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Research Article

THE ANTIMICROBIAL EFFECT OF *LIPIDIUM SATIVUM* EXTRACT ON SOME CLINICAL ISOLATED BACTERIA FROM UNIVERSITY OF GONDAR TEACHING HOSPITAL

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ABSTRACT

The phenomenon of antibiotic resistance exhibited by the pathogenic microorganisms has led to the need for screening of several medicinal plants for their potential antimicrobial activity. Thus the aim of this study was to assess the antibacterial effect of *Lepidium sativum* seed extracts against some selected clinically isolated, pathogenic bacteria; namely: *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli* and standard microorganisms such as; *Staphylococcus aureus* (ATCC25923), *Streptococcus pneumoniae* (ATCC63), *Escherichia coli* (ATCC2592), *Shigella flexineri* (ATCC12022) as standard controls. Ethanolic, methanolic and chloroform crude extracts of *Lepidium sativum* was evaluated against tested pathogenic bacteria using agar well diffusion method; the inhibitory zones were recorded in millimeters. Tetracycline and Vancomycin were used as positive controls while sterile distilled water was served as negative control. The minimal inhibitory concentration (MIC) of the plant extracts against test bacteria were assessed using agar well dilution and micro-dilution method; and then MBC (minimum bacterial concentration) was evaluated. The inhibition zone of ethanolic, methanolic and chloroform crude seed extracts of *Lepidium sativum* ranged from (19.64-25.68 mm) against *S. pneumoniae* and *S. aureus* (clinical isolates) and were significantly ($p \leq 0.05$) greater than the inhibition zone of other resistant clinical isolates. In the present study, it was observed that ethanolic extract used against almost all tested organism had shown better antimicrobial effect than methanolic and chloroform extracts. Therefore, the findings strongly support the claim of the local community to use *Lepidium sativum* for the treatment of different pathogenic bacterial infections. Further optimization and scientific study is warranted to recommend this extract as antimicrobial agent to be utilized by the public.

Keywords: ***Lepidium sativum*, Agar well diffusion method, MIC, MBC, Antimicrobial activity.**

INTRODUCTION

Our knowledge concerning plants found in our planet is not remaining limited only by identifying them and taxonomically classifying. Beyond this, it goes far in understanding their physiology, biochemistry, mode of reproduction, genetics and their adaptation to new environments as well. In all these achievements and for proper medical utilization of plants by human beings, several scientists from different disciplines and perspectives have contributed a lot. Hence, researchers have so far discovered more than 10,000 medicinal plants with biologically active compounds of microbial origin (Shahidi *et al*., 2004). According to the World Health Organization (WHO), about 65-80% of the world's population in developing nations relies essentially on traditional medicinal plants and traditionally processed products for their primary healthcare because of poverty and lack of access to modern medicine. Particularly in Ethiopia, about 80% of the total population is depending on traditional medicine to treat different types of human ailments. Traditional medicinal practices are common in our country in which about this much of the population in the country use plant based traditional medicine by indigenous knowledge as their major primary health care system. Seemingly therefore, it is possible to say that traditional knowledge of medicinal plants and their use by indigenous healers is serving as an input and a walking step for many researchers to see and go for checking it through their scientific work which is supported by experimental evidences (Berhanu, 2002).

Studies have shown that the use of traditional medicines is not restricted only in developing nations where modern treatment methods are poorly recognized, but also used in developed countries where conventional medicine is predominant in the national health care system (Ansari *et al.*, 2006). It is because of many accounts; traditional systems of medicine continue to be widely practiced all over the world; high population growth, limited supply of drugs, prohibitive cost of treatments, side effects of the existing drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. It is expected that leaves, roots, flowers, and seed extracts of plants are rich in a wide variety of secondary metabolites which are generally appreciated in their anti-microbial activities (Abat, 2001).

