Research Article *http://dx.doi.org/10.4314/mejs.v16i1.10*

Antimicrobial Activities of Stem Bark and Fruit Extracts of *Rhamnus prinoides*L'Herit against Selected Multiple Drug Resistance Human Bacterial and Fungal Pathogens

Hayelom Debalkew and Berihu Tekluu*

Department of Chemistry, College of Natural and Computational Sciences, Aksum University, P. O. Box 1010, Axum, Ethiopia (*tekluberihu@gmail.com, hayelom21@gmail.com).

ABSTRACT

Rhamnus prinoides has an impressive range of medicinal uses with high nutritional value. It is considered a natural product and environmentally friendly material. The objective of the study was to evaluate the *in vitro* antibacterial and antifungal activities of methanolic and aqueous stem bark and fruit extracts against human bacterial and fungal pathogens. The result showed that the qualitative phytochemical analysis of the stem bark of *R. Prinoides* revealed the presence of a maximum number of secondary metabolites. UV and FTIR analysis showed the presence of different organic constituents with functional groups of hydroxyl group, C=O, and C-O. The presence of these groups is responsible for the broad spectrum of antimicrobial activities. Antibacterial activity of methanol and aqueous extracts of R. *prinoides* (stem bark) against *B. subtilis, S. aureus, S. pneumoniae, E. coli, S. flexneri,* and *S. typhi,* revealed inhibition zone diameter ranging from 22.17-24.67 mm and 14-28 mm, respectively at 200 µg/ml concentration, compared to a positive control Cipro (25µg) ranged from 22.67-27.80 mm. Similarly, it was reported that the methanol and aqueous extracts of R. *prinoides* (fruit)showed at the range of 20-22 mm and 10-16, respectively at 200 µg/ml concentration, compared to a positive control Cipro (25µg) ranged from 22.17-24.17 mm. The antifungal activity of methanol and aqueous extracts of R. *prinoides* (stem bark) against *A. flavus* and *C. Albicans* revealed inhibition zone ranging from 14-19 mm and 13-15 mm, respectively at 200 µg/ml concentration, compared to a positive control Nystatin ranged from 18.3-21 mm. antifungal activity of methanol and aqueous extracts of R. *prinoides* (stem bark) against *A. flavus* and *C. Albicans,* revealed inhibition zone ranging from 14-19 mm and 13-15 mm, respectively at 200 µg/ml concentration, compared to a positive control Nystatin ranged from 18.3-21 mm. Therefore, ethanol extract of *R. prinoides* have the most active antibacterial components than antifungal.

Keywords: *Rhamnus prinoides,* Phytochemical analysis, Zone of inhibition, antimicrobial agents, Minimum inhibitory concentration.

1. INTRODUCTION

Plants have been known since ancient times and therefore scientists have found them to be a better choice in such for bioactive compounds (Khan et al., 2011). Microbial resistance to the currently used antibiotics has greatly increased in the last four decades despite efforts by pharmaceutical industries to produce new antibiotics. Many researchers have been working on the development of

Submitted on: 14th August 2023 ***Revised and Accepted on: 27th February 2024***

novel therapeutic antibiotics to combat multidrug-resistant (MDR) bacterial and fungal clinical pathogens, particularly by using medicinal plants (Tortorella et al., 2018). According Araya et al., (2015), about 80% of the individuals from developing countries use known traditionally plants as medicine. In Ethiopia, about 80% of the human population has been used medicinal plants for the treatment of different ailments and complementary and alternative traditional medicine is an integral part of the culture, belief structure and lifestyle of Ethiopian people (Bekalo et al., 2009; Yirga, 2010).

Antibiotic substances either kill or stop the development of other bacteria. Changes are currently being made to reflect the reality that bacteria are not the only source of antibiotics. By either eradicating the harmful microbe directly, stopping it from multiplying, or weakening it so that the host's immune system can more successfully combat and eradicate it, medications help the host defend against bacteria, viruses, and other microorganisms (Onaran and Saglam, 2016). The rich content of antifungal substances in plants have been used bio pesticide since up to the beginning of human civilization. Antifungal substances which are obtained from plants have no side effect against environment thus, giving a significant advantage. Antifungal compositions having enhanced antifungal activity comprising an antifungal agent and a food additive are described (Chen et al., 2018).

B. subtilis is a gram-positive, catalase-positive bacteria that is typically found in soil and dust as well as in the digestive tracts of people and ruminants. It is sometimes referred to as the hay bacillus or grass bacillus (Dessalegn, 2014). *S. aureus* is a facultatively anaerobic, gram-positive and is the most common cause of staph infections. It is a common inhabitant of part of the human nasal and the upper respiratory tract. *S. pneumonia* is the most common cause of bacterial meningitis in adults, meningitis in which there is leakage of spinal fluid. Many people carry *S. pneumoniae* bacteria in their nose and throat (Birgitta et al., 2013) *E. coli* is a gram-negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. *E. coli* is a common organism involved in diarrhea and urinary tract infection of bacterial origin (Singleton, 1999). *S. flexneri* is a facultative anaerobic, non-motile, gram-negative bacterium. It is a lactose-fermenting rod-shaped bacteria that causes dysentery (Yang and Yang, 2005). *Salmonella* infection (*salmonellosis*) is a common bacterial disease that affects the intestinal tract. The second most frequent species seen in human infections is *A. flavus*. Naturally, it is found typically in dead plants, animals, and soil. The most prevalent species of the genus *Candida*, *C.*

albicans, continues to cause many opportunistic yeast infections worldwide (Peromingo et al., 2016).

Rhamnus prinoides L'Hérit is a native edible plant of Ethiopia and Eritrea belongs to the family of Rhamnaceae, widely distributed in African countries (Germplasm Resources Information Network, 2010). In some regions of the African countries, *Rhamnus prinoides* (*R. prinoides*) leaves have been traditionally used for the treatment of both infectious and non-infectious diseases such as diarrhea, gonorrhea, ringworm infections, common cold, and malaria. Stomach complications and joint pain respectively (Molla et al., 2016). The traditional use of stem bark and fruits of *R. prinoides* for the treatment of infectious pathogens motivates us to select those parts to be screened against MDR human bacterial and fungal pathogens and inadequate antimicrobial activities were reported on this selected medicinal plant *R. prinoides*, the therapeutic potential of antimicrobial activities is not thoroughly carried out. This study aimed to investigate the antibacterial and antifungal activities of crude ethanol and aqueous extracts of *R. prinoides* medicinal plant, namely, stem bark and fruit.

