceutical significance. Preliminary phytochemical analysis revealed that the presence of alkaloids, flavonoids, Triterpenoids, saponins, volatile oil,

phenolics, resin were present in high amounts in some of the plants (Tables 16-18). It is not surprising that there are differences in the antimicrobial effects of plant groups, due to phytochemical properties and differences among species. The results of the study also support the folklore claim along with the development of new antimicrobial drugs from both the plant parts.

#### DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

#### DISCLAMIER

The authors take full responsibility for any error.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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