Evolution of highly resistant bacterial strains has compromised the use of newer generations of antibiotics (Levy, 2002). Several chemical compounds; synthetic or natural sources show an indirect effect against many species of bacteria, by enhancing the activity of a specific antibiotic, reversing the natural resistance of specific bacteria to given antibiotics, promoting the elimination of plasmids from bacteria such as *Escherichia coli*, and inhibiting transport functions of the plasma membrane in regard to given antibiotics. The enhancement of antibiotic activity or the reversal of antibiotic resistance by natural or synthetic non-conventional antibiotics affords the classification of these

compounds as modifiers of antibiotic activity (Mathias *et al.,* 1994).

Although the active constituents may occur in lower concentrations, plant extracts may be a better source of antimicrobial compounds than synthetic drugs. The phenomenon of additive or synergistic effects is often crucial to bioactivity (Aqil *et al.,* 2006) in plant extracts. The use of extracts as antimicrobial agents shows a low risk of increasing resistance to their action, because they are complex mixtures, making microbial adaptability very difficult and it is also reported to have minimal side effects (Nwankwo, 2010).

Herbs and Spices; the most important parts of human diet are among the traditional medicinal plants used in the world. They synthesize substances that are useful to the maintenance of health in humans and other animals. One of the drawbacks of the fast food life is that everything is stripped away, from the flavor to the nutrition, when they are cooked. People who generally cook at home and use fresh spices and herbs tend to be healthier and have less disease. In addition to boosting flavor, they are also known for their preservative and medicinal value (DeSouza *et al*., 2005). These attributes are useful in the development of snack foods and meat products, and in the production of new drugs with new properties (Shelef, 1983; Giese, 1994).

The hills and mountains of Ethiopia are covered with great number of plant species of which only few are noted for their uses as medicinal herbs or as botanical pesticides. Spices in different parts of the world, are widely employed by the food, pharmaceutical, perfume and cosmetic industries. Here, in Ethiopia, spices and herbs are widely used by the public, they are found almost in every home. In Ethiopia, many herbs and spices are used as home remedies in common illnesses such as cold, cough, fever, influenza, aches and pains, burns and wounds. Except the few mere traditional usage, in Ethiopia majority of these spices and herbs have not yet been studied scientifically for their individual or combined (synergistic) effect against various infections. From herbs: *Lepidium sativum* of which this study has focused on the most widely used traditional herb for the treatment of various bacterial infections. Several reports are available in literature regarding the antimicrobial activity of plant crude extracts from this plant (Kaushi and Dhiman, 2002).

Lepidium sativum, also known as "Pepper cress" or "Elrashad", belongs to the family Brassicaceae (Cruciferae). The vernacular name of *Lepidium sativum* is "Feto" in Amharic and different names are given in different localities at different parts of the country. The leaves are variously lobed and entire, flowers are white small and found in racemes and fruits are obviating pods, about 5 mm long, with two seeds per pods. The plant is eaten and seed oils are used in treating dysentery and diarrhea (Broun and Massey, 1992) and migraine (Merzouki *et al.,* 2000). Its leaves and seeds are considered as one of the popular medicinal herbs used in the community of many countries as a good mediator for bone fracture healing in the human skeleton. A number of recent studies pointed out the antibacterial uses of *Lepidium sativum* seed

extracts in controlling many clinical problems. They were also used as anti-asthmatic antiscorbutic, aperient, diuretic, galactogogue, poultice and stimulant (Merzouki *et al*., 2000).

Materials and Method

Study Area and period

The study was conducted from November 2013 to June 2014 in University of Gondar, Molecular Biology laboratory at the Department of Biotechnology in North West Amhara region, Ethiopia.

Study design

The study design was experimental using appropriate methods such as determination of antibacterial activities, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Collection and Identification of Plant Material

A dried and healthy seed sample of *Lepidium sativum* was collected from a local market of Gondar town, the so called "Arada" in November, 2013. The plant was selected based on the indigenous knowledge of the local traditional medicinal plant healers. A voucher specimen of these plant seed was identified and confirmed by a Botanist in Department of Biology, University of Gondar. The cleaned, washed, dried, and mechanically grinded plant seeds were brought to the research laboratory, Department of Biotechnology, University of Gondar for further investigation.