2. MATERIALS AND METHODS

2.1. Chemicals and apparatus

Con. HCl, con.H_2SO_4, Mayer's reagent, Wagner's reagent, 10 %$FeCl_3$, Ethanol, iodine, 10% Lead acetate, 40% NaOH solution, Potassium iodide, Acetic anhydride, glacial acetic acid, Dimethyl Sulfoxide (DMSO) (Uni-Chem, India), Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB), brain heart infusion agar, blood agar base and XLD medium (Oxoid Ltd, Basingstoke, Hampshire, England), mannitol salt agar, brain heart infusion broth (Becton Dickinson and Cockeysville), violet red bile glucose agar (Research-lab Fine Chem. Industries, India), Potato Dextrose Broth (PDB) (HiMedia Laboratories Pvt. Ltd. India), Potato Dextrose Agar (PDA) (Sisco Research Laboratories Pvt. Ltd. India), nystatin 100 units/disc, ciprofloxacin 25 units/disc and sterile sheep blood. All the chemicals and solvents used were of analytical grade procured from sigma Alderich and were purchasing from Mekelle, Tigray and Addis Ababa, Ethiopia.

2.2. Plant Material and Sample Preparation

The fresh and stem barks and fruits of *R. prinoides* were collected randomly from a 2500-3500m^2 area of southeastern part of Ahferom central zone of Tigray, Ethiopia in November 2019. Afterward, the specimens of those plant materials were kindly authenticated in College of Natural

and Computational Sciences, Aksum and Mekelle Universities, Ethiopia respectively. A voucher specimen was stored in the biology department Laboratory Aksum University with the number HD/001/19.

After collecting the samples peeling (stripping) barks from stems and picking the fruits from the *Rhamnus prinoides*, it was cleaned properly using tap water and then with distilled water. The plant's bark and fruits were immediately dried for about three weeks at 25°C. After it is completely dried, ground using an electric miller (grinder) into powder. The powders were stored in sealed glass containers. 100gm of the powders were used for the subsequent extraction process. The solvent fractionation was conducted by using both Soxhlet and maceration techniques. In a succession of extraction methods from low polarity to high polarity utilizing the soxhlet extraction method. Soxhlet extraction was carried out by sequential extraction of the powdered leaves in solvents of increasing polarity viz. methanol and water as described by a study (Navneet et al., 2012). The extraction procedures continued until the Soxhlet equipment's siphon tube's solvent turns colorless. The solvent was evaporated using rotary vacuum evaporator with a water bath at 40ºC. Finally, the dried marc of methanol fraction was macerated with distilled water and dried in lyophilizer to get the aqueous fraction.

2.3. Phytochemical Analysis

The extracts were subjected to phytochemical screening for the presence of metabolites such as terpenoids, alkaloids, saponins, tannins and flavonoids. Quantitative analysis of major secondary metabolites of *Rhamnus prinoides* was carried out using the standard methods (Roopashree et al., 2008; Trease and Evans, 1996).

2.4. Statistical Analysis

The results were presented as the standard error of the mean ± standard deviation. The statistical difference between the tested extracts were analyzed by one way analysis of variance followed by Turkey 'multiple comparison test Graph pad prism 6.0 software and followed by Dunnett's t-test. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ represents a significant difference between the extracted test group.

2.5. Ultraviolet - visible Spectroscopy

About 0.1g of bark and fruit powder was dissolved in 200 mL of distilled water and from the solution 2ml was taken and diluted in 200 mL distilled water for analysis using Ultraviolet-visible spectrophotometer (Shimadzu UVd-1800 PC, Japan). The extract of bark was scanned in the range

of 200-600 nm and the extract of fruit scanned in the range of 200-400 nm at 1 nm interval against the solvent blank and the peaks were recorded.

2.6. Fourier Transform Infrared (FTIR) Spectroscopy

The samples were analysed using JASCO FT–IR spectrometer. A thin microfilm of the extracted samples using a solvent paraffin oil was prepared, placed in the optical path of the instrument, and scanned over the range of 545–4000 $cm^{-1}$for the fruit extracts and 1000–4000$cm^{-1}$for the bark extracts at 1 cm^{-1} interval. The absorption bands were obtained and recorded as frequency (cm^{-1}) against percent transmittance (%T). The spectrum was interpreted to deduce the functional groups present in the compounds (Sabri et al., 2012).

2.7. In *vitro* Antimicrobial Activity

2.7.1. Antibacterial and Antifungal Activity of the Crude Extract (Zone of Inhibition)

Agar well diffusion was used to assess the antibacterial properties of *R. prinoides* fruit and bark extracts. A 150 mm diameter agar petri plate was filled with approximately 1000 mL of freshly made, sterile Mueller-Hinton Agar (MHA) and approximately 700 mL of Potato Dextrose Agar (PDA) media, which were then allowed to cool at room temperature. A sterile cotton swab was dipped into the modified microbial suspension within 15 min of increasing the turbidity of the inoculum solution to 0.5 McFarland standard, twisted gently, and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. To ensure a uniform dispersion of the inoculum, the MHA/PDA plate was streaked with the swab three times while rotating it by around 600 degrees each time (Buller, 2014). The petri plates were then kept at room temperature for 3 to 5 min. Then, an equal distance hole with a diameter of 6 mm was punched aseptically using sterile cork borer tip. A 100 µl volume of all solvent extracts at concentration of (100, 150 and 200 µg/ml) were introduced to fill the wells using micropipette after dissolved in DMSO. The next step was the addition of positive and negative controls so as the positive antibacterial discs like Ciprofloxacin (25 µg/disc), was placed as a positive control disc for the bacterial species. The standard antibacterial discs were selected based on the vulnerability of bacterial species. A 100 µl of DMSO was used as a negative control. After placement of the plant extracts and negative control into prepared wells and positive control into label area of the agar plate, the plates were placed undisturbed at room temperature for 2 hours. Then, plates were incubated at 37 ºC for 18-24 hours (Kuta et al., 2015).

Similar to this, the fungal strains were exposed to *R. prinoides'* bark and fruit extract at three different concentrations (100, 150, and 200 g/mL). As a positive control, nystatin (100 units/well) was utilized. The plates were then incubated for 48 h at 30 ºC. Both bacterial and fungal strains were negatively controlled using 100µLof DMSO. After incubation, the antibacterial and antifungal activities were assessed by measuring the diameter of the inhibitory zone in millimeters on a millimeter scale (Perez et al., 1990). Triplicates of each experiment were performed. The metrics for the findings were Mean and Standard Deviation.

