

Antibacterial Activity of Rhamnus prinoides extracts against Staphylococcus aureus and Escherichia coli

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Research article

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Abstract

Background: Infectious diseases caused by bacteria have a large impact on public health. Increasing in resistant microbial infections intensified the search for new, safer, and more efficacious agents to combat serious microbial infections. Plants have played a central part in combating many diseases in human and domestic animal in many local communities, including Africa. *Rhamnus prinoides* is Ethiopian medicinal plants that have been used traditionally for the treatment of diverse infectious diseases. Therefore, this research was carried out to evaluate antibacterial activity *Rhamnus prinoides* extracts against pathogenic bacteria.

Methods: The antimicrobial activity tests were carried out by the agar well diffusion method. Broth dilution and streak plate methods were used to determine MIC and MBC of extracts respectively.

Results: The results revealed that ethanol extracts of fruits showed the highest antibacterial activity against standard strains of *S.aureus* with mean inhibition zone of 26 ± 0.58 , 28.33 ± 1.2 and 33.33 ± 0.9 mm at 25, 50 and 100mg/ml respectively. The lowest mean MIC value (1.04 mg/ml) was recorded with ethanol extract of fruits against standard strains of *S. aureus* and the lowest mean MBC value (2.08 mg/ml) was recorded with ethanol extract of fruits against standard strains and clinical isolates of *S. aureus*.

Conclusion: On the basis of the current findings, *Rhamnus prinoides* could be a good candidate in the search for new antibacterial agents from natural products against bacterial pathogens. Therefore, further studies are needed to study their toxicology and isolate the bio- active components from this plant.

Key words: Antibacterial activity, *Escherichia coli*, MBC, MIC, *Rhamnus prinoides*, *Staphylococcus aureus*

1. Background

Currently, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. *Staphylococcus aureus* and *Escherichia coli* were observed to be the most frequent pathogenic bacteria. Worldwide 50% of medicines are inappropriately prescribed while 50% of the medicines are inappropriately used [40]. Prolonged use of antimicrobial agents led to microbial adaptation, resulting in the development of resistance in microorganisms and the consequent failure of antibiotic therapy, has led to hundreds of thousands of deaths annually [34]. These developments and increasing in resistant microbial infections intensified the search for new, safer, and more efficacious agents to combat serious microbial infections [43].

Plants have been used as medicinal bases since ancient times and even today plant-based pharmacopoeias continue to play an essential role in world health care [44]. In recent times focus on plant research has increased all over the world and there is widespread of belief that the green medicines are healthier and harmless than the synthetic ones [28]. Plants are good sources for new, safe, biodegradable and renewable drugs however the use of plants as therapeutic agents in addition to being

used as food is age long [19]. Medicinal plants are recognized for their capacity to produce a wealth of bioactive compounds and mankind has used many species for centuries to treat a variety of diseases (Kaur *et al.*, 2013). Herbal medicines are prepared from a variety of plant materials-leaves, stems, roots, barks, fruits, seeds, flowers and so on. They usually contain most of the biologically active ingredients and are used primarily for treating mild to chronic ailments.

Plants have played a central part in combating many diseases in human and domestic animal in many local communities, including Africa [10]. Numerous plants containing volatile oils, polyphenols and alkaloids as active constituents are utilized as popular folk medicines, whereas others gained popularity in the form of phytomedicines [5].

In Ethiopia, medicinal plants have been used from time immemorial to treat different human and livestock ailments [45]. People have been using both plant and animal species for medication of different animal and human diseases over centuries when there was no modern health service. The practice has been not stopped with introduction of the modern pharmacotherapy and plant remedies are still the most important and sometimes the only sources of therapeutics for nearly more than 90% livestock population [41, 17].

Familiarity of medicinal plants of Ethiopia and their uses provides vital role to human and domestic animals health care needs throughout the country [7]. The majority of Ethiopian people especially in rural communities still depend on traditional medicinal plants to treat several diseases because their derived products can be exploited with sustainable, comparative and competitive advantage including reduced cost, less dangerous, more effective and readily available [27].

Rhamnus prinoides L'Herit (Rhamnaceae) is one of Ethiopian medicinal plant that has been used traditionally for the treatment of different infectious diseases. It has different ethno medicinal 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 [32, 30]. In Ethiopia, the leaves, fruits or roots of *Rhamnus prinoides* is used to treat tonsillitis [9, 24,15, 16, 21]. In addition, the different parts of the plant have been used in the management of scabies, hepatitis, tinea capitis, 'chiffa' (Eczema), ringworm and dandruff [42, 25, 16,13]. Moreover, the leaf of the plant is used for the management of waterborne and related diseases [38].