Preparation of plant extracts

The collected plant seed materials were thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water. The seed sample was dried in the laboratory in an open air at room temperature for about five days and dried in an oven at 45^0C. Once completely dry, these were grounded to a fine powder using mechanical grinder, and the powder was stored in a sterile bottle at room temperature in dark place. The dried and powdered seed of the plant (50g) was extracted with 300 ml ethanol, chloroform and methanol using Soxhlet extractor for 48 hrs at temperature not exceeding the boiling point of the solvent. The extracts were filtered through a sterile Whatman No. 1 filter paper and then were concentrated in a vacuum at 40^0C using a rotary evaporator. Each extract was transferred to glass vials and kept it at 4^0C until use (Shahidi *et al*., 2004).

Sources and Preparation of the Tested Organisms

Microorganisms used in this study included nine different bacterial strains, four strains of Gram-positive (*Staphylococcus aureus* (ATCC25923), *Streptococcus pneumoniae* (ATCC63), Resistant *Staphylococcus aureus* (clinical isolate), and *Streptococcus pneumoniae* (clinical isolate) and five strains of Gram-negative (*Klebsiella pneumoniae* (clinical isolate), *Escherichia coli* (ATCC2592), *Escherichia coli* (clinical isolate), *Klebsiella pneumoniae (*ATCC13883*),* and *Shigella flexneri* (ATCC 12022). These samples were obtained from Department of Biotechnology, University of Gondar and different clinical resistant pathogenic bacteria isolates were collected from Gondar University teaching hospital. The bacterial cultures were maintained in their appropriate agar slants at 4^0C until use.

Media, Chemicals and Solvents Used in the Study

The culture media employed for antibacterial screening in this study were Mueller Hinton Agar, MHA (Oxoid, England), Nutrient Agar Media, tryptic soy broth (TSB), and Nutrient broth (Himedia laboratory, Pvt. Ltd., India). The preparation and treatment of all the culture media were according to the specific manufacturer's instructions. Moreover, the following chemicals and solvents were used during this laboratory work; Barium chloride ($BaCl_2$), distilled water, Methanol (Abron chemicals, batch No. AB 130507), Chloroform (BDH chemicals Ltd, Poole, lot No.27710, England) and Ethanol (Lab Merck chemicals (Ltd batch No.020411, India).

Preparation of Inoculums or bacterial suspensions

The tested microorganisms were separately cultured on nutrient agar at 37^0C for 24 hrs. This was achieved by streaking the inoculating loop containing the bacteria at the top end of the agar plate moving in a zig-zag horizontal pattern until 1/3 of the plate was covered. Then, three to five well-isolated overnight cultured colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a sterile bent wire-loop and the growth was transferred into a screw-capped tube containing 5ml of tryptic soy broth (TSB). The broth culture (test tubes) was incubated without agitation for 24 hrs at 37^0C until it achieves or exceeds the turbidity of the 0.5 McFarland standards. The turbidity of the actively growing broth culture was adjusted with sterile saline to obtain a turbidity optically comparable to that of the 0.5 McFarland turbidity standard 1.5×10^8 colony-forming units (CFU)/ml. To perform this step properly, there was an adequate light to visually compare the inoculum tube and the 0.5 McFarland turbidity standards (Murray *et al*., 2004).

Media Preparation

Mueller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. The mixture of Mueller-Hinton agar powder and sterile distilled water was stirred with a sterilized glass rod and covered with a cotton wool, over which an aluminum foil was tightly wrapped and then autoclaved for 15 min at 121^0C. Soon after autoclaving, the agar was allowed to cool and placed inside a water bath at about 50^0C to maintain the media in a molten stage (to minimize the amount of condensation that forms). Then, the agar medium was allowed to cool to room temperature in the laminar flow hood prior to pouring it into the petri-plate. Plates were dried faster in lower humidity by keeping them in a laminar flow hood. The freshly prepared and cooled medium was poured into flat-bottomed petri-dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This was achieved by pouring 40ml of the large sized plates with diameters of around 200 mm.

