Evaluation of *in-vitro* antibacterial and antifungal activities of crude extracts and solvent fractions of methanol extract of leaves of *Ricinus communis* Linn (Euphorbiaceae) against selected pathogens

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Research article

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Abstract

Background Impacts of infectious diseases are reduced due to development of antimicrobial agents. However, the effectiveness of the antimicrobial agents is reduced over time because of the emergence of antimicrobial resistance.To overcome these problems scholars have been searching for alternative medicines, particularly focusing on traditionally used medicinal plants. *Ricinus communis* Linn is used as a traditional treatment for bovine mastitis, wound infection, and other medicinal purposes. Moreover, the antimicrobial activity of *Ricinus communis* Linn leaf at crude extract level has been confirmed against human originated pathogens in the previous studies. The objective of the present study was to further evaluate the antimicrobial activities of *Ricinus communis* Linn leaf extracts and fractions. The *Ricinus communis* Linn leaves were macerated in absolute methanol and acetone solvents. The methanol crude extract was shown best antimicrobial activity and exposed to further fractionation via increasing polarity of solvents (n-hexane, chloroform, ethyl acetate, and aqueous). Test microorganisms included in the study were six laboratory reference bacteria (*E. coli, S. aureus, S. agalactiae, K. pneumoniae, P. aeruginosa,* and *S. Pyogenes*), two clinical isolate bacteria (*E. coli* and *S. aureus*), and *Candida albicans*. The agar well diffusion method was employed to determine antimicrobial activity. The minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) were determined through broth microdilution method.

Results The results indicated that the best antimicrobial activity(based on zone of inhibition) for ethyl acetate fraction ranging from 14.67 mm (clinical *E. coli*) to 20.33 mm (*S. aureus*) at 400mg/ml however, n-hexane exhibited lowest antimicrobial activity. Among tested fractions, ethyl acetate fraction was produced lowest MIC values ranging from 1.5625mg/ml (*S. aureus*) to 16.67 mg/ml (*Candida albicans*). The ethyl acetate fraction showed bactericidal activity against all tested microorganisms.

Conclusion Methanol extract of *Ricinus communis* Linn leaf exhibited better antimicrobial activity than acetone extract. Hence, ethyl acetate fraction of crude methanol extract exhibited best antimicrobial activity indicating its potential for development of antimicrobial products.

Background

Infectious diseases are exacerbated due to the existence of zoonotic diseases and antimicrobial resistance [1, 2]. Several surveillances have been conducted on antimicrobial resistance. The augmentations of antimicrobial resistances have harmed both human and animal health, exposing to longer periods of hospitalization, and affect treatment costs [3, 4, 5]. Alternative medicines have been screening from a variety of plants for their pharmacological potential as secondary metabolites are less in drug adverse effects, resistances, and residues [6, 7, 8, 9].

Ricinus communis Linn in Ethiopia is used for the treatment of blackleg and actinomycosis [10], diarrhoea, wound and skin rashes or dermatitis [11,12], and bovine mastitis [13]. The studies are conducted on validation of antimicrobial activity of *Ricinus communis* Linn leaf crude extracts using different solvents in Pakistan and Ghana, and methanol crude extract was reported to have a promising antimicrobial potential [14,15]. Besides, in Ethiopia *Ricinus communis* Linn leaf has been demonstrated to have antibacterial activity for an organic solvent solution, but the aqueous extract has shown less activity [16]. However, methanol solvent was not used for extraction in Abew *et al*. [16] study and none has been done on the antimicrobial activities of solvent fractions of *Ricinus communis* Linn leaf.

Antimicrobial activities of medicinal plants are not only determined by plant species. There are also other factors such as altitude, temperature, illumination, and moisture. These factors have regulated accumulation and metabolism of secondary metabolites in medicinal plants. Additionally, differences in the location of medicinal plants have contributed to presence of different active ingredients and their concentrations [17]. Therefore, the current study is intended to evaluate antimicrobial activities of methanol and acetone crude extracts, and solvent fractions of methanol extract that exhibited the best antimicrobial activity.

Methods

Extraction of the plant

The plant leaves were collected in October, 2019 from Sululta district, surrounding of Addis Ababa, Ethiopia. The specimen was authenticated by a taxonomist at Herbarium of the college of natural and computational sciences, Addis Ababa University, and voucher number (002/BK) was deposited for future reference.

The extraction of leaves was performed according to Ogbiko *et al*. [18]. The powdered leaves of 200 grams were weighed on an analytical balance (Mettler Toledo, Switzerland) and macerated in 1000 ml of absolute methanol and acetone in Erlenmeyer flask at the ratio of 1:5; after three days extract was collected and marc was re-macerated. Collection of the extract was carried out at the interval of three days, so leaves were macerated totally for 9 days with the intermittent shaking on the rotary-shaker (VWR DS-500; The Lab World Group, Boston, MA, USA). The pooled extract was first filtered through sieve mesh then followed by filtration via Whatman no.1 by using filtration apparatus or unit. A filtrate of extracts was concentrated in a rotary evaporator (Buchii model R-200, Switzerland) at 40^{o}C temperature and 40 revolutions per minute (RPM) until solvents were completely removed and solid extracts were formed.

Crude extract solvent fractionation

The crude methanol extract was subjected to further solvent fractionation by increasing polarity including n-hexane, chloroform, ethyl acetate, and aqueous. Voukeng *et al*. [19] method was used for solvent fractionation with modification on the concentration of extract residue between fractionation intervals. The methanol crude extract was not completely dissolved in water, therefore, 90% methanol solvent was used as diluent. The methanol crude extract 60 grams were weighed on an analytical balance and subjected to dissolve completely in 100ml of 90% methanol (10ml water and 90ml methanol) in the beaker. The completely dissolved 100ml methanol crude extract was mixed with 100ml n-hexane for solvent partitioning in separatory funnel having a capacity of 250ml. The mixture in separatory funnel was fixed to the standing stage pole and waited until a clear and separated layer formed between two solvents. Once a clear layer formed, the methanol part was taken first carefully to beaker and n-hexane partition to another container. This procedure was repeated three times, the n-hexane partition was collected together for future concentration. While the remaining crude methanol extract solution was subjected to evaporation in a rotary evaporator at 40^{o} C and 40 rpm to remove methanol. Then, 90ml of water was added to the concentrated crude methanol extract to form a 100ml aqueous solution. The 100ml aqueous solution of crude methanol extract was mixed with 100ml of chloroform in the separatory funnel. The separatory funnel was fixed on the standing stage pole and waited until a clear layer formed between the aqueous solution of crude methanol extract and chloroform. The chloroform portion was held lower layer and collected first in the container and aqueous portion in another container. It was replicated three times and the chloroform portion pooled in the container for later concentration. The remnant aqueous portion of crude methanol extract was concentrated on a