However, *Rhamnus prinoides* has such advantages; the use of locally made medicines prepared as infusions in hot water or mixed with food to treat infection and the majority of the evidence on the antimicrobial activity of this plant was anecdotal and lacked scientific validity. Moreover, the alarming side effects of synthetic drugs and increasing the resistance of microorganisms towards the present-day drug leads to failure in the treatment of infectious diseases demand a new antimicrobial drug with selective action against new targets in the microbial cells, without irreversible side effects in the host [43]. Even though the antibacterial activity of the crude extract of *Rhamnus prinoides* leaves had been studied

by previous works in Ethiopia, Gondar [8]; Mekelle [6] and Debre Markos [26]; the extract was also evaluated in this study to assure its antibacterial activity as there was differences in geographical areas of plant collection, bacterial stains, solvent system, method of test and the parts of the plant used for extraction purpose. More over there was no study related to antimicrobial activity of *Rhamnus prinoides* fruits have been published previously. Therefore, this study was aimed to evaluate antimicrobial activity of *Rhamnus prinoides* extracts against pathogenic bacteria.

2. Results

2.1. Antibacterial activity of *Rhamnus prinoides* extracts

The antibacterial activity of water, ethanol and chloroform extracts of *Rhamnus prinoides* were tested against standard strains and clinical isolates of *S. aureus* and *E. coli* by agar well-diffusion method. The results showed that plant extracts were active against all the test bacteria to variable extent in concentration dependent manner. Ethanol extracts of fruits showed the highest antibacterial activity against standard strains of *S.aureus* with mean inhibition zone of 26 ± 0.58 , 28.33 ± 1.2 and 33.33 ± 0.9 mm at 25, 50 and 100mg/ml respectively. The lowest antibacterial activity was seen in ethanol, chloroform and water extracts of leaves against clinical isolates of *E. coli* with inhibition zone of 11 ± 0.58 , 9.67 ± 0.33 and 13.67 ± 0.67 mm at 25, 50 and 100mg/ml respectively. However, water extract of *Rhamnus prinoides* leaves were devoid of an antibacterial activity against any of the test bacterium and chloroform extracts of leaves were devoid of an antibacterial activity against standard strains and clinical isolates of *E. coli* at 25 mg/ml. Negative control exhibited no zone of inhibition as expected whereas, positive control (disc containing antibiotics) exhibited zones of inhibition against all strains studied (Table 1). The mean zone of inhibition among bacteria showed statistically significant difference at all extracts of fruits and leaves of the plant except water extracts of leaves ($P<0.05$) at 25mg/ml.

The statistical analysis of the data showed that there were significant differences among positive control, water, ethanol and chloroform extracts of *Rhamnus prinoides* fruits and leaves on mean zone of inhibition within each tested bacterium at all concentrations.

Table 1: Antibacterial activity of *Rhamnus prinoides* extracts against pathogenic bacteria using Agar well diffusion method.