Inoculation of Test Plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a small volume about 0.1ml of the bacterial suspension was inoculated onto the dried surface of Mueller-Hinton agar plate and streaked by the sterile cotton swab over

the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60°C each time to ensure an even distribution of inoculum and finally the rim of the agar was swabbed. The lid was left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the crude extracts on the respective well.

Determination of the Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) values of seed extracted from *Lepidium sativum,* was determined based on agar well dilution and broth macro-tube dilution methods. For the determination of MIC, sterile screw-capped test tubes were arranged on a suitable rack in a number of rows and labeled each of them including the negative and positive control test tubes. The seed extract was diluted to concentrations ranging from 3.125% to 50%. Each test tube was containing 10 ml of extract and nutrient broth and 50% meaning that (5 ml of crude extract and 5 ml of nutrient broth), 25% (2.5 ml of extract and 7.5 ml of nutrient broth), 12.5% (1.25 ml of extract and 8.75 ml of nutrient broth), 6.25% (0.625 ml of extract and 9.375 ml of nutrient broth), and 3.125 (0.3125ml of extract and 9.6875 ml of nutrient broth) to bring 10 ml. The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (turbidity) after overnight incubation was recorded as the MIC.

Determination of the Minimum Bactericidal Concentration (MBC)

To determine the MBC, all the agar wells, macro-test tubes used in the MIC, which did not show any visible growth of bacteria after the incubation period were sub-cultured on to the surface of the freshly prepared Mueller Hinton Agar (MHA) plates and incubated at 37°C for 24 hrs. The MBC was recorded as the lowest concentration (highest dilution) of the extract that did not permit any visible bacterial colony growth on the agar plate after the period of incubation (Mueller and Mechler, 2005).

Data Analysis

All the data were analyzed using the program SPSS software package version 16.0 for windows. Means and standard deviations of the triplicates analysis were calculated (analyzed) by one-way analysis of variance (ANOVA) to determine the significant differences between the means followed by Duncan's multiple range test ($P \leq 0.05$). The statistically significant difference was defined as $P \leq 0.05$.

RESULTS

Evaluation of *L. sativum* seed crude extracts against tested bacteria

The diameter of inhibition zone of *Lepidium sativum* extracts of ethanolic, methanolic and chloroform solvents were evaluated against standard and clinical isolated pathogenic bacteria and are given in Table 1.

The mean inhibition zone of chloroform crude seed extract of *L. sativum* (9.60 mm) against *E. coli* (ATCC2592) was significantly ($P \leq 0.05$) less than the ethanolic and methanolic extracts; whereas the inhibition zone (11.05 mm) of the ethanolic extract against *E. coli* (clinical isolate) was significantly ($P \leq 0.05$) greater than the inhibition zone of other extracts (which are

10.19 mm and 9.40 mm). There was no statistically significant difference between the zone of inhibition of methanolic and chloroform extracts of *L. sativum* against *S. aureus* (ATCC25923) but the inhibition zone of ethanolic extract (24.40 mm) against the tested bacteria was significantly (P≤0.05) greater than ethanolic and chloroform extracts. The inhibition zone (13.52 mm) of chloroform extract of *L. sativum* against *S. flexineri* (ATCC12022) was significantly (P≤0.05) less than the rest extracts, but there was no statistically significant difference between the inhibition zones (18.72 mm) of methanolic and ethanolic crude extracts against this test organism. Moreover, there was no statistically significant difference among inhibition zone (17.88-20.25 mm) and (18.35-20.75 mm) of all extracts against *K. pneumoniae* (clinical isolate) and *K. pneumoniae* (MTCC13883), respectively. Whereas the mean inhibition zone (27.38 mm) of ethanolic crude extract of *L. sativum* against *S. pneumoniae* (ATCC63) was significantly (P≤0.05) greater than the methanolic and chloroform solvents which both have no statistically significant difference in each other with inhibition zone of 24.17 mm against this organism. The inhibition zone (25.68 mm) of ethanolic seed extract of *L. sativum* against *S. pneumoniae* (clinical isolate) was significantly (P≤0.05) greater than the inhibition zone of methanolic and chloroform extracts.