rotary evaporator to remove the remaining chloroform. The concentrated 100ml aqueous portion of crude methanol extract was mixed with 100ml ethyl acetate in the separatory funnel. The separatory funnel was fixed on the standing stage pole and waited till a clear layer appeared between aqueous fraction and ethyl acetate fraction. It was repeated three times, and aqueous fraction and ethyl acetate fraction were collected in different containers. The aqueous fraction was lyophilized by lyophilizer (Operon, Korea vacuum limited, Korea), but n-hexane, chloroform, and ethyl acetate fractions were concentrated in a rotary evaporator.

Preliminary phytochemical screening

The crude extracts and fractions were screened for phytochemical constituents or secondary metabolites using standard methods. Tannins and cardiac glycosides were screened as described by Ayoola et al.2008(20), steroids and anthraquinones were screened as described by Nwadiaro et al.2015 (21), flavonoids, terpenoids, phenolic compounds were detected as described by Shetty et al.2016(22), while alkaloids were detected as described by Santhi et al.2016(23) .

Test organisms

Microorganisms selected for the experiment were standard strains including *Staphylococcus aureus* (ATCC 25923), *Streptococcus agalactiae* (ATCC 12386), *Streptococcus pyogenes* (ATCC 19615), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella* pneumoniae (ATCC 700603) brought from Ethiopian Public Health Institute, and clinical isolates *Staphylococcus aureus* and *Escherichia coli* obtained from Animal Products, Veterinary Drug and Animal Feed Quality Assessment Centre of Veterinary Drug and Animal Feed Administration and Control Authority, and *Candida albicans* (ATCC10231) brought from Ethiopian Biodiversity Institute. The gram staining, selective media, haemolysin, and catalase test were conducted to confirm test microorganisms according to [24, 25].

Standard drugs

Gentamicin 10µg disc was used as positive control against bacteria and brought from Animal Products, Veterinary Drug, and Animal Feed Quality Assessment Centre of Ethiopian Veterinary Drug and Animal Feed Administration and Control Authority. Amphotericin-B 20µg was used as positive control against fungus and obtained from the Ethiopia Food and Drug Administration and Control Authority. The standard drugs were used based on sensitivity against selected microorganisms and availability in the laboratory.

Antibacterial activity

The brain heart infusion (BHI) broth was prepared for streptococcal species and nutrient broth for other test bacteria. Overnight cultured 3-5 distinct colonies of bacteria based on their colony size were inoculated into 4ml broth media and incubated at 37^{o}C overnight. The nutrient or BHI broth was added to the overnight incubated bacterial suspension, and vortexed on a vortex mixer (Fisher Scientific. Ltd., England) for one minute to attained uniformly distribution. The vortexed bacterial suspension was adjusted to 0.5 McFarland standard (Remel, Lenexa Kansas 66215, USA) (equivalent to 1-2x10^{8} CFU/mL) through contrasting against white paper black line striped and was used for experiment within 15 minutes [26].

The 100µl of adjusted bacterial suspension was pipetted using a micropipette and applied on the surface of Mueller Hinton agar, and was swabbed at 60^{o} rotation to uniformly distribute bacteria throughout media surface using a cotton swab. The swabbed Mueller Hinton agar was allowed to stand for 15 minutes to provide time for the attachment of bacteria on the media. Then, the sterilized cork borer of 6mm diameter was perforated with the swabbed media to create 6mm diameter wells. At the time of punching media for different test bacteria, cork borer was sterilized by immersing in alcohol and burning with bunsen burner flames [27, 28]. The concentration of extracts for the experiment was determined based on a previous study on the plant [16]. The created wells were filled with 50µl extracts or fractions at a concentration of 400mg/ml, 200mg/ml and 100mg/ml, and negative control, but positive control disc (gentamicin) was placed on the media surface. After all wells on petri dishes were filled, and positive control was placed on petri dishes, then petri dishes were placed in the refrigerator at 4^{o}C for 2h to facilitate diffusion of extracts or fractions in the media. Subsequently, petri dishes were incubated at 37^{o}C for 24h in the incubator (BioTechnics India). The inhibition zone diameter after 24h incubation was measured by a ruler in millimetre and recorded [15, 16,29] . The experiment was done in triplicate.

Antifungal activity

The *Candida albicans* was cultured on sabouraud dextrose agar and incubated overnight. The overnight incubated yeast culture was inoculated into normal saline (0.85%). The inoculated normal saline was vortexed and adjusted to 0.5 McFarland standard (equivalent to 1-5x10^{6}cells/ml) by contrasting against white paper black line striped [30]. The 100µl adjusted *Candida albicans* suspension was pipetted using a micropipette and applied on the surface of sabouraud dextrose agar and swabbed at 60^{o} rotation to uniformly distribute yeast throughout media surface using a cotton swab. The swabbed sabouraud dextrose agar stands for 15 minutes to provide time for the attachment of yeast on the media. After that, the sterilized 6mm diameter cork borer was perforated swabbed media to create a 6mm diameter of wells [29]. The concentration of extracts for the experiment was determined based on a previous study on the plant [15]. The created wells were filled with the 50µl extracts or fractions at concentration 400mg/ml, 200mg/ml and 100mg/ml, negative, and positive control. The inoculated petri dishes were placed in the refrigerator at 4^{o}C for 2h to facilitate diffusion of extracts or fractions in the media. Next to that, Petri dishes were incubated at 37^{o}C for 24h in the incubator. The inhibition zone diameter after 24h incubation was measured by a ruler in millimetre and recorded [15,16,29]. The experiment was done in triplicate.