Plant species	Plant Part	Mean Zone of inhibition(mm) at 25mg/ml			
		Test bacteria			
		<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
		Standard	Clinical	Standard	Clinical
Water	Leaves	0.00±0.00 ^{a1}	0.00±0.00 ^{a1}	0.00±0.00 ^{a1}	0.00±0.00 ^{a1}
	Fruits	13.67±0.33 ^{b3}	12±00 ^{ab3}	12.33±0.33 ^{a2}	11.33±0.67 ^{a2}
Ethanol	Leaves	13.67±0.67 ^{bc3}	12±0.58 ^{ab3}	14.33±0.33 ^{c3}	11±0.58 ^{a2}
	Fruits	26±0.58 ^{d6}	20.67±0.33 ^{c5}	17.33±0.33 ^{b4}	14.33±0.67 ^{a3}
Chloroform	Leaves	6.33±0.33 ^{b2}	5.67±0.33 ^{b2}	00 ^{a1}	00 ^{a1}
	Fruits	19.33±0.9 ^{b4}	15.33±0.33 ^{a4}	18.33±0.33 ^{b5}	14.67±0.33 ^{a3}
Gm	0.01	21.67±0.33 ^{b5}	19.67±0.33 ^{a5}	24.67±0.33 ^{c6}	22.67±0.33 ^{b4}
Mean Zone of inhibition(mm) at 50mg/ml					
Water 50	Leaves	14.67±0.33 ^{c2}	12.67±0.33 ^{ab2}	13.67±0.67 ^{bc2}	11.67±0.67 ^{a2}
	Fruits	21±0.58 ^{c4}	18.33±0.33 ^{b4}	18±1 ^{b3}	15.67±0.33 ^{a4}
Ethanol	Leaves	18.33±0.67 ^{b3}	14.67±0.33 ^{a3}	17.33±0.33 ^{b3}	14±0.58 ^{a3}
	Fruits	28.33±1.2 ^{c6}	24.33±0.67 ^{b6}	22.33±0.33 ^{ab4}	21.33±0.33 ^{a6}
Chloroform	Leaves	11.67±0.33 ^{c1}	10.33±0.33 ^{ab1}	11.33±0.33 ^{bc1}	9.67±0.33 ^{a1}
	Fruits	25±0.58 ^{c5}	20.33±0.67 ^{a5}	22.33±0.33 ^{b4}	19±0.58 ^{a5}
Gm	0.01	21.67±0.33 ^{b4}	19.67±0.33 ^{a45}	24.67±0.33 ^{c5}	22.67±0.33 ^{b6}
Mean Zone of inhibition(mm) at 100mg/ml					
Water 100	Leaves	18.33±0.67 ^{c1}	14.67±0.33 ^{ab1}	16.33±0.33 ^{b2}	13.67±0.67 ^{a2}
	Fruits	29.33±0.67 ^{d3}	26.67±0.9 ^{c4}	22.67±0.33 ^{b4}	20±0.58 ^{a3}
Ethanol	Leaves	22.33±0.67 ^{b2}	19.67±0.33 ^{a2}	20±0.58 ^{a3}	19.33±0.9 ^{a3}
	Fruits	33.33±0.9 ^{c4}	29.33±0.33 ^{b5}	27.67±0.33 ^{ab6}	26.67±0.33 ^{a5}
Chloroform	Leaves	17.33±0.9 ^{b1}	16±0.58 ^{b1}	13±0.58 ^{a1}	11.33±0.33 ^{a1}
	Fruits	30.67±0.33 ^{d3}	25±0.58 ^{c3}	27.67±0.33 ^{b6}	22.33±0.9 ^{a4}

GM	0.01	21.67±0.33 ^{b2}	19.67±0.33 ^{a2}	24.67±0.33 ^{c5}	22.67±0.33 ^{b4}
Negative control	Water	-	-	-	-

Values are expressed as mean of three replicates ± S.E.M. Values with different letters in the same row and numbers in the same column at each concentration indicate statistically significant differences (Duncan's test, $p < 0.05$), GM= Gentamicin, - = no bacterial activity.

2.2. Minimum inhibitory concentration of *Rhamnus prinoides* extracts

Table 2 represented the MIC value of water, ethanol and chloroform extracts of *Rhamnus prinoides* fruits and leaves against standard strains and clinical isolates *S. aureus* and *E. coli*. As shown from the Table, ethanol extract of *Rhamnus prinoides* fruits showed the lowest MIC value of 1.04 mg/ml against standard strains of *S. aureus*. Water extract of *Rhamnus prinoides* leaves showed the highest MIC values 50mg/ml against all test bacteria and the chloroform extracts showed the highest MIC values 50mg/ml against *E. coli*.

The MIC values among bacteria showed statistically significant difference at chloroform extracts of leaves ($P < 0.05$). The statistical analysis of the data showed that there were significant differences among water, ethanol and chloroform extracts of *Rhamnus prinoides* fruits and leaves on MIC within each tested bacterium (Table 2).

Table 2: MIC (mg/ml) of *Rhamnus prinoides* extracts against pathogenic bacteria using broth dilution.

Solvent	Plant Part	MIC				Control	
		Test bacteria				Positive	Negative
		<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>			
		Standard	Clinical	Standard	Clinical		
Water	Leaves	50 ^{a6}	50 ^{a6}	50 ^{a4}	50 ^{a4}	+	-
	Fruit	25 ^{a5}	25 ^{a5}	25 ^{a3}	25 ^{a3}	+	-
Ethanol	Leaves	3.13 ^{a3}	3.13 ^{a3}	4.17 ^{a2}	5.21 ^{a2}	+	-
	Fruit	1.04 ^{a1}	1.3 ^{a1}	2.08 ^{a1}	2.08 ^{a1}	+	-
Chloroform	Leaves	12.5 ^{a4}	12.5 ^{a4}	50 ^{b4}	50 ^{b4}	+	-
	Fruit	2.08 ^{a2}	2.08 ^{a2}	3.13 ^{a12}	3.13 ^{a1}	+	-

Values are expressed as mean of three replicates. Values with different letters in the same row and numbers in the same column at each solvent indicate statistically significant differences (Duncan's test, $p < 0.05$), +=growth, - = no growth.