Table1.The mean inhibition zone of *Lepidium sativum L.* seed crude extracts with ethanolic, methanolic and chloroform solvents against standard and clinical isolated pathogenic bacteria.

Test Organisms	Solvents for Extraction	Inhibition Zone of Extracts (mm)
E.coli. (ATCC2592)	Et	(11.20 ± 0.35) [a]
	Met	(10.29 ± 0.96) [a]
	Chl	(9.60 ± 1.60) [a]
E. coli (clinical isolate)	Et	(11.05 ± 0.22) [a]
	Met	(10.19 ± 0.94) [a]
	Chl	(9.40 ± 1.65) [a]
S. aureus (ATCC25923)	Et	(24.40 ± 4.10) [bc]
	Met	(21.68 ± 1.64) [bc]
	Chl	(21.68 ± 1.64) [bc]
S. aureus (clinical isolate)	Et	(23.62 ± 3.41) [b]
	Met	(20.65 ± 1.77) [b]
	Chl	(19.64 ± 1.72) [b]
S. flexineri (ATCC12022)	Et	(18.72 ± 0.12) [b]
	Met	(18.72 ± 0.12) [b]
	Chl	(13.52 ± 0.89) [b]
K. pneumoniae (clinical isolate)	Et	(20.25 ± 2.66) [b]
	Met	(18.30 ± 1.83) [b]
	Chl	(17.88 ± 1.60) [b]

S. pneumoniae (ATCC63)	Et	(27.38 ± 1.98) [c]
	Met	(24.17 ± 1.25) [c]
	Chl	(24.17 ± 1.25) [c]
K. pneumoniae (13883)	Et	(20.75 ± 2.66) [b]
	Met	(19.15 ± 2.00) [b]
	Chl	(18.35 ± 1.99) [b]
S. pneumoniae (clinical isolate)	Et	(25.68 ± 1.48) [d]
	Met	(24.17 ± 1.25) [c]
	Chl	(23.54 ± 1.34) [c]

*Values were means of triplicate determinations. Values of the same column followed by different letters are significantly different at ($p \leq 0.05$).

The inhibition zone of ethanolic, methanolic and chloroform crude seed extracts of *L. sativum*, and commercial antibiotic discs against Gram negative and Gram positive pathogenic bacteria is shown on Table 2.

The inhibition zone of antibiotic discs Tetracycline 30µg and Vancomycin 30µg (ZOI 0.00 mm) against the organisms *K. pneumoniae* (clinical isolate), and *E. coli* (clinical isolate) were statistically ($p \leq 0.05$) less than the mean inhibition zones of ethanolic, methanolic and chloroform crude seed extracts of *L. sativum.* Also the inhibition zone of Tetracycline 30µg and Vancomycin 30µg (ZOI 0.00 mm) against *S. pneumoniae* (clinical isolate) as well as the zone (ZOI 13.50 mm) of Vancomycin against *S. aureus* (clinical isolate) were significantly ($p \leq 0.05$) less than the inhibition zone of *L. sativum.*

Table 2. Comparison of inhibition zone of ethanolic, methanolic, and chloroform crude seed extracts of *Lepidium sativum* and commercial antibiotic discs against Gram-negative test bacteria.