Determination of minimum inhibitory concentration (MIC) for pathogenic bacteria

The minimum inhibitory concentrations were determined using the broth microdilution technique for extracts or solvent fractions as their inhibition zones equal to or greater than 7 mm in agar well diffusion techniques [31]. The overnight cultured 3-5 distinct bacterial colonies were inoculated into 4ml Mueller Hinton broth and incubated at 37^{o}C overnight. Overnight incubated bacterial suspension had been adjusted (0.5McFarland standard) was diluted at a ratio of 1:20 with Mueller Hinton broth (0.5ml bacterial suspension was added to 9.5ml broth) and vortexed to have uniformly distributed bacterial suspension (5x10^{6} CFU/ml). The UV radiated sterile microtiter plate (Greiner Bio-One, Germany) wells were filled with 100µl Mueller Hinton broth which commenced from well one to twelve. The serial double dilution technique was employed for extracts and fractions in broth filled wells. The serial double dilution was performed as 100µl extracts or fractions were added to the first well and thoroughly mixed for five times by rinsing using micropipette and 100µl of the mixture was transferred to the second well using a new micropipette tip and thoroughly mixed as above. A 100µl of the second well mixture was pipetted using a new micropipette tip and transferred to third well then was thoroughly mixed. The process was continued until the tenth

well and 100µl mixture of the tenth well was pipetted and discarded to have an equal volume of fluid in wells [26]. The twofold serially diluted concentrations of extract for the experiment were determined from a previous study on the plant. The serially diluted concentrations used in the experiment were (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, 1.5625mg/ml, 0.78125mg/ml, and 0.3906mg/ml) [16]. The 100µl broth filled eleventh and twentieth wells were used as growth and sterility control, respectively. The 10µl diluted bacterial suspension (10% of 100µl well volume) was pipetted to wells from eleventh to first wells to reduce contamination to sterility control and attained final concentration of $5x10^5$CFU/ml bacteria in each well, but 10µl broth was pipetted to twelfth well. Finally, microtiter plates were sealed using parafilm and incubated at 37^{o}C for 24h [26]. The incubated microtiter plate wells were filled with 0.01% resazurin sodium salt indicator from twelfth to first well and incubated for 2h at 37^{o}C. The resazurin sodium salt reaction with actively growing microorganisms produces colour changes which are important to determine the MIC of extracts or fractions based on colour changes. The blue or purple colour appears if the growth of microorganisms is inhibited, while pink or colourless change is observed for those actively growing cells which reduced resazurin sodium salt to resorufin. Resazurin sodium salt solution was prepared by dissolving 0.01gram in 100ml sterile distilled water and filtered through 0.2µ pore size filter paper and stored in a dark container at 4^{o}C refrigerator until use [29,32]. The experiment was performed in triplicate.

Determination of minimum inhibitory concentration for pathogenic fungi

Overnight cultured three colonies of yeast were inoculated into sabouraud dextrose broth and incubated at 37^{o}C overnight. Overnight incubated yeast suspension had been adjusted (0.5McFarland standard) was diluted at a ratio of 1:20 with sabouraud dextrose broth (0.5ml yeast suspension was added to 9.5ml broth) and vortexed to have uniformly distributed yeast suspension (0.5-2.5x10^5 CFU/ml). The sterile microtiter plate wells were filled with 100µl sabouraud dextrose broth started from well one to twelve. The serial double dilution technique was employed for extracts and fractions in broth filled wells commenced from first to tenth wells. The serial double dilution was performed as 100µl extracts or fractions were added to the first well and thoroughly mixed five times by rinsing using micropipette and 100µl of the mixture was transferred to the second well using a new micropipette tip and thoroughly mixed as above. A 100µl of the second well mixture was pipetted using a new micropipette tip and transferred to third well then was thoroughly mixed as above. The process was continued until the tenth well and 100µl mixture of the tenth well was pipetted and discarded to have an equal volume of fluid in wells [34]. The twofold serially diluted concentrations of extract for the experiment were determined from a previous study on the plant. The serial double dilution concentrations used in the experiment were (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, 1.5625mg/ml, 0.78125mg/ml, and 0.3906mg/ml) [15]. The 100µl broth filled eleventh and twentieth wells were used as growth and sterility control, respectively. The 10µl diluted yeast suspension (10% of 100µl broth volume) was pipetted to wells from eleventh to first wells to reduce contamination on sterility control and attained final concentration of yeast suspension (2.5x10^4CFU/ml) in each well, but 10µl broth was pipetted to twelfth well. The filled microtiter plate wells were sealed by parafilm and incubated at 37^{o}C for 24h [26,34]. The incubated microtiter plate wells were filled with 0.01% resazurin sodium salt indicator from twelfth to first well and incubated for 2h at 37^{o}C. The MIC of extracts and fractions were determined as blue or purple resazurin colour changed to pink or colourless [29,32]. The experiment was done in triplicate.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined through sub-culturing of 10µl content of microtiter plate well which is greater or equal to the lowest minimum inhibitory concentration on the Mueller Hinton agar and incubated for 24h. After 24h incubation, the petri dish was assessed for the presence of growth, and the minimum concentration of extracts or fractions with no visible growth was taken as minimum bactericidal concentration [33]. The experiment was done in triplicate.

Determination of Minimum Fungicidal Concentration (MFC)

The minimum fungicidal concentration was determined through sub-culturing of 10µl content of microtiter plate well which is greater or equal to the lowest minimum inhibitory concentration on the sabouraud dextrose agar and incubated for 24h. After 24h incubation, the Petri dish was assessed for the presence of growth, and the minimum concentration of extracts or fractions with no visible growth was taken as minimum fungicidal concentration [33]. The experiment was done in triplicate.

Data Analysis

The data were entered into an excel spreadsheet for statistical analysis using Statistical Package for Social Science (SPSS) version 20. The descriptive statistics, one-way ANOVA, Tukey Post Hoc test, and linear regression R^2 were utilized for statistical analysis and inference. The descriptive statistics were employed for calculation of group mean of inhibition zone diameter as Mean ± SEM. The one-way ANOVA was performed to determine the significant difference among group means. One-way ANOVA was followed by Tukey Post Hoc test to determine where the significant difference occurred between groups. The linear regression R^2 was calculated to determine the concentration dependence of crude extracts and solvent fractions on antimicrobial activities against test microorganisms. Statistically significant differences were declared at a p-value of less than 0.05.