2.7.2. Determination of Minimum Inhibitory Concentration (MIC) of Extracts

The tested bacterial strains, such as *B. subtilis, S. aureus, S. pneumonia, E. coli, S. flexneri*, and *S. typhi*, as well as two fungal strains, such as *A. flavus and C. albicans*, all demonstrated significant antimicrobial activity against the selected methanol and aqueous extracts of the bark and fruit of *R. prinoides* was calculated using the Clinical and Laboratory Standards Institute's M07-A8 AS technique utilizing Mueller-Hinton broth and Potato Dextrose broth microdilution (Wikler, 2009). A 10% DMSO solution was used to prepare the original stock solution of several plant extracts, which contained 100 g of extract per milliliter. Each stock solution was diluted to obtain final concentrations of 100, 50, 25, 12.5, 6.25, 3.125 and 1.56µg/mL with the DMSO solution. Minimum inhibitory concentrations (MIC) of the methanol stem bark and fruit extracts were determined in a well on a 96-welled plate. Then, 100 µL bacterial and fungal cell suspensions containing 10^8CFU ml^{-1}were inoculated in each well/plate (Balouiri et al., 2016).

3. RESULTS AND DISCUSSION

3.1. Preliminary Phytochemical Screening

Preliminary analysis of phytochemicals from stembarks and fruits of *Rhamnus prinoides indicated* the presence of major secondary metabolite groups. These included flavonoids, Saponins, steroids, tannins, terpenoids, phenolic compounds, alkaloids and cardiac glycosides which were found in both the methanolic and aqueous extracts of stem barks and fruits (Table 1). However, terpenoids were absent in the methanolic and aqueous extracts of fruits.

The results are in agreement with Teklit (2015) where the aqueous and methanolic leaf extract of *R. prinoides* showed the presence of alkaloids, steroids, triterpenes, tannins, flavonoids, and phenols.The mode of action of these secondary metabolites possibly associated to their ability to inactivate several metabolic enzymes, microbial adhesion and cell envelope transport proteins

(Alan and Miller, 1996) and also have reported that the antibacterial activity of tested plant extract might be responsible for their ability to form a complex with membrane proteins, cytosolic proteins, and bacterial cell wall (Divakar et al., 2000; Tazelaar et al., 2009; Tsuchiya et al., 1996) Therefore, the phytochemical screening revealed that methanolic and aqueous stem bark and fruit extracts of *R. prinoides* were good sources of secondary metabolites for the treatment of both infectious and noninfectious agents (Rauha et al., 2000). The antifungal activity of the plant could be attributed to the presence of important phytochemicals as reported by Mandal et al. (2013) in which the extracts of methanol and aqueous were found to contain alkaloids, tannins, saponins, terpenoids, flavonoids, and phenolic compounds.

Table1. Preliminary phytochemical screening of methanolic and aqueous stem barks and fruits extracts of *Rhamnus Prinoides.*

Phytochemicals	***Stem bark***		***Fruits***	
	Methanol	***Aqueous***	***Methanol***	***Aqueous***
Alkaloids	++	+	+	+
Flavonoids	+++	+	++	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Tannins	++	++	++	+
Cardiac glycosides	+	+	+	+
Terpenoids	++	+	-	-
Phenolic compounds	+++	++	++	+

3.2. Ultraviolet - visible Spectroscopy

The secondary metabolites present in the methanolic bark and fruit of the *R. Prinoide* were characterized by UV- spectroscopy. A sharp peak at 290 nm is observed in stem bark (Fig 1a) and other peaks at 256 nm were observed (Fig 1b) for methanolic stem bark and fruit extract of *R. prinoides*. Therefore, this UV- visible spectroscopy result indicated that the presence of possible functional groups of secondary metabolites such as flavonoids, phenolic compounds, and tannins in the crude extract of the stem bark and fruit of *R. Prinoides* responsible for showing significant *in vitro* antibacterial and antifungal activities, and similar with previous reports of (Sahu and Saxena, 2014)

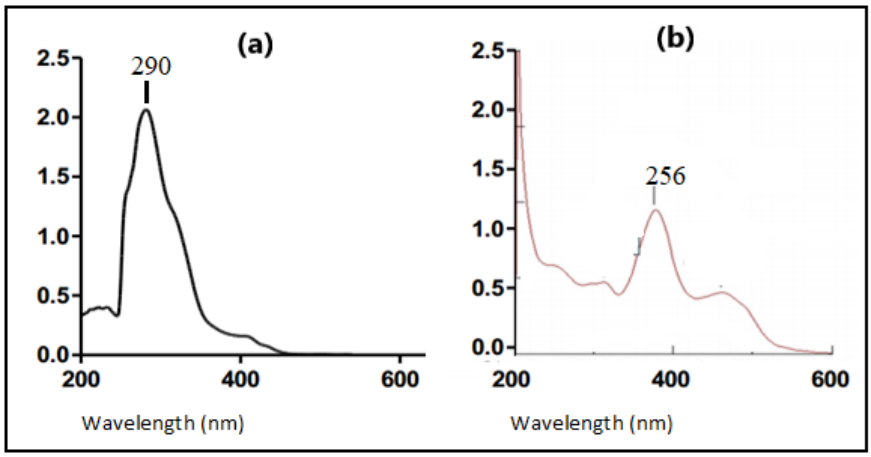

Figure 1. UV-Visible spectrum of methanol extract of the stem bark (a) and fruit (b) of *R. Prinoides.*

3.3. Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectroscopy is used to determine the functional group present in the sample. It is used to confirm the identity of a particular compound and as a tool to determine the crude extracted of *Rhamnus prinoides*. A weak absorption peak at 3361 cm^{-1} shown in the FTIR spectrum can be corresponded to the OH stretching of the hydroxyl group. At 2922cm^{-1} can be assigned to the C-H stretching. This indicates the existence of vinyl carbon attached to the hydrogen. Meanwhile, another important strong band is observed at 1602 cm^{-1}, which can be attributed to the C=O stretching of acyl group. In addition, a further remarkable absorption band was observed at 1336.67 cm^{-1} belonging to the C-O bond. In the end, the two peaks at 1151 cm^{-1} can be related to C-C bond was also observed (Fig 2).