2.3. Minimum bactericidal concentration of *Rhamnus prinoides* extracts

As depicted in Table 3, MBC values of water, ethanol and chloroform extracts of *Rhamnus prinoides* against standard strains and clinical isolates *S. aureus* and *E. coli*. As represented in the Table, ethanol extract of *Rhamnus prinoides* fruits showed the lowest MBC value (2.08 mg/ml) against *S. aureus*. Water extract of *Rhamnus prinoides* leaves showed the highest MIC value of 100mg/ml against all test bacteria and the chloroform extracts showed the highest MIC value of 100mg/ml against *E. coli*.

The MBC values among bacteria showed statistically significant difference at ethanol extracts of fruits and chloroform extracts of leaves ($P < 0.05$). The statistical analysis of the data showed that there were significant differences among water, ethanol and chloroform extracts of *Rhamnus prinoides* fruits and leaves on MBC within each tested bacterium.

Table 3: The MBC (mg/ml) of the *Rhamnus prinoides* extracts against pathogenic bacteria.

Solvent	Plant part	MBC			
		Test bacteria			
		<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
		Standard	Clinical	Standard	Clinical
Water	Leaves	100 ⁴	100 ⁵	100 ⁴	100 ³
	Fruits	50 ³	50 ⁴	50 ³	50 ²
Ethanol	Leaves	5.21 ^{a1}	5.21 ^{a2}	6.25 ^{a2}	8.33 ^{a1}
	Fruits	2.08 ^{a1}	2.08 ^{a1}	4.2 ^{ab1}	5.2 ^{b1}
Chloroform	Leaves	20.83 ^{a2}	25 ^{a3}	100 ^{b4}	100 ^{b3}
	Fruits	4.2 ^{a1}	5.2 ^{a2}	6.25 ^{a2}	8.33 ^{a1}

Values are expressed as mean of three replicates. Values with different letters in the same row and numbers in the same column at each solvent indicate statistically significant differences (Duncan's test, $p < 0.05$).

3. Discussion

Phytochemicals resulting from plant products serve as a trial to develop less toxic and more effective medicines in controlling the growth of microorganism [19].

In the present investigation water, ethanol, and chloroform extracts of *Rhamnus prinoides* were evaluated for their antimicrobial activity against standard strains and clinical isolates of *E. coli*, *S. aureus*. The results showed that all water, ethanol, and chloroform extracts of the plant were active against tested bacteria in concentration dependent manner.

According to results obtained in the present investigation, water extract of *Rhamnus prinoides* leaves showed good antibacterial activity against tested bacterial species. On the other hand, *Rhamnus prinoides* fruits showed the highest antibacterial activity against tested bacterial species. However, water extract of *Rhamnus prinoides* leaves were devoid of antibacterial activity against any of the test bacterium at low concentration (25 mg/ml).

These results were supported by [33] who reported that, water extracts of *Ecbalium elaterium* fruit showed antimicrobial activity against all test microorganisms except MRSA, *Eminium spiculatum* and seed extracts of *Lupinus varius* exhibited antibacterial activity against all test microorganisms. In a research conducted by [22], aqueous extract of *Sambucus ebulus* has effects against *S. aureus* with inhibition zone diameter of 14 mm at 500mg/ml in the agar well diffusion method. aqueous extract of fruits of *Terminalia bellerica* also showed significant activity against all the bacterial and fungal isolates tested with zone of inhibition ranged from 15 -23 mm [14]. These findings support our results even if the plants are different.

On the other hand, the results of current study were contrasted with that of [6] and [26] who reported aqueous extract of *Rhamnus prinoides* leaves was found to be devoid of antibacterial activities against all the test bacterial strains regardless of the concentrations. Another study conducted by [3] reported that, water extracts of *Avicennia marina* did not give any inhibition against *Staphylococcus* sp. and *Proteus* sp. Aqueous extracts of *Lippia citriodora*, *Plantago major*, *Althaea officinalis*, *Tilia bengonifolia* and *Adiantum capillus-veneris* showed no significant antibacterial effect [22]. Antibacterial activities of plant extracts in the current study were differing from study conducted by those authors. The probable reason for this difference may be attributed to concentration of extracts, types of plants, methods of extraction, methods of test as well as bacterial strains used.