Results

Antibacterial activity

Agar well diffusion assay

The inhibition zone diameter was observed for crude extracts, solvent fractions, and positive control, but not for negative control. The highest inhibition zone diameter of crude methanol extract determined against gram-positive bacteria (17.33 mm against *S. pyogenes*) and gram-negative bacteria (14.67 mm against *P. aeruginosa*). However, the highest concentration of crude methanol extract against *K. pneumoniae* produced the lowest inhibition zone diameter of 12.67 mm. The highest inhibition zone diameter of crude acetone extract observed against gram-positive bacteria were 14.33 mm against *S. pyogenes* and gram-negative bacteria (13.33 mm against *E. coli*). Nevertheless, the highest concentration of crude acetone extract against *K.* pneumonia produced the lowest inhibition zone diameter of 11.67 mm (Table 1 and 2). The highest inhibition zone diameter of ethyl acetate fraction observed were against gram-positive (20.33 mm against *S. aureus*) and gram-negative bacteria (16.67 mm against *P. aeruginosa*). Whereas, n-hexane fraction produced the lowest inhibition zone diameter and no antibacterial activity against *P. aeruginosa*. Additionally, ethyl acetate fraction produced the highest inhibition zone diameter than the crude extract of methanol, acetone, and other solvent fractions (Table 1, 2, 3, and 4). The highest inhibition zone diameter of ethyl acetate fraction observed against clinical isolate bacteria of gram-positive (17.67 mm against *S. aureus*) and gram-negative (14.67 mm against *E. coli*) (Table 5).

Antibacterial activity

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Table 1: Mean inhibition zone diameter (mm) of *Ricinus communis* Linn leaf crude extracts against gram-positive bacteria.

le extracts		*S. aureus*		*S. agalactiae*		*S. pyogenes*	
		Mean ± SEM	R^2	Mean ± SEM	R^2	Mean ± SEM	R^2
anol	100mg/ml	10.67 ± 0.333^{a3c1d3}	0.876	10.33 ± 0.333^{a3d3}	0.893	12.67 ± 0.333^{a3c2d3}	0.831
	200mg/ml	12.67 ± 0.333^{a3b1d1}		12.00 ± 0.577^{a3d2}		15.67 ± 0.333^{b2d1}	
	400mg/ml	15.00 ± 0.577^{a3b3c1}		14.67 ± 0.333^{a1b3c2}		17.33 ± 0.333^{b3c1}	
rol	10µg	20.67±0.333		16.67±0.333		16.67±0.333	
	100mg/ml	9.67 ± 0.333^{a3c1d3}	0.916	9.67 ± 0.333^{a3c1d2}	0.809	9.67 ± 0.333^{a3b1d3}	0.932
one	200mg/ml	11.33 ± 0.333^{a3b1d2}		12.00 ± 0.577^{a3b1}		11.67 ± 0.333^{a3b1d2}	
	400mg/ml	13.67 ± 0.333^{a3b3c2}		$13.67\pm.333^{a2b2}$		14.33 ± 0.333^{a2b3c2}	
rol	10µg	20.67±0.333		17.00±0.577		16.67±.333	

Values expressed as Mean±SEM for n=3. The mean comparisons for different extracts and control (Gentamicin 10µg) were performed by one-way ANOVA followed by Tukey HSD Post Hoc multiple comparison test. Where, compared to [a]positive control, [b]100mg/ml, [c]200mg/ml and [d]400mg/ml. [1]P<0.05, [2]P<0.01, [3]P<0.001.

Table 2: Mean inhibition zone diameter (mm) of *Ricinus communis* Linn leaf crude extracts against gram-negative bacteria.

e extract		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
		Mean ± SEM	R^2	Mean ± SEM	R^2	Mean ± SEM	R^2
anol	100mg/ml	10.67 ± 0.333^{a3d2}	0.790	9.67 ± 0.333^{d2}	0.875	11.33 ± 0.333^{a3d3}	0.889
	200mg/ml	12.00 ± 0.577^{a3}		10.67 ± 0.333^{d1}		12.67 ± 0.333^{a3d1}	
	400mg/ml	13.67 ± 0.333^{a3b2}		$12.67\pm.333^{a2b2c1}$		14.67 ± 0.333^{a1b3c1}	
rol	10µg	19.67±0.333		10.00±0.577		16.67±0.333	
	100mg/ml	9.67 ± 0.333^{a3c1d3}	0.893	8.33 ± 0.333^{c1d2}	0.813	9.67 ± 0.333^{a3d2}	0.843
ɔne	200mg/ml	11.33 ± 0.333^{a3b1d1}		10.33 ± 0.333^{b1}		10.67 ± 0.333^{a3d1}	
	400mg/ml	13.33 ± 0.333^{a3b3c1}		11.67 ± 0.333^{b2}		12.33 ± 0.333^{a3b2c1}	
rol	10µg	19.33±0.333		10.00±0.577		16.33±0.333	

Values expressed as Mean±SEM for n=3. The mean comparisons for different extracts and control (Gentamicin 10µg) were performed by one-way ANOVA followed by Tukey HSD Post Hoc multiple comparison test. Where, compared to [a]positive control, [b]100mg/ml, [c]200mg/ml and [d]400mg/ml. [1]P<0.05, [2]P<0.01, [3]P<0.001.

Table 3: Mean inhibition zone diameter (mm) of *Ricinus communis* Linn leaf crude solvent fractions of methanol extract against gram-positive bacteria.

nt fractions		*S. aureus*		*S. agalactiae*		*S. pyogenes*	
		Mean ± SEM	R^2	Mean ± SEM	R^2	Mean ± SEM	R^2
ane	100mg/ml	7.33 ± 0.333^{a3d2}	0.746	7.33 ± 0.333^{a3d2}	0.764	7.33 ± 0.333^{a3d1}	0.723
	200mg/ml	8.67 ± 0.333^{a3}		8.33 ± 0.333^{a3}		8.33 ± 0.333^{a3}	
	400mg/ml	9.67 ± 0.333^{a3b2}		10.00 ± 0.577^{a3b2}		9.33 ± 0.333^{a3b1}	
ol	10µg	20.33±0.333		17.33±0.333		16.67±0.333	
oform	100mg/ml	7.33 ± 0.333^{a3c1d2}	0.750	7.67 ± 0.333^{a3c1d2}	0.754	7.33 ± 0.333^{a3c2d3}	0.750
	200mg/ml	9.67 ± 0.333^{a3b1}		9.67 ± 0.333^{a3b1}		9.67 ± 0.333^{a3b2}	
	400mg/ml	10.67 ± 0.333^{a3b2}		10.67 ± 0.333^{a3b2}		10.67 ± 0.333^{a3b3}	
ol	10µg	21.00±0.577		17.00±0.577		17.33±0.333	
te	100mg/ml	15.33 ± 0.333^{a3c2d3}	0.928	12.67 ± 0.333^{a3c1d3}	0.898	13.33 ± 0.333^{a2c2d3}	0.864
	200mg/ml	17.67 ± 0.333^{a2b2d2}		15.00 ± 0.577^{a1b1d2}		16.33 ± 0.333^{b3c1}	
	400mg/ml	20.33 ± 0.333^{b3c2}		17.67 ± 0.333^{b3c2}		18.33 ± 0.333^{b3c1}	
ol	10µg	20.67±0.333		17.33±0.333		17.00±0.577	
ous	100mg/ml	12.67 ± 0.333^{a3d2}	0.893	10.67 ± 0.333^{a3c1d3}	0.893	13.67 ± 0.333^{a3c1d3}	0.916
	200mg/ml	14.33 ± 0.333^{a3d1}		12.33 ± 0.333^{a3b1d1}		15.33 ± 0.333^{a1b1d1}	
	400mg/ml	16.33 ± 0.333^{a2b2d1}		14.33 ± 0.333^{a2b3c1}		17.33 ± 0.333^{b3c1}	
ol	10µg	20.67±0.667		17.33±0.333		17.33±0.333	