Test organisms	Solvents	Diameter of inhibition zone (mm)			
		L. sativum	+ve control		- ve control
			TE	VAN	DW
E. coli (ATCC2592)	Et	(11.20±0.35) [a]	(15.83±0.76)[c]	(29. 67±0.29)[e]	0.00±0.00
	Met	(10.29±0.96) [a]	(15.83±0.76)[c]	(29.67±0.29)[e]	0.00±0.00
	Chl	(9.60±1.60) [a]	(15.83±0.76)[c]	(29.67±0.29)[e]	0.00±0.00
K.pneumoniae (13883)	Et	(20.75±2.66) [b]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00
	Met	(19.15±2.00) [b]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00
	Chl	(18.35±1.99) [b]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00
E.coli (clinical isolate)	Et	(11.05±0.22) [a]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00
	Met	(10.19±0.94) [a]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00
	Chl	(9.40±1.65) [a]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00
S.flexineri (ATCC12022)	Et	(18.72±0.12) [b]	(14.67±0.58)[b]	(25.33±0.58)[d]	0.00±0.00
	Met	(18.72±0.12) [b]	(14.67±0.58)[b]	(25.33±0.58)[d]	0.00±0.00
	Chl	(13.52±0.89) [b]	(14.67±0.58)[b]	(25.33±0.58)[d]	0.00±0.00
K. pneumoniae (clinical isolate)	Et	(20.25±2.66) [b]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00
	Met	(18.30±1.83) [b]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00
	Chl	(17.88±1.60) [b]	(0.00±0.00)[a]	(0.00±0.00)[a]	0.00±0.00

TE: Tetracycline; Van: Vancomycin; DW: Distilled water

Apart from that, the inhibition zone of Tetracycline 30µg and Vancomycin 30µg (ZOI 0.00 mm) against *S. pneumoniae* (clinical isolate) as well as the zone (ZOI 13.50 mm) of Vancomycin against *S. aureus* (clinical isolate) were significantly ($p \leq 0.05$) less than the inhibition zone of *L. sativum* as shown below in Table 3.

Table 3. Comparison of inhibition zone of ethanolic, methanolic, and chloroform crude seed extracts of *Lepidium sativum* and commercial antibiotic discs against Gram positive test bacteria.

Test organisms	Solvents	Diameter of inhibition zone (mm)			
		L. sativum	positive control		negative control
			TE	VAN	SDW
S. aureus (ATCC25923)	Et	24.40±4.10 ^{bc}	(19.00±1.00)^{d}	(21.00±1.00)^{c}	0.00±0.00
	Met	21.68±1.64 ^{bc}	(19.00±1.00)^{d}	(21.00±1.00)^{c}	0.00±0.00
	Chl	21.68±1.64 ^{bc}	(19.00±1.00)^{d}	(21.00±1.00)^{c}	0.00±0.00
S. pneumoniae (clinical. isolate)	Et	25.68±1.48 ^{d}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	0.00±0.00
	Met	24.17±1.25 ^{c}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	0.00±0.00
	Chl	24.17±1.25 ^{c}	(0.00±0.00)^{a}	(0.00±0.00)^{a}	0.00±0.00
S. pneumoniae (ATCC63)	Et	27.38±1.98 ^{c}	(20.67±1.61)^{e}	(25.50±1.50)^{d}	0.00±0.00
	Met	24.17±1.25 ^{c}	(20.67±1.61)^{e}	(25.50±1.50)^{d}	0.00±0.00
	Chl	24.17±1.25 ^{c}	(20.67±1.61)^{e}	(25.50±1.50)^{d}	0.00±0.00
S. aureus (clinical isolate)	Et	23.62±3.41 ^{b}	(19.00±1.00)^{d}	(13.50±0.32)^{b}	0.00±0.00
	Met	20.65±1.77 ^{b}	(19.00±1.00)^{d}	(13.50±0.32)^{b}	0.00±0.00
	Chl	19.64±1.72 ^{b}	(19.00±1.00)^{d}	(13.50±0.32)^{b}	0.00±0.00

Key: Te= Tetracycline, Van= Vancomycin, Dw=Distilled water

Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different concentration of crude seed extract of *L. sativum*,(v/v) is given in Figure 1.