In ethanol extracts, the highest antibacterial activity was showed in *Rhamnus prinoides* fruits at all concentration followed by *Rhamnus prinoides* leaves at 100mg/ml against standard strains of *S.aureus*. The lowest antibacterial activity was seen against clinical isolates of *E. coli* in *Rhamnus prinoides* leaves at 25mg/ml. The results of antibacterial activity of *Rhamnus prinoides* leaves in this study was in accordance with that of [8] who reported that ethanol and methanol extracts have been shown antimicrobial activity against different species of pathogenic bacteria with clear zone diameter of the wells greater than 10 mm in all pathogenic bacteria. Results of the current study were also in line with the

study of [26] who reported that methanol fraction of *Rhamnus prinoides* leaves showed the highest antibacterial activity against standard strains of *S.aureus* and the lowest antibacterial activity against clinical isolates of *E. coli*. According to the study conducted in Algeria, *Punica granatum* bark ethanol macerate showed to be potent inhibitors against all bacterial strains [20] and methanol water extract of *Rhamnus prinoides* leaves was active against all bacteria strains [6].

Another study conducted in Jordan showed that ethanol extracts of *Mandragora autumnalis* fruits exhibited antibacterial activity against all test microorganisms and ethanol extracts of *Mandragora autumnalis* fruits showed the highest significant antibacterial activity against *E. coli* and MRSA with inhibition zone of 26.0 ± 1.0 mm and 28.7 ± 1.5 mm respectively [33]. Ethanol extraction of *Lippia citriodora* and *Plantago major* showed a remarkable antibacterial effect against *S. aureus* with the inhibition zone diameter of 26mm and 33mm respectively at 500mg/ ml [22].

On the other hand, ethanol extract of *Mimosa pudica* twig [4], *Lippia citriodora* and *Plantago major* [22] and *Ecbalium elaterium* [33] were not active against *E. coli*. Previous study conducted in Bangladesh also documented that ethanol extract of *Mimosa pudica* and *Lawsonia inermis* twig showed the highest effect against *S. aureus* with zone of inhibition 11.2 ± 0.14 mm and 17.1 ± 0.14 mm respectively whereas, *Lawsonia inermis* showed the zone of inhibition 7.20 mm against *E. coli* in Disc diffusion method [4] which were lower than the result of our study. The probable reason for this difference may be attributed to concentration, type and parts of plant, methods of test, methods of extraction as well as bacterial strains used.

In chloroform extract the highest antibacterial activity was showed in *Rhamnus prinoides* fruits at 50mg/ml and 100mg/ml followed by *Rhamnus prinoides* at 100mg/ml against standard strains of *S.aureus*. The lowest antibacterial activity was seen against clinical isolates of *E. coli* in *Rhamnus prinoides* leaves at 25mg/ml. chloroform extract of *Moringa oleifera* leaves showed antibacterial activity against *S. aureus* (11.0 ± 0.5 mm) at 200mg/ml [1] which was lower than the result of the present study and chloroform extracts of *Cassia auriculata* leaf showed good activity against *S. aureus* (12 mm) and *E. coli* (14 mm) [29].

According to the study conducted in India, chloroform extract of *Terminalia bellerica* fruits showed moderate activity against *E. coli* with zone of inhibition 13.6 ± 1.5 mm [14] and chloroform extract *Lawsonia inermis* showed antibacterial activity against *S.aureus* with zones of inhibition 8.30 ± 0.14 mm [4]. Chloroform of *Trichopus zeylanicus* fruits also showed pronounced antibacterial activity at concentration 250mg/ml against all tested microorganisms [37].

According to the study conducted in Debre Markos, Ethiopia, the chloroform fraction of *Rhamnus prinoides* leaves showed that the highest antibacterial activity against standard strains of *S.aureus* at 19.5, 39 and 78mg/ well concentrations with inhibition zone 11 ± 0.67 , 13 ± 0.58 and 14.33 ± 0.33 respectively and the lowest antibacterial activity was seen against clinical isolates of *E. coli* with no antibacterial activity [26]. However antibacterial activity of current study for *S.aureus* at all concentration

and *E. coli* at 50 and 100mg/ml were differing from study conducted by those authors, the antibacterial activity of current study for *E. coli* at 25mg/ml was in accordance with study conducted by those authors.