Values expressed as Mean±SEM for n=3. The mean comparisons for different crude methanol extract's solvent fractions and control (Gentamicin 10µg) were performed by one-way ANOVA followed by Tukey HSD Post Hoc multiple comparison test. Where, compared to [a]positive control, [b]100mg/ml, [c]200mg/ml, and [d]400mg/ml. [1]P<0.05, [2]P<0.01, [3]P<0.001.

Table 4: Mean inhibition zone diameter (mm) of *Ricinus communis* Linn leaf crude solvent fractions of methanol extract against gram-negative bacteria.

ent fractions		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
		Mean ± SEM	R^2	Mean ± SEM	R^2	Mean ± SEM	R^2
xane	100mg/ml	7.33 ± 0.333^{a3d2}	0.764	7.33 ± 0.333^{a2d2}	0.795	--	
	200mg/ml	8.33 ± 0.333^{a3}		8.33 ± 0.333		--	
	400mg/ml	10.00 ± 0.577^{a3b2}		9.67 ± 0.333^{b2}		--	
rol	10µg	19.67 ± 0.333		9.67 ± 0.333		17.33 ± 0.333	
roform	100mg/ml	7.67 ± 0.333^{a3c1d2}	0.816	8.67 ± 0.333^{d2}	0.875	8.67 ± 0.333^{a3d2}	0.875
	200mg/ml	9.33 ± 0.333^{a3b1}		9.67 ± 0.333^{d1}		9.67 ± 0.333^{a3d1}	
	400mg/ml	10.67 ± 0.333^{a3b2}		11.67 ± 0.333^{b2c1}		11.67 ± 0.333^{a3b2c1}	
rol	10µg	19.33 ± 0.333		10.00 ± 0.577		17.33 ± 0.333	
l ate	100mg/ml	10.67 ± 0.333^{a3c1d3}	0.898	10.67 ± 0.333^{c1d3}	0.945	10.67 ± 0.333^{a3c2d3}	0.949
	200mg/ml	13.00 ± 0.577^{a3b1d2}		12.67 ± 0.333^{a2b1d2}		13.33 ± 0.333^{a2b2d2}	
	400mg/ml	15.67 ± 0.333^{a2b3c2}		15.67 ± 0.333^{a3b3d2}		16.67 ± 0.333^{b3c2}	
rol	10µg	19.67 ± 0.333		9.67 ± 0.333		17.00 ± 0.577	
eous	100mg/ml	10.33 ± 0.333^{a3d2}	0.764	10.33 ± 0.333^{d2}	0.795	10.67 ± 0.333^{a3d2}	0.843
	200mg/ml	11.33 ± 0.333^{a3}		11.33 ± 0.333^{a1}		11.67 ± 0.333^{a3}	
	400mg/ml	13.00 ± 0.577^{a3b2}		12.67 ± 0.333^{a2b2}		13.33 ± 0.333^{a2b2}	
rol	10µg	19.67 ± 0.333		9.67 ± 0.333		17.00 ± 0.577	

Values expressed as Mean±SEM for n=3. The mean comparisons for different crude methanol extract's solvent fractions and control (Gentamicin 10µg) were performed by one-way ANOVA followed by Tukey HSD Post Hoc multiple comparison test. Where, compared to [a]positive control, [b]100mg/ml, [c]200mg/ml and [d]400mg/ml. [1]P<0.05, [2]P<0.01, [3]P<0.001. No activity = --

Table-5: Mean inhibition zone diameter (mm) of *Ricinus communis* Linn leaf crude extracts and solvent fractions of methanol extract against clinical isolate bacteria.

Crude extracts and Solvent fractions		Clinical *E. coli* isolate		Clinical *S. aureus* isolate	
		Mean ± SEM	R^2	Mean ± SEM	R^2
Methanol	100mg/ml	7.67 ± 0.333^{a3d2}	0.875	9.33 ± 0.333^{a3d2}	0.735
	200mg/ml	8.67 ± 0.333^{a3d1}		10.67 ± 0.333^{a3}	
	400mg/ml	10.67 ± 0.333^{a3b2c1}		12.00 ± 0.577^{a3b2}	
Control	10µg	19.67±0.333		20.33±0.333	
Acetone	100mg/ml	7.67 ± 0.333^{a3d2}	0.875	7.33 ± 0.333^{a3d2}	0.860
	200mg/ml	8.67 ± 0.333^{a3d1}		8.67 ± 0.333^{a3d1}	
	400mg/ml	10.67 ± 0.333^{a3b2c1}		11.00 ± 0.577^{a3b2c1}	
Control	10µg	19.33±0.333		20.33±0.333	
n-hexane	100mg/ml	7.33 ± 0.333^{a3d1}	0.723	7.33 ± 0.333^{a3d2}	0.795
	200mg/ml	8.33 ± 0.333^{a3}		8.33 ± 0.333^{a3}	
	400mg/ml	9.33 ± 0.333^{a3b1}		9.67 ± 0.333^{a3b2}	
Control	10µg	19.33±0.333		19.67±0.333	
Chloroform	100mg/ml	7.33 ± 0.333^{a3d1}	0.723	8.33 ± 0.333^{a3d2}	
	200mg/ml	8.33 ± 0.333^{a3}		9.33 ± 0.333^{a3}	
	400mg/ml	9.33 ± 0.333^{a3b1}		10.67 ± 0.333^{a3b2}	
Control	10µg	19.33±0.333		19.67±0.333	
Ethyl acetate	100mg/ml	11.33 ± 0.333^{a3d2}	0.804	13.00 ± 0.577^{a3c1d3}	0.842
	200mg/ml	13.00 ± 0.577^{a3}		15.67 ± 0.333^{a2b2d1}	
	400mg/ml	14.67 ± 0.333^{a2b2}		17.67 ± 0.333^{b3c2}	
Control	10µg	19.00±0.577		19.33±0.333	
Aqueous	100mg/ml	7.67 ± 0.333^{a3d2}	0.843	12.00 ± 0.577^{a3d2}	0.766
	200mg/ml	8.67 ± 0.333^{a3d1}		14.00 ± 0.577^{a3}	
	400mg/ml	10.33 ± 0.333^{a3b2d1}		15.67 ± 0.333^{a2b2}	
Control	10µg	19.67±0.333		19.67±0.333	