A weak absorption peak at 3354.93 cm^{-1} shown in the FTIR spectrum can be corresponded to the OH stretching of the hydroxyl group. At 2947.23 cm^{-1} can be assigned to the C-H stretching. This indicates the existence of vinyl carbon attached to the hydrogen. Meanwhile, another important strong band is observed at 1656.85 cm^{-1}, which can be attributed to the C=O stretching of acyl group. In addition, a further remarkable absorption band was observed at 1377 cm^{-1} belonging to the C-O bond was also observed (Fig 3). Therefore, this FTIR result showed the presence of secondary metabolic group in the crude extract of the stem bark and fruit of *R. prinoides* and similar with another researchers (Gahlaut and Chhillar, 2013).

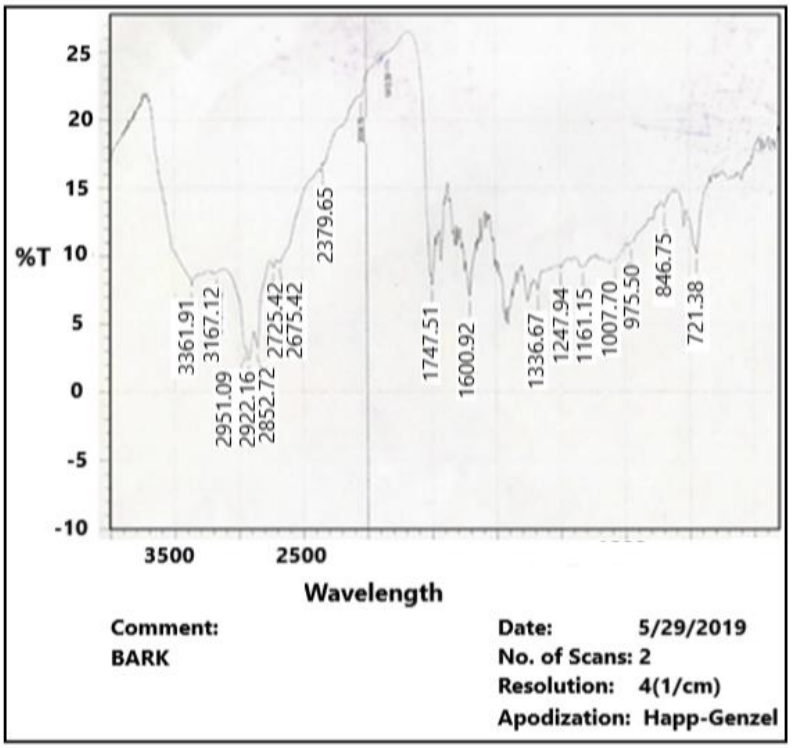

Figure 2. FT-IR functional group peaks analyze of stem bark of *Rhamnus prinoides.*

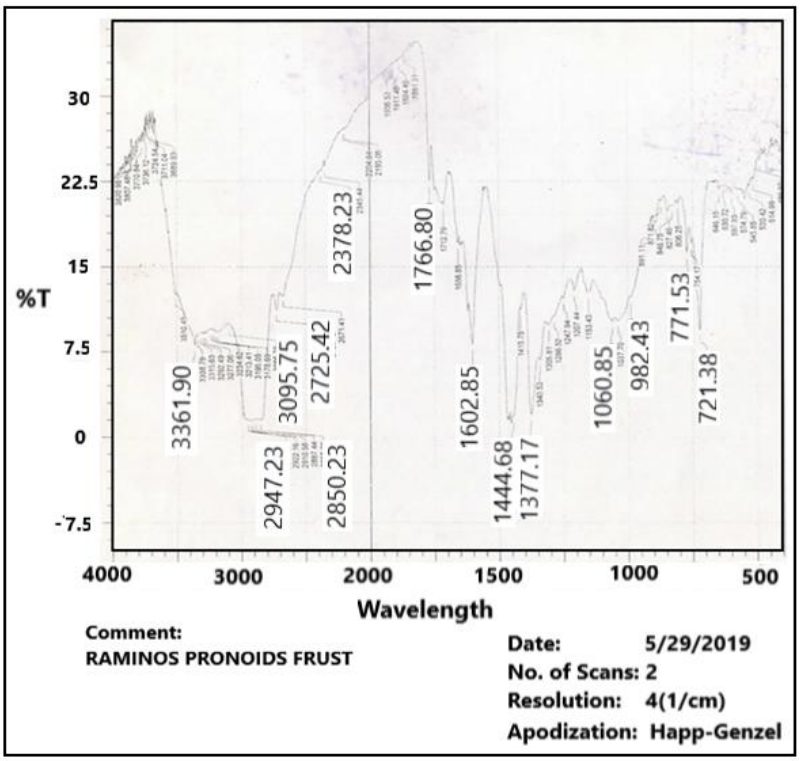

Figure 3. FT-IR functional group peaks analyze of fruit of *Rhamnus prinoides.*

3.4. *In vitro* Antibacterial and Antifungal Activity of Stem Bark and Fruit Extract of *R. Prinoides*

The antimicrobial activity of crude extracts from the plant of *R prinoides* against bacteria (Gram-negative and Gram-positive) examined in the present study was assessed qualitatively by measuring the inhibition zone diameters. Zone of inhibition of different concentration of bark and fruit extracts (100, 150 and 200µg/mL) were significantly different ($p \leq 0.05$) from their respective positive control discs. Antibacterial activity of methanol and aqueous extracts of R. *prinoides* (stem bark) against *B. subtilis*, *S. aureus*, *S. pneumoniae*, *E. coli*, *S. flexneri*, *S. typhi*, (Table 2, see page 202) revealed inhibition zone diameter ranging from 22.17-24.67 mm and 14-28 mm respectively at 200 µg/mL concentration, compared to a positive control Cipro (25µg) ranged from 22.67-27.80 mm. Similarly, it was reported that the methanol and aqueous extracts of R. *prinoides* (fruit) showed at the range of 20-22 mm and 10-16 mm, respectively at 200 µg/mL concentration, compared to a positive control Cipro (25µg) ranged from 22.17-24.17 mm (Fig 4).