On the other hand, chloroform extract *Lawsonia inermis* not active on *E. coli* and extract of *Mimosa pudica* twig was devoid of antibacterial activity against *S. aureus* and *E. coli* in disc diffusion method [4] which was disagree with this study. The difference may be attributed to plant type and parts, concentration of extracts and bacteria, methods of extraction, methods of antibacterial test as well as bacterial strains used.

The results obtained during this investigation elucidated clearly that, Gram-positive bacteria were more sensitive than Gram-negative bacteria towards plant extracts tested. These are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with Gram-negative outer membrane acting as a barrier to antibiotics [46]. This result was agreed to that previously recorded by [48] who reported that Gram-negative bacteria were not susceptible to plant extracts when compared to Gram-positive bacteria, and this has been attributed to the external lipopolysaccharide (LPS) wall that surrounds the peptidoglycan cell wall of the former. Among the test bacteria, the most susceptible bacterium at all concentration of all plant extracts was standard strains of *S. aureus* and the most resistant was clinical isolates of *E. coli* with different mean zone of inhibition depending on type of plants and concentration of extracts. This data is in close agreement with [2] who report that out of seventy-five extracts; almost all extracts exhibited the highest antibacterial activity against *S. aureus*. On the other hand, ethanol extract of *Lawsonia inermis* was found to exhibit most effective antibacterial activity against Gram-negative organisms [4]. The mean zone of inhibition among bacteria within the same solvent and concentration for each plant showed statistically significant difference ($P<0.05$).

All Plant extracts have higher zone of inhibition against standard strains than clinical isolates. These differences in the potency of the plants extract against the strains of the same bacterial species might be associated to the susceptibility differences between the strains in which the clinical isolates could have a higher chance of developing a resistance mechanism of reducing the access of the bioactive metabolites to the target sites since they had been isolated from the clinic settings in which resistant strains are common [47].

Among extracts of medicinal plants used in the study, the ethanol extract of *Rhamnus prinoides* fruits showed the maximum zone of inhibition which was in line with the study of [33] who reported that ethanol extracts of *Mandragora autumnalis* fruits showed the highest significant antibacterial activity against *E. coli* (26.0 ± 1.0 mm) and MRSA (28.7 ± 1.5 mm). The mean zone of inhibition among plants with in the same solvent and concentration for each bacterium showed statistically significant difference ($P<0.05$). This difference may due to the presence of different secondary metabolites in the plants.

The present study showed that, among water extract of plants the lowest MIC value (25mg/ml) was obtained with *Rhamnus prinoides* fruits and the highest MIC value (50mg/ml) of was obtained with *Rhamnus prinoides* leaves against all tested bacteria. The MIC value of water extract of *Ecbalium elaterium* fruits was 64 mg/ml against *E. coli* [33] which was higher than our study, while aqueous extract

of *Terminalia bellerica* fruit could inhibit the growth of *E. coli* at minimum concentration of 6.25mg/ml [14] which was lower than this study. The present result was also contrasted with that of [6] who reported that aqueous extract of *Rhamnus prinoides* leaves was not active against any of the bacterial strains that were tested.

Regarding to ethanol extracts of plants, the lowest MIC value was found with *Rhamnus prinoides* fruits (1.04mg/ml) against standard strains *S. aureus*. ethanol extracts of *Mandragora autumnalis* fruits showed very strong activity against MRSA with the best MIC (4 mg/ml) and 8 mg/ml against *E. coli* [33] which were higher than MIC values of *Rhamnus prinoides* fruits (*S. aureus*) of the current study whereas the values were in line with *Rhamnus prinoides* leaves. He also reported that MIC of ethanol extracts of *Ecbalium elaterium* fruits was 64mg/ml against *E. coli* which was higher than this study.

The current results were disagree with that of [26] who reported that the MIC value for methanol fraction of *Rhamnus prinoides* leaves ranged from 32.5 mg/ml (against *E. coli* species) to 8.13 mg/ml (against *S. aureus*); and [8] who reported that the MIC of ethanol and methanol gesho extract against standard strains of *E. coli* and *S. aureus* was 97.5 mg/ml, while MIC of 390 was found for clinical isolates of *S. aureus*. MIC of *Rhamnus prinoides* leaves methanol water extract against *E. coli* and *S. aureus* were 400mg/ml and 200 mg/ml respectively [6].