Values expressed as Mean±SEM for n=3. The mean comparisons for different extracts, crude methanol extract's solvent fractions, and control (Gentamicin 10µg) were performed by one-way ANOVA followed by Tukey HSD Post Hoc multiple comparison test. Where, compared to [a]positive control, [b]100mg/ml, [c]200mg/ml, and [d]400mg/ml.[1]P<0.05, [2]P<0.01, [3]P<0.001.

Antifungal activity

Agar well diffusion assay

The assay determined inhibition zone diameter for crude extracts, solvent fractions, and positive control, but not for negative control. The aqueous fraction exhibited the highest inhibition zone diameter of 21 mm, but no inhibition zone diameter observed for n-hexane and chloroform fractions against *C. albicans* (Table 6).

Table 6: Mean inhibition zone diameter (mm) of *Ricinus communis* Linn leaf crude extracts and solvent fractions of methanol extract against fungi.

Test extract and fraction		*Candida albicans*	
		Mean ± SEM	R^2
Methanol extract	100mg/ml	7.33 ± 0.333^{a3d2}	0.735
	200mg/ml	8.67 ± 0.333^{a3}	
	400mg/ml	10.00 ± 0.577^{a3b2}	
Control	20µg/ml	23.33±0.333	
Acetone extract	100mg/ml	7.33 ± 0.333^{a3d2}	0.746
	200mg/ml	8.67 ± 0.333^{a3}	
	400mg/ml	9.67 ± 0.333^{a3b2}	
Control	20µg/ml	22.67±0.333	
n-hexane fraction	100mg/ml	--	
	200mg/ml	--	
	400mg/ml	--	
Control	20µg/ml	23.33±0.333	
Chloroform fraction	100mg/ml	--	
	200mg/ml	--	
	400mg/ml	--	
Control	20µg/ml	23.33±0.333	
Ethyl acetate fraction	100mg/ml	11.33 ± 0.333^{a3b1d3}	0.890
	200mg/ml	13.33 ± 0.333^{a3b1d1}	
	400mg/ml	15.33 ± 0.333^{a3b3c1}	
Control	20µg/ml	22.67±0.333	
Aqueous fraction	100mg/ml	14.67 ± 0.333^{a3c1d3}	0.928
	200mg/ml	17.00 ± 0.577^{a3b1d2}	
	400mg/ml	21.00 ± 0.577^{a1b3c2}	
Control	20µg/ml	23.33±0.333	

Values expressed as Mean±SEM for n=3. The mean comparisons for different extracts, crude methanol extract's fractions, and control (Amphotericin-B 20µg/ml) were performed by one-way ANOVA followed by Tukey HSD Post Hoc multiple comparison test. Where, compared to [a]positive control, [b]100mg/ml, [c]200mg/ml, and [d]400mg/ml. [1]P<0.05, [2]P<0.01, [3]P<0.001. No activity = --

Determination of minimum inhibitory concentration of crude extracts and solvent fractions of methanol extract against pathogenic bacteria.

The minimum inhibitory concentration of methanol crude extract ranging from 6.25mg/ml (*S. aureus*) to 25mg/ml (*E. coli*, *K. pneumoniae*, *P. aeruginosa*) and acetone crude extract ranging from 8.33mg/ml (*S. pyogenes*) to 100mg/ml (*K. pneumoniae*). Also, minimum inhibitory concentration ethyl acetate fraction ranging from 1.5625mg/ml (*S. aureus*) to 12.5mg/ml (*P. aeruginosa*) and for aqueous fraction ranging from 6.25mg/ml (*S. aureus* and *S. pyogenes*) to 66.67mg/ml (*K. pneumoniae*) (Table 7 and 8). The minimum inhibitory concentration for clinical isolate bacteria ranging from 3.125mg/ml of ethyl acetate fraction (*S. aureus*) to 100mg/ml of n-hexane and chloroform fractions (*S. aureus* and *E. coli*) (Table 9).

Determination of minimum bactericidal concentration of crude extracts and solvent fractions of methanol extract.

The MBC of methanol extract ranging from 100mg/ml (gram-positive bacteria) to 200mg/ml (*P. aeruginosa*) and was not detected in *E. coli* and *K. pneumoniae*. The minimum bactericidal concentration of acetone extract was 200mg/ml in gram-positive bacteria, but not detected in gram-negative bacteria. The minimum bactericidal concentration of n-hexane and chloroform fractions were not detected. However, minimum bactericidal concentration ethyl acetate fraction ranging from 25mg/ml (*S. aureus* and *S. agalactiae*) to 200mg/ml (*E. coli* and *K. pneumoniae*), and in clinical isolate bacteria ranging from 88.33mg/ml (*S. aureus*) to 200mg/ml (*E. coli*) (Table 7, 8, 9).

Table7: MIC and MBC of crude extracts and solvent fractions against gram-positive bacteria.