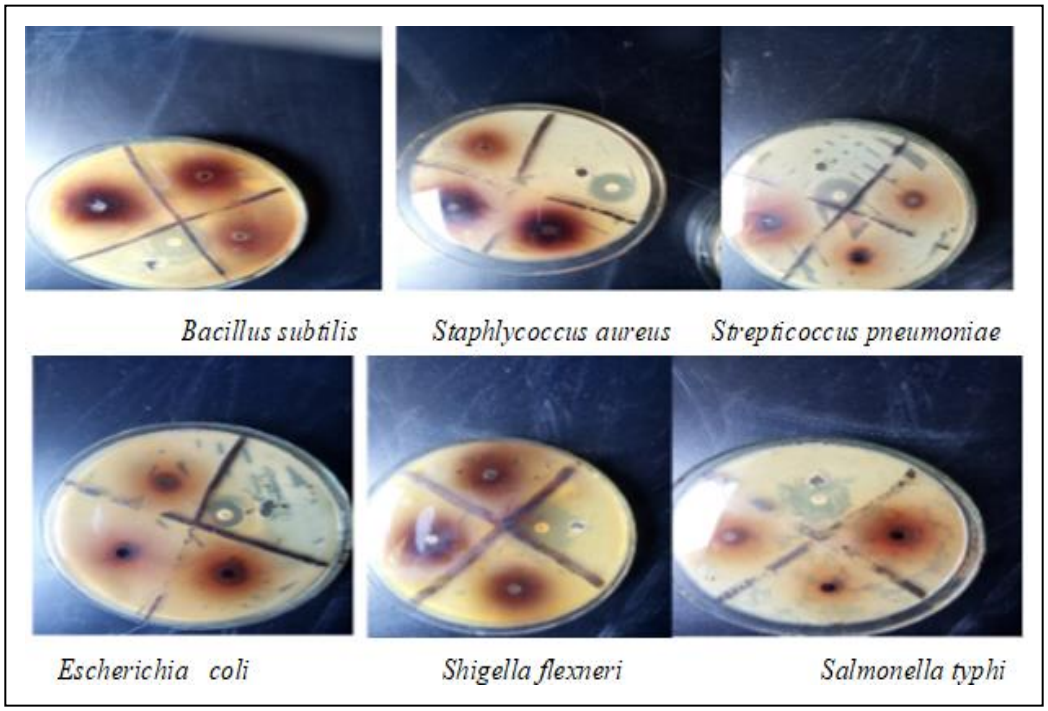

Figure 4. Diagrammatic representation of different Zone of inhibitions caused by the crude plant extracts from the stem bark of *Rhamnus prinoides*.

Stem bark methanol extract showed greater activity in the majority of tested bacterial strains when compared with aqueous extract. Fruit methanol extract showed greater inhibition zone

than fruit aqueous extract. This greater activity of methanol fraction against gram-positive bacterial strains might be attributed to the presence of secondary metabolites like flavonoids and steroids as the mixture of these two bioactive compounds had more activity against gram-positive bacteria (Osuntokun et al., 2017). On the other hand, the aqueous stem bark extract showed better activity against gram-negative (*S. flexneri and S. typhi*) tested bacterial strains compared to the aqueous fruit extract. Therefore, the higher activity of methanol and aqueous extracts in gram-negative bacterial strains might be associated with the presence of phenolic metabolite additional to terpenoids, saponins, tannins, and alkaloids. Harmala et al. (1992) reported that the higher activity of extracts in these groups of organisms was associated with the detection of phenolic compounds since phenol owns the ability to form a complex in gram-negative bacteria outer membrane (Lipopolysaccharide layer) which act as a strong barrier.

According to Getahun and Masresha (2017), in the majority of test microorganisms, the methanol extraction from the bark showed better activity while low activity in the case of fruit extraction where the former might be associated with number of bioactive metabolites, and presence of lower quantity of metabolites in the later. In addition, the higher activity of bark extraction was due to the polar nature of the plant bioactive constituents with antimicrobial activity found to be the best solvent for the extract of polar biological active compounds (Chawawisit et al., 2015). The results obtained in this study match well with Ramazani et al. (2013), who stated that bioactive compounds obtained from bark showed better activity against gram-positive bacteria as opposed to gram-negative bacteria and yeast.

The antifungal activity of methanol and aqueous extracts of R. *prinoides* (stem bark) against *A. flavus* and *C. Albicans* (Table 3, see page 203) revealed that the inhibition zone diameter is ranging from 14 to19 mm, and 13 to 15 mm respectively at 200 µg/mL concentration, compared to a positive control Nystatin ranged from 18.3 to 21 mm. Bark extract showed significant zone inhibition against the tested fungal strains such as *A. flavus* and *C. Albicans* displayed significant ($p < 0.05$). The maximum antifungal activity of the fruit extract could be credited to the presence of flavonoids, tannins, and phenols (Singh et al., 2018). Higher activity of methanol extraction in fungal species might be associated with the presence of secondary metabolites like terpenoids, saponins, tannins, alkaloids, and phenols (Ige and Mebude, 2015). Besides, this showed that the antifungal activities against both tested fungal species are indicative of the presence of broad-spectrum bioactive metabolites.

3.5. Determination of MIC of the Plant Extract

The antimicrobial activity of crude extracts from the plant of *R prinoides* against bacteria (Gram-negative and Gram-positive) examined in the present study was assessed quantitatively by determining minimal inhibitory concentrations. The MIC value of methanol and aqueous extracts from the barks and fruits of *Rhamnus prinoides* were determined for extracts which shows zone of inhibition in agar well diffusion test greater than or equal to 7 mm in diameter for all test bacterial species. Some antimicrobial activities of the plant which are diverse between the aqueous and methanol extracts on gram negative bacteria usually require a higher concentration of extract for activity than the gram-positive bacteria. Therefore, the MIC value extracts were in agreement with its initial antibacterial activities in agar well diffusion that is the gram-negative bacteria in the present study showed a higher MIC than the gram-positive bacteria. On the other hand, the methanol extract of the plant was more powerful against gram positive bacteria than that of gram-negative bacteria with the exception of *E. coli*. The maximum MIC (less diluted) obtained was 50 µg/ml against *E. coli* and the minimum MIC (highest dilution) was 1.56 µg/mL against *S. flexneri* (Table 4).

Table 4. MIC in (µg/ml) of methanol and aqueous extracts from the bark and fruit of the *R. Prinoides* against gram positive and gram-negative bacteria.

Tested bacterial species	***Solvents and parts of the plant***			
	Bark		***Fruit***	
	Methanol	***Aqueous***	***Methanol***	***Aqueous***
Bacillus subtilis	12.5±0.00	3.13±0.00	6.25±0.00	1.56±0.00
Staphylococcus aureus	25.00±0.00	1.56±0.00	12.5±0.00	0.78±0.00
Streptococcus pneumoniae	6.25±0.00	12.5±0.00	3.13±0.00	6.25±0.00
Escherichia coli	50±0.00	3.13±0.00	12.50±0.00	1.56±0.00
Shigella flexneri	1.56±0.00	1.56±0.00	12.5±0.00	6.25±0.00
Salmonella typhi	25.00±0.00	6.25±0.00	6.25±0.00	1.56±0.00

Note: Data are expressed as mean ± SD. The experiment was performed in a minimum of three replicates.