In the case of chloroform extract, the lowest MIC value was found with *Rhamnus prinoides* fruits (2.08±0.26mg/ml) against standard strains and clinical isolates of *S. aureus* and *Rhamnus prinoides* leaves (50mg/ml) against standard strains and clinical isolates of *E. coli*. These results were disagree with that of [26] who reported that the MIC value for chloroform fraction of gesho against standard strains and clinical isolates of *S. aureus* were 8.13mg/ml and 16.25mg/ml respectively, while this fraction was found to be devoid of MIC against standard strains and clinical isolates of *E. coli* at the same concentrations. Chloroform extracts of *Polygonum aviculare stem and leaf* showed MIC value of 18 mg/ml and 20 mg/ml against *S. aureus* and 15 mg/ml and 18 mg/ml against *E. coli* respectively [36]. The probable reason for difference may be attributed to plant type and parts, concentration of bacteria, methods of extraction, methods of antibacterial test as well as bacterial strains used.

The present study also showed that, the lowest MBC value was found with water extracts of *Rhamnus prinoides* fruits (50mg/ml) and the highest MBC value was found with and *Rhamnus prinoides* leaves (100mg/ml) against all tested bacteria. MBC of water extract of *E. elaterium* fruit was (128) against *E. coli* [33] which was higher than our study. Ethanol extract of *Rhamnus prinoides* fruit was showed the lowest MBC (2.08mg/ml) against standard strains and clinical isolates of *S. aureus*, [33] reported that ethanol extract of *Mandragora autumnalis* fruits showed MBC value of 16mg/ml and 8mg/ml against *E. coli* and MRSA respectively. He also reported that ethanol extracts of *Ecbalium elaterium* fruits was 64mg/ml against *E. coli*.

MBC value of ethanol extract of *Lippia citriodora* was 62.5 and 250 against *S. aureus* and *E. coli*, while MBC value of *Plantago major* was 31.25 and 125 against *S. aureus* and *E. coli* [22]. Previous study by [26] showed that the MBC value for methanol fraction of *Rhamnus prinoides* leaves against *S. aureus*

was 16.25 mg/ml where as 32.5mg/ml and 65 mg/ml were found against standard and clinical isolates of *E. coli* respectively. Similar research conducted in Gondar, Ethiopia reported that the MBC gesho extract against standard strains of *E. coli* and *S. aureus* was 195 and MBC of 780 mg/ml was found for clinical isolates of *S. aureus* [8]. These results were higher than the result of the present study.

Regarding to chloroform extracts of plants, the lowest MBC value (4.2 ± 1.04 mg/ml) was found with *Rhamnus prinoides* against standard strains of *S. aureus* while the lowest MBC value (100mg/ml) *Rhamnus prinoides* leaves against standard strains and clinical isolates of *E. coli*. According to [26] the MBC value for chloroform fraction of *Rhamnus prinoides* leaves was 16.25 mg/ml against *S. aureus* and devoid of MBC against standard strains and clinical isolates of *E. coli* at the same concentrations. The probable reason for difference may be attributed to plant type and parts, concentration of bacteria, methods of extraction, methods of antibacterial test as well as bacterial strains used. The results obtained during this investigation elucidated clearly that ethanol extract exhibited higher activity followed by chloroform and water extracts showed least activity against all the tested organisms.

3.1. Conclusion

The results of present study clearly indicated that *Rhamnus prinoides* extracts have potential antibacterial activity against standard and clinical isolates of *S.aureus* and *E. coli* in concentration dependent manner. This support the traditional application of the plant extracts which posse compounds with antimicrobial properties that can be used in novel drugs for the treatment of bacterial diseases. Plant extracts investigated in this study showed higher antibacterial activity on standard strains than clinical isolates and on gram positive bacteria than gram negative bacteria. The results of this study also clearly indicated that the antibacterial activities vary with plant part used, solvent type, concentration of extracts and microorganism tested. Plant extracts have great potential as antimicrobial compounds against *S.aureus* and *E. coli*. Thus, they can be used in the treatment of infectious diseases caused by these bacteria.

Based on the findings of the present study, it can be recommended that further studies are needed to study their toxicology, phytochemical analysis and isolate the bio- active components from this valuable plant to innovate new natural antibiotics and minimize the spread of drug resistant bacteria. Since current study was conducted on antibacterial activity of *Rhamnus prinoides* extracts against bacterial pathogens, it is recommended that further study be made on antifungal and anti viral activity of this plant against fungal and viral pathogens.

4. Methods

4.1. Study design and area

Cross sectional experimental study design was conducted to evaluate antimicrobial activity of *Rhamnus prinoides* extracts against some antimicrobial resistant skin pathogens at Bahir Dar University post

graduate Microbiology laboratory, Bahir Dar town from May to June 2018.