Test extract and fraction	Activities	*S. aureus*	*S. agalactiae*	*S. pyogenes*
		Mean±SEM (mg/ml)	Mean±SEM (mg/ml)	Mean±SEM (mg/ml)
Methanol extract	MIC	6.25±0.000	12.500±0.000	6.25±3.1250
	MBC	100.00±0.000	100.00±0.000	100.00±0.000
Acetone extract	MIC	18.75±6.25	16.67±4.167	8.33±2.083
	MBC	200.00±0.000	200.00±0.000	200.00±0.000
n-hexane fraction	MIC	100.00±0.00	100.00±0.00	100.00±0.00
	MBC	ND	ND	ND
Chloroform fraction	MIC	83.33±16.667	16.67±4.167	16.67±4.167
	MBC	ND	ND	ND
Ethyl acetate fraction	MIC	1.5625±0.00	4.17±1.0417	3.125±0.000
	MBC	25.00±0.000	25.00±0.000	50.00±0.000
Aqueous fraction	MIC	6.25±0.00	12.50±0.000	6.250±0.000
	MBC	200.00±0.000	200.00±.000	200.00±.000

Mean value expressed as Mean±SEM (n=3), ND = not detected

Table 8: MIC and MBC of crude extracts and solvent fractions against gram-negative bacteria.

Test extract and fraction	Activities	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
		Mean±SEM (mg/ml)	Mean±SEM (mg/ml)	Mean±SEM (mg/ml)
Methanol extract	MIC	25.00±0.000	25.00±0.000	25.00±0.000
	MBC	ND	ND	200.00±0.000
Acetone extract	MIC	66.67±16.667	100.00±0.000	66.67±16.667
	MBC	ND	ND	ND
n-hexane fraction	MIC	100.00±0.000	100.00±0.000	NT
	MBC	ND	ND	NT
Chloroform fraction	MIC	83.33±16.667	83.33±16.667	50.00±0.000
	MBC	ND	ND	ND
Ethyl acetate fraction	MIC	4.17±1.0417	6.250±0.000	12.50±0.000
	MBC	200.00±0.000	200.00±0.000	50.00±0.000
Aqueous fraction	MIC	50.00±0.000	66.67±16.667	25.00±0.000
	MBC	ND	ND	200.00±0.000

Mean value expressed as Mean±SEM (n=3) ND = not detected, NT = not tested

Table 9: MIC and MBC of crude extracts and solvent fractions against clinical isolate bacteria.

Test extract and fraction	Activities	Clinical *E. coli* isolate	Clinical *S. aureus* isolate
		Mean±SEM (mg/ml)	Mean±SEM (mg/ml)
Methanol extract	MIC	25.00±0.000	12.50±0.000
	MBC	ND	ND
Acetone extract	MIC	25.00±0.000	12.50±0.000
	MBC	ND	ND
n-hexane fraction	MIC	100.00±0.000	100.00±0.000
	MBC	ND	ND
Chloroform fraction	MIC	100.00±0.000	100.00±0.000
	MBC	ND	ND
Ethyl acetate fraction	MIC	6.250±0.000	3.125±0.000
	MBC	200.00±0.000	83.33±16.667
Aqueous fraction	MIC	66.67±16.667	25.00±0.000
	MBC	ND	ND

Mean value expressed as Mean±SEM (n=3), ND = not detected

Determination of minimum inhibitory and fungicidal concentration of crude extracts and solvent fractions of methanol extract against *Candida albicans*.

The minimum fungistatic concentration of crude extracts and solvent fractions of methanol extract ranging from 3.125mg/ml of the aqueous fraction to 66.67mg/ml of methanol crude extract against *C. albicans*. The minimum fungicidal concentration of solvent fractions of methanol extract ranging from 50mg/ml of the aqueous fraction to 200mg/ml of ethyl acetate fraction. However, the minimum bactericidal concentration of methanol and acetone crude extracts were not detected against *C. albicans* (Table 10).

Table 10: MIC and MFC of crude extracts and solvent fractions against fungi.

Test extract and fraction	Activities	*Candida albicans*
		Mean±SEM (mg/ml)
Methanol extract	MIC	66.67±16.667
	MFC	ND
Acetone extract	MIC	41.67±8.333
	MFC	ND
n-hexane fraction	MIC	ND
	MFC	ND
Chloroform fraction	MIC	ND
	MFC	ND
Ethyl acetate fraction	MIC	16.67±4.167
	MFC	200.00±0.000
Aqueous fraction	MIC	3.125±0.000
	MFC	50.00±0.000

Mean value expressed as Mean±SEM (n=3), ND, = not detected

Preliminary screening of phytochemical constituents of *Ricinus communis* Linn leaf

The results of preliminary phytochemical investigations of *Ricinus communis* Linn leaf crude extracts and solvent fractions is indicated on table11. The phytochemical screening indicated alkaloids, flavonoids, terpenoids, tannins, cardiac glycosides, steroids, anthraquinones, saponins, and phenols in crude methanol extract and ethyl acetate fraction of *Ricinus communis* Linn leaf (Table 11 and 12).

Table 11: Phytochemical constituents of crude extracts and solvent fractions of methanol extract of *Ricinus communis* Linn leaf.

Secondary metabolites	Crude extract		Solvent fractions			
	Methanol	Acetone	n-hexane	Chloroform	Ethyl acetate	Aqueous
Flavonoids	+	+	−	+	+	+
Alkaloids	+	+	+	+	+	+
Saponins	+	−	−	+	+	+
Cardiac glycosides	+	+	−	−	+	+
Terpenoids	+	+	−	−	+	+
Tannins	+	+	+	+	+	+
Steroids	+	−	−	−	+	+
Phenols	+	+	+	+	+	+
Anthraquinones	+	−	−	−	+	−

-, absence, +, presence

Discussion

The current study aimed to investigate antimicrobial activities of crude extracts and solvent fractions of methanol extract of *Ricinus communis* Linn leaf against pathogenic bacteria and *Candida albicans.* However, antibacterial activity had been done by a previous study from Gonder, Ethiopia, but this study did not include antifungal activity and methanol solvent for extraction [16]. Furthermore, the previous findings have been reported that methanol solvent extract exhibited best antimicrobial activities from Ghana and Pakistan [14,15]. Both methanol and acetone crude extracts were also assessed for their antimicrobial activities to select the one which exhibited better antimicrobial activity for further solvent fractionation. There was a difference in the antimicrobial activities of the two extracts for presence and concentration of secondary metabolites could be affected by the type of solvent used for extraction [17].