The MIC value of bark extraction using methanol and aqueous were larger than the fruit extracted using both solvents, i.e., the bark extracted using methanol is greater than the fruit extracted using the same solvent and the bark extracted using aqueous is greater than the fruit extracted using aqueous. Therefore, zone of inhibition of the bark and fruit extracts against the

prepared bacteria were inversely proportional to their MIC values, i.e., the more susceptible the organisms to the extract in agar well diffusion method, the less is its corresponding MIC values.

In other words, the greater inhibition zone of extract in agar well diffusion method was corresponding with smaller values of extracts in (µg/mL) required to inhibit or kill organisms, this suggests the reproducibility of the experiments. The antibacterial activity of the selected plant extracts and their potency was assessed by the presence or absence of inhibition zone. According to Solomon (2018), the maximum number of secondary metabolites such as tannins and phenolic compounds, terpenoids, flavonoids and saponins were found in methanol stem bark than the fruit methanol extracts. This is attributed to the recorded promising broad spectrum of antibacterial activities which is similar with the findings of the present study.

4. CONCLUSION

In the present study, methanolic extract of stem bark and fruit *R. prinoides* were tested against some pathogenic bacteria and fungi. Antibacterial activity of the extract was quantitatively assessed by the presence or absence of inhibition zone. *R. prinoides* extract showed good activity against various organisms at 200 µg/mL concentration while less activity at 150 µg/mL and 100µg/mL concentrations. Therefore, the plant is a potential source of antibacterial and ant-fungal bioactive secondary metabolites used for the treatment of bacterial infections caused by the vulnerable bacteria and fungi infections. The antibacterial and ant-fungal activities of the plant are linked with the presence of polar or/and intermediately polar bioactive secondary metabolites in the methanol and aqueous extractions including alkaloids, terpenoids, tannins, steroids, Cardiac glycosides, phenolic compounds, saponins and flavonoids that can act either individually or in combination. Among the fungi *A. flavus* showed significant activity at highest concentration 200 µg/ml comparable to positive control (Nystatin).

5. ACKNOWLEDGMENTS

The authors duly acknowledge Aksum University for the financial support as postgraduate student and Department of Chemistry, Aksum University, Ethiopia for providing laboratory facilities.

6. CONFLICT OF INTEREST

The authors agree there are no conflicts to declare.

7. REFERENCE

Alan, L & Miller, N. D. 1996. Antioxidant flavonoids: Structure, function and clinical Usage, *Alternative Medicine Review*,**1**: 103–111.

Araya, S., Abera, B & Giday, M. 2015. Study of plants traditionally used in public and animal health management in Seharti Samre District, Southern Tigray, Ethiopia. *Journal of ethnobiology and ethnomedicine*, **11**: 1-25. https://doi.org/10.1186/ s13002-015-0015-5.

Balouiri, M., Sadiki, M. & Ibnsouda, S. K.2016. Methods for *in vitro* evaluating antimicrobial activity: A review. *J. Pharmaceutical Analysis*. **6**:71-79, doi.org/10.1016/j.jpha.2015.11.005.

Bekalo, T., Demissew, S & Asfaw, Z. 2009. An ethno botanical study of medicinal plants used by local people in the lowland of Konta special district, SNNPRS, Ethiopia. *Journal of Ethnobiology and Ethno. Medicine*, **4**: 107-122,doi:10.1186/1746-4269-5-26.

Birgitta, H. N & Elaine, I. T. 2013. The Pneumococcus: Epidemiology, Microbiology, and Pathogenesis. *Cold Spring Harb Perspect Medicine*, **3(7):** 10215-10219.

Buller, N., Thomas, A & Barton, M. 2014. Antimicrobial Susceptibility Testing. *Australia and New Zealand standard diagnostic procedures*,1-13.

Chawawisit, K., Bhoopong, P., Phupong, W & Lertcanawanichakui, M. 2015. Antimicrobial and cytotoxic activities of bioactive compounds produced by Streptomyces Sp.KB1. *International J. Pharmacology and Pharmaceutical Sciences*, **7(11)**: 338-344.

Chen, C., Long, L., Zhang, F., Chen, Q., Chen, C., Yu, X., Liu, Q., Bao, J & Long, Z., 2018. Antifungal activity, main active components and mechanism of Curcuma longa extract against Fusarium graminearum. *PloS one*, **13(3)**: 697-716, doi.org/10.1371/journal.pone.0194284.

Clinical and Laboratory Standards Institute (2015) Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard. 10th Edition. CLSI Document M07-A10, Clinical and Laboratory Standards Institute, Wayne, PA.

Das, K., Tiwari, R. K. S & Shrivastava, D. K. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of medicinal plants research*, **4(2)**:104-111.

Dessalegn, A. 2014. Antimicrobial Activity of Medicinal Plant Extracts and Their Synergistic Effect on Some Selected Pathogens. *American Journal of Ethno medicine*, **1(1)**: 018-029.

Divakar, M. C., Devi, L. S., Kumar, S & Rao, S. B. 2000. Studies on wound healing property of Polysciasscutellaria leaf saponins. *Indian Journal of Natural Products*, **17**:37–42.

Gahlaut Chhillar, A. K. 2013. Evaluation of antibacterial potential of plant extracts using Resazurin based microtiter dilution assay. *International Journal of Pharmaceutical Sciences*, **5**: 372-376.

Germplasm Resources Information Network (GRIN) 2010. Agricultural Research Service (ARS), United States Department of Agriculture (USDA).

Getahun, Y & Masresha, F. 2017. In vitro evaluation of the antibacterial activity of some medicinal Plant extracts against *Xanthomonascampestris PV. Musacearum*. *Ethiopian Journal of Science and Technology*, **10(1)**: 17-32,doi.org/10.4314/ejst.v10i1.2.

Harmala, P., Vuorela, H., Tornquist, K & Hiltunen, R. 1992. Choice of solvent in the extraction of *Angelica archangelica* roots with reference to calcium blocking activity. *Plant Medicine*. **58**:176-183, doi: 10.1055/s-2006-961424.

Ige, A & Mebude, O. 2015. Antidermatophytic activities of Musa sapientum methanol leaf extract in-vitro. *British Microbiology Research Journal*, **10(1):** 1-7.