4.2. Plant collection and preparation

Rhamnus prinoides leaves and fruits were collected from around Bahir Dar town in June 2018. These plant parts were washed with tap water, dried in an open air, separately powdered to suitable size and made ready for extraction [39].

4.3. Extraction of plant materials

Plant materials were extracted by maceration technique with chloroform, ethanol and water solvents with occasional shaking at room temperature for three days. Grinded plant materials were soaked with each solvent separately at 10:1 solvent-to sample ratio (v/w). The extracts were separately filtered by Whatman No. 1 filter paper and concentrated with dry oven. Further, fresh solvents were added to the residue at the same ratio until required amount of extracts were obtained. The dry extracts were stored in sample bottles at refrigerator for further use [39].

4.4. Antimicrobial activities of *Rhamnus prinoides* extracts

4.4.1. Source of the test bacterial pathogens and inoculums preparation

Bacteria strains (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923) and clinical isolates of *S. aureus* and *E. coli* were obtained from Amhara regional state central laboratory. The bacteria will be activated by streaking on grown in nutrient agar (Himedia) and incubated at 37°C for 24 h. The bacteria used for the study were prepared by inoculating isolates into nutrient broth and incubated at 37°C for 24 hrs. The culture turbidity was adjusted to a 0.5 McFarland standard (approximately 1×10^8 cfu/ml) using sterile normal saline [12].

4.4.2. The antimicrobial assays

The antimicrobial activity tests of the plant extracts against bacteria were carried out by the agar well diffusion method, which is commonly used for screening of the antimicrobial activities of herbal drugs [35]. The standardized bacterial broth cultures prepared in section 2.4.1 were swabbed on Mueller-Hinton agar (Himedia) plates using sterile cotton swabs. After thirty minutes, on each plate, five equidistant wells were made with a 6 mm diameter sterilized cork borer. Stock solution of each plant extract was prepared using sterilized distilled water at different concentration (25, 50 and 100) mg/ml and 100µl of each plant

extracts were added into the wells aseptically. The plates were allowed to diffuse at room temperature for 2hrs and incubated at 37°C for 18-24 h in triplicates. Sterile distilled water without plant extract used as negative control and Gentamicin (0.01mg/ml) will be used as a positive control respectively. The antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition. Zone of inhibition was measured in mm using ruler [31].

4.4.3. Determination of minimum inhibitory concentration (MIC)

The two-fold broth dilution method was used to determine minimum inhibitory concentration (MIC) of extracts, which showed high antimicrobial activity with the agar well diffusion method [12]. The plant extract solutions (100mg/ml) were serial dilution with nutrient broth and 20µl of a standard suspension of test organisms were added to each concentrations of the extract. Extra test tubes containing peptone water and peptone water plus the test organisms were prepared to serve as a negative and positive control respectively. The broth plates were incubated along with the batch of the test tubes at 37°C for 24 hours. The MIC value of the extract was taken as the lowest concentration that shows no visible growth or turbidity in the test tube [31].

4.4.4. Minimum bactericidal concentration (MBC)

From all tubes showed no visible signs of growth / turbidity (MIC and higher dilutions), loop full of inoculums were streaked on nutrient agar plates and incubated at 37°C for 24 hours. The least concentration that shows no visible growth after incubation was considered the MBC value of the tested extracts against the tested bacterial species [12].

4.5. Data analysis

The statistical differences of the mean zone of inhibition, MIC and MBC of extracts for individual bacterium and among bacteria was carried out by employing one-way analysis of variance followed by Duncan's multiple comparison tests using Statistical Package for the Social Sciences (SPSS) Version 20 software. P-values less than 0.05 were considered statistically significant.

Abbreviations

ATCC:	American type cell collection
CLSI:	Clinical and Laboratory Standards Institute
<i>E. coli:</i>	<i>Escherichia coli</i>
MBC:	Minimum bactericidal concentration
MIC:	Minimum inhibitory concentration
MRSA:	Methicillin-resistant <i>Staphylococcus aureus</i>
NCCLS:	National Committee for Clinical Laboratory Standards
<i>S.aureus:</i>	<i>Staphylococcus aureus</i>
WHO:	World health organization

Declarations

Ethics approval and consent to participate

Not applicable

Consent to publish

Not applicable

Availability of data and materials

The data is available in the manuscript

Conflicts of Interest

The author declares no conflicts of interest

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