The current study indicated that ethyl acetate fraction exhibited the highest antimicrobial activities in all tested microorganisms. Crude extracts were tested for their effects against gram-positive and gram-negative bacteria for their antimicrobial activities. Methanol crude extract revealed higher antimicrobial activity than acetone crude extract at the same concentrations. This finding agrees with that of the previous studies of Chandrasekaran and Venkatesalu [34], Naz and Bano [14], and Suurbaar *et al*. [15]. It is payable to the capability of methanol dissolving more secondary metabolites [34]. Methanol and acetone crude extracts exhibited greater antibacterial activities against gram-positive bacteria than gram-negative bacteria in a concentration-dependent manner. This could be because of differences in cell surface structure between gram-positive and negative bacteria. The outer membrane of gram-negative bacteria possesses lipopolysaccharides and lipoproteins. The lipopolysaccharides are amphipathic compounds that comprised hydrophilic polysaccharide at the core that makes up a more rigid outer membrane which slows down the diffusion of hydrophobic compounds through gram-negative bacteria cell membranes and acts as a barrier of permeability [6,7,8].

The solvent fractions of methanol extract exhibited antimicrobial activity in a concentration-dependent manner except for n-hexane fraction that showed no antibacterial activity against *P. aeruginosa.* Hexane and chloroform fractions revealed the lowest antibacterial activity and no antifungal activity. This could be due to variations in the concentration of secondary metabolites present in the solvent fractions [35,36]. Ethyl acetate fraction revealed highest antibacterial activity than crude extracts and solvent fractions which is in agreement with the finding of the previous study done by Voukeng *et al.* [19]. The aqueous fraction exhibited the highest antifungal activity followed by ethyl acetate fraction perhaps due to the capability of ethyl acetate solvent in concentrating a greater number of secondary metabolites from partitioning of methanol crude extract and interaction of these phytochemical constituents. Secondary metabolites with antifungal activity are concentrated more in aqueous solvent of methanol crude extract [35,36,37].

Mean of inhibition zone diameter of crude extracts and solvent fractions were significantly ($P < 0.05$) lower than the mean of inhibition zone diameter of positive control. The reason could be crude extracts and solvent fractions possessed both pharmacologically active and non-active substances whereas, control positive possessed purified and concentrated active ingredient [38]. Contrary to this, mean of inhibition zone diameter of crude methanol extract and aqueous fraction against *S. pyogenes*, crude acetone extract and chloroform fraction against *K. pneumoniae*, and ethyl acetate fraction against all tested gram-positive bacteria, *P. aeruginosa* and clinical *S. aureus* at 400mg/ml displayed inhibition zone comparable to the positive control. Furthermore, the mean inhibition zone diameter of crude methanol extract, ethyl acetate fraction, and aqueous fraction against *K. pneumoniae* at 400mg/ml was significantly ($P < 0.05$) higher than the mean of inhibition zone diameter of positive control. Mean of inhibition zone diameter of crude extracts and solvent fractions against clinical isolate *E. coli* and *S. aureus* were slightly lower than that of laboratory strains *E. coli* and *S. aureus* which is in agreement with the finding of Molla *et al.* [39].The resistance mechanisms such as efflux pumps, β-lactamase production, and biofilm formation could have hindered the effectiveness of antibacterial in clinical isolates than laboratory strains [40,41].

The broth microdilution technique revealed the lowest minimum inhibitory concentration for ethyl acetate fraction against pathogenic bacteria whereas, aqueous fraction against yeast. The experiment indicated that the minimum inhibitory concentration of the broth microdilution technique was inversely proportional to the inhibition zone of the agar well diffusion technique. This is an indication of the reproducibility of an experiment [42]. The ethyl acetate fraction also exhibited minimum bactericidal and fungicidal concentration against all tested microorganisms. Apart from this, n-hexane and chloroform fractions were devoid of bactericidal and fungicidal activity. This could be due to the concentration of higher number secondary metabolites in ethyl acetate fraction than crude extract and solvent fractions despite the detection of phytochemical constituents [36,37].

The maceration technique was performed for the extraction of *Ricinus communis* Linn leaf and yielded a higher percent for methanol crude extract than acetone crude extract. The solvent fractionation yielded a higher percent for aqueous fraction than other solvent fractions. The phytochemical constituents screening revealed the presence of flavonoids, alkaloids, saponins, cardiac glycosides, terpenoids, tannins, steroids, phenols, and anthraquinones in methanol crude extract and ethyl acetate fraction whereas, anthraquinones were not detected in the aqueous fraction. Saponins, steroids, and anthraquinones were absent in acetone crude extract, but only alkaloids, tannins, and phenols were presented in n-hexane fraction. The capacity of methanol solvent in extracting more percent of extract yield and phytochemical constituents is in agreement with the findings of Felhi *et al.* [9] from Tunisia and Truong *et al*. [43] from Vietnam. The variation in types and concentration of phytochemical constituents and percent of extract yield is because of the difference in substance solubility among solvents. The difference in solubility of a substance might be based on the physical and chemical properties of solvents and phytochemical

constituents. Types, quantity, and interactions of secondary metabolites present in crude extracts and solvent fractions are determinants of antimicrobial activities [9,36,44,45]. This study was conducted using small number of pathogenic bacteria and one fungal species which could be a limitation

Conclusion

The methanol extract of *Ricinus communis* Linn leaf constituted all screened secondary metabolites and exhibited the best antimicrobial properties against all tested microorganisms in a concentration-dependent manner. Among the solvent fractions from crude methanol extract, ethyl acetate fraction constituted all screened secondary metabolites and revealed the most pronounced antimicrobial activity than crude extracts and other solvent fractions, but aqueous fraction exhibited better anticandidal activity. The current study supports the claims of use of *Ricinus communis* Linn leaf as traditional medicine for the treatment of infectious diseases caused by bacterial and fungal pathogens. Further studies should be conducted on ethyl acetate fraction to isolate, purify, and identify bioactive principle(s) responsible for antibacterial and antifungal activities of the plant.

List Of Abbreviations

ANOVA, Analysis of Variance; ATCC, American Type Culture Collection; BHI, Brain Heart Infusion; CFU, Colony Forming Units; DMSO, Dimethyl Sulfoxide; MBC, Minimum Bactericidal Concentrations; MFC, Minimum Fungicidal Concentration; MHB, Muller Hinton Broth; MIC, Minimum Inhibitory Concentration; MHA, Muller Hinton Agar; CLSI, Clinical Laboratory Standard Institute; SEM, Standard Error of Mean; SPSS, Statistical Package for Social Sciences

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

The study was approved by Ethics Review Board of School of Pharmacy in Addis Ababa University but no consent was needed.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BK developed proposal; designed and conducted all laboratory experiments; analyzed and interpreted experimental results; developed manuscript.WS supervised the study, and involved in proposal development and manuscript preparations. Authors read and approved the final manuscript.

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