Khan, A. V., Ahmed, Q. U., Mir, M. R., Shukla, I & Khan, A. A. 2011. Antibacterial efficacy of the seed extracts of Melia Azedarach against some hospital isolated human pathogenic bacterial strains. *Asian Pacific J. Tropical Biomedicine*, **16**: 452-455, doi.org/10.1016/S2221-1691(11)60099-3.

Kuta, F. A., Tsado, D. J., Garba, S. A & Saidu, A. N., 2015. Antibacterial activity of the leaf and stem bark crude extracts of Khaya senegalensis. *Medicinal and Aromatic Plant Research Journal*, **3(1):** 9-15.

Mandal, S., Patra, A., Samanta, A., Roy, S., Mandal, A., Maha Patra, T. D., Pradhan, S., Das, K & Nandi, D. K. 2013. Analysis of phytochemical profile of *Terminalia Arjuna* bark Extract With ant oxidative and antimicrobial properties. *Asian Pacific Journal of Tropical Biomedicine*,**3(12)**:960-966, doi.org/10.1016/S2221-1691(13)60186-0.

Molla, Y., Nedi, T., Tadesse, G., Alemayehu, H & Shibeshi, W. 2016. Evaluation of the in vitro antibacterial activity of the solvent fractions of the leaves of *Rhamnus prinoides* L'Herit (Rhamnaceae) against pathogenic bacteria. *BMC complementary and alternative medicine*. **16(1)**: 287.

Navneet Kumar, N. K. Ruchi Kant, R.K. Melese Sinaga, M. S. Belay Yimame, B. Y & Tefera Belachew, T. B. 2012. Preliminary phytochemical screening and in vitro antibacterial evaluation of the leaf and root extract of Azadirachta indica plant.

Onaran, A & Sağlam, D. H. 2016. Antifungal Activity of Some Plant Extracts against Different Plant Pathogenic Fungi. *International Journal of Advances in Agricultural and Environmental Engineering*, **3(2)**:284-293, doi.org/10.15242/IJAAEE.ER0616208.

Osuntokun, O. T., Ajayi Ayodele, O., Adeoye, M. I & Odufunwa, A. E. 2017. Assessment of antimicrobial and phytochemical properties of crude leaf and bark extracts of *Ceiba pentandra*on selected clinical isolates found in Nigerian teaching hospital. *Journal of Bacteriology and Mycology*, **4(1)**:17–23.

Perez, C., Paul, M & Bazerque, P. 1990. Antibiotic assay by agar well diffusion method. *Acta Biologyat Medicinal Experiment*, **15**: 113-115.

Peromingo, B., Rodríguez, A., Bernáldez, V., Delgado, J & Rodríguez, M. 2016. Effect of Temperature and Water activity on growth and aflatoxin production by *Aspergillus Flavus* and *Aspergillus Parasiticus* on cured meat model systems. *Meat Science*, **122**: 76-83, doi.org/10.1016/j.meatsci.2016.07.024.

Ramazani, A., Moradi, S., Sorouri, R., Javani, S & Garshasbi, M. 2013**.** Screening for Antibacterial activity of streptomyces species isolated from zanjan province, Iran *International J. Pharmaceutical, Chemical and Biological Sciences*, **3**(**2**): 342-351.

Rauha, J. P., Remes, S., Heinonen, M., Hopia, A., Kkahkonen, M., Kuyala, T., Pihlaya, K., Vuorela, H & Vuorela, P. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International J. Food Microbiology*, **56**: 3-12.

Roopashree, T. S., Dang, R., Rani, S. R. H & Narendra,C. 2008. Antibacterial Activity of Anti Psoriatic Herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *International Journal of Applied Research in Natural Products*,**1**:20-28.

Sabri, F. Z., Belarbi, M., Sabri S & Alsayadi, M. M. S. 2012. Phytochemical Screening and Identification of some compounds from Mallow. *Journal of Natural Products and Plant Resources*, **2**: 512-513.

Sahu, R & Saxena, J. 2014. Ultraviolet-Visible and Fourier Transform Infrared Spectroscopic Studies on Non-Conventional Species of Curcuma. *Indian Journal of advances in Chemical Science*, **2 (4)**: 300-302.

Singh, V., Bhardwaj, A., Wahi, N & Tripathi L.V. 2018. Phytochemical analysis and *in vitro* antimicrobial activities of Terminalia Arjuna leaf, bark, and fruit extracts in different solvents. *Current Trends in Biotechnology and Pharmacy*, **12 (3)**: 286-294.

Singleton, P. 1999. *Bacteria in Biology, Biotechnology and Medicine*. 5th Edition, ISBN-10: 0471988804, Wiley, (444–454), 500p.

Solomon, Y. 2018. In vitro anti-bacterial and immune protective evidence of sidaschimperiana and Verbascum sinaiticum root extracts on selected bacterial pathogens and *E. coli* Induced Peritonitis in albino wistar rats. M.Sc. Thesis, Aksum University, Ethiopia.

Tazelaar, E., Bruinsma, J., Veenhuizen, B & van den Bosch, P. 2009. Driving cycle characterization and generation, for design and control of fuel cell buses. *World Electric Vehicle Journal*, **3**: 1–8.

Teklit, G. A. 2015. Evaluation of Phytochemical, Chemical Composition, Antioxidant and Antimicrobial Screening Parameters of *Rhamnus prinoides* (Gesho) Available in the Market of Mekelle, Tigray, Ethiopia.*Natural Products Chemistry & Research*, **4 (1)**:1-10.

Tortorella, E., Tedesco, P., Esposito, P. F., January, G. G., Fani, R., Jaspars, M & de Pascale, D. 2018. Antibiotics from deep-sea microorganisms: Current discoveries and perspectives. *Marine Drugs*, **16**: 355.

Trease, G.E & Evans, W. C. 1996. *A Textbook of Pharmacognosy*. 14th Edition, London: Bailliere Tindall Ltd.

Tsuchiya, H., Sato, M & Miyazaki, T. 1996. Comparative studyon the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology*, **50 (1)**: 27–34, doi.org/10.1016/0378-8741 (96) 85514-0.

Wikler, M. 2009. *Clinical and Laboratory Standards Institute Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard*. 10th Edition, Wayne, USA, **35**: 9-110.

Yang, F & Yang, J. 2005. Genome dynamics and diversity of Shigella species, the etiologic agents of bacillary dysentery. *Nucleic Acid Research*, **33 (9)**: 6445-6458, doi.org/10.1093/nar/gki954.

Yirga, G. 2010. Ethnobotanical Study of Medicinal plants in and around Alamata, Southern Tigray, Northern Ethiopia, and Ethiopia. *Journal of Biological Sciences.***2**: 338.

Table 2. Zone of inhibitions (in mm) of different concentrations of methanolic and aqueous extracts against selected bacterial strains.

Source of crude extract		***Concen-tration (µg)***	***Extract***	***Zone of inhibition (mm) (mean±SD)***					
				B. subtilis	***S. aureus***	***S.pneumoniae***	***E. coli***	***S.flexneri***	***S. typhi***
Rhamnus prinoides	Stem barks	100	Methanol	16.83±0.29^{a1b3d3}	15.67±0.58^{a1c2}	15.00±0.00 ^{c2d3}	16.00±0.00^{a2d2}	16.50±0.58^{a3c2d3}	17.17±0.29^{a1c2d2}
			Aqueous	9.00±0.30^{a3d3}	-----	10.67±0.58^{c3d1}	-----	15.17±0.29^{a3c2b3}	13.10±0.17^{b2c3}
		150	Methanol	23.00±0.00$^{a2b\ b1c3}$	21.67±0.58^{a2c2d3}	19.67±0.58^{a1c2d2}	19.33±0.58^{a1c3d3}	20.67±0.50^{a3c2}	20.00±0.00^{c2b3}
			Aqueous	11.00±0.50^{a1d3}	10.00±0.00	13.17±0.29^{a2c2b3}	9.00±0.40^{a2c3}	17.33 ± 0.58^{c2b3}	14.17±0.29^{a3b33}
		200	Methanol	24.67±0.58^{b2c3}	23.33±0.58^{a1c1d2}	24.00±0.00 ^{a1c2d1}	23.17±0.29^{a3c3}	23.50±0.50^{a3}	22.17±0.29^{a3b2c2}
			Aqueous	14.00± 0.50^{a1c2}	28.00±0.80^{a1b2c2}	17.67±0.20 ^{a1c2d1}	15.00±0.60^{a1c3d1}	20.33± 0.58^{a3b2c3}	19.20±0.35^{a2b1c2}
		Control	Cipro25µg	22.67±0).58	25.00±0.00	24.33±0.58	26.83±0.76	24.00±0.00	27.80±0.35
	Fruits	100	Methanol	10.33±0.58^{a2d3}	12.33±0.58^{b1c2d3}	11.33±0.58^{b3d2}	12.33±0.58^{a3d3}	12.83±0.29^{a3b2}	16,99±0.00^{a3b1c3}
			Aqueous	9.00±0.40^{a1c2}	12.00±0.50^{a1b3c1}	6.67±0.58 a^{1b2c1}	----	3.50±0.50^{b3c2d2}	8.83±0.29^{a1c2b1}
		150	Methanol	16.33±0.58^{a1c2d3}	17.00±0.00^{a1c2d1}	16.00±0.00^{b2c3}	15.17±0.29^{a3c2d3}	16.67±0.58^{a3b2d2}	19.83±0.29^{a3c2d3}
			Aqueous	10.00±0.40^{c2d3}	15.00±0.60^{a1b2c3}	10.17±0.29^{a3b1d3}	9.00±0.30^{a3c3d3}	10.17±0.29^{a3b3}	12.00±0.00^{a3b3d3}
		200	Methanol	20.67±0.58^{b3d3}	20.00±0.00^{a2d3}	20.67±0.58^{a2c3d2}	20.67±0.58^{a2c3}	20.83±0.29^{a3c2}	22.00±0.00^{a2c3d1}
			Aqueous	13.00±0.30^{a2b3d3}	16.00± 0.50^{a1b2}	13.33±0.58 ^{a3d2}	10.00±0.30^{c1d2}	14.00±0.00^{a1b2}	15.80±0.35^{a1b3c2}
		Control	Cipro25µg	22.33±0.58	23.00±0.00	22.67±0.58	24.17±0.29	22.17±0.29	23.17±0.29

Note: Values are expressed as Mean ± S.D (n=3), The comparison of means of different concentration of extracts and control was analyzed using one-way ANOVA followed by Tukey's Post hoc test; a -compared with control value, b to 100 µg/mL, c to 150 µg/mL, d to 200 µg/mL, 1P<0.05, 2 P<0.01, 3P<0.001; positive control (Cipro).

Table 3. Zone of inhibition (in mm) of the antifungal activities of methanol and aqueous extracts of the barks and fruits of *R. prinoides* against tested fungal strains.

Fungi	***Types of solvent***	***Bark***			***Fruit***		
		100µg/ml	***150 µg/ml***	***200 µg/ml***	***100µg/ml***	***150µg/ml***	***200 µg/ml***
Aspergillus flavus	Methanol	11.83 ± 0.29^{a3d2}	15.83 ± 0.29^{a2}	19.00 ± 0.00^{b2c1}	11.90 ± 0.17^{a2d1}	13.00 ± 0.00^{a1}	15.00 ± 0.00^{a1b1c2}
	Aqueous	9.2 ± 0.35^{a1d2}	11.00 ± 0.00^{d1}	14.00 ± 0.00^{a1b2c1}	8.00 ± 0.00^{d2c1}	10.13 ± 0.23^{a1b1}	14.00 ± 0.00^{a2b2}
	Nystatin (100 units)	21.00±0.00			18.30±0.30		
Candida albicans	Methanol	13.87 ± 0.23^{a3d1}	16.00 ± 0.00^{a3}	19.80 ± 0.35^{a3b1c2}	10.00 ± 0.00^{a3c1d1}	13.00 ± 0.00^{a3b1d1}	14.30 ± 0.30^{b3c1}
	Aqueous	8.87 ± 0.23^{a3d3}	13.00 ± 0.00^{a3d1}	16.00 ± 0.00^{b3c1}	7.00 ± 0.00^{a1b2c3}	10.10 ± 0.17^{a1b2c3}	13.00 ± 0.00^{a3b2c1d2}
	Nystatin	19.20±0.30			18.50±0.30		

Note: Values are expressed as Mean ± S.D (n=3), The comparison of means of different concentration of extracts and control was analyzed using one way ANOVA followed by Tukey's Post hoc test; a -compared with control value, b to 100 µg/mL, c to 150 µg/mL, d to 200 µg/mL, 1P<0.05, 2 P<0.01, 3P<0.001; (+) = positive control (Nystatin).