The minimum bactericidal concentration (MBC) of ethanolic, methanolic and chloroform crude seed extracts of *L. sativum* against both *E.coli* (ATCC2592) and *E.coli* (clinical isolate), and chloroform extract of this seed against *S. aureus* (ATCC25923) were 25%.

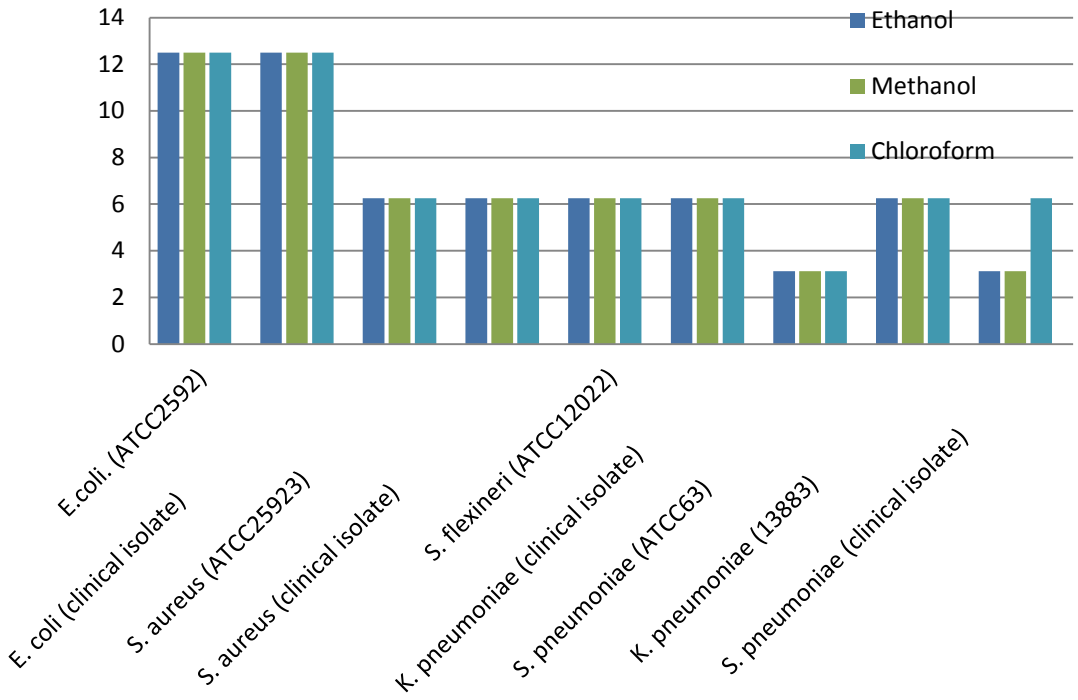

Fig. 1.MIC determination of ethanolic, methanolic and chloroform crude seed extracts of *L. sativum* against standard and drug resistant clinical isolated pathogenic bacteria.

Key**:** *E. coli=Escherichia coli, S. aureus=Staphylococcus aureus, S. flexineri=Shigella flexineri, K. pneumoniae=Klebsiella pneumoniae, S. pneumoniae=Streptococcus pneumoniae.*

The MBC of the three solvents of *L. Sativum* is given in figure 2 below. The MBC of all solvents of *L sativum* seed extracts against *K. pneumoniae* (ATCC13883), *K. pneumoniae* (clinical isolate), and *S. aureus* (clinical isolate), ethanolic and methanolic extracts against *S. aureus* (ATCC25923), methanolic and chloroform extracts against *S. flexineri* (ATCC12022), and chloroform extract of this seed against *S. pneumoniae* (clinical isolate) were 12.5%. Moreover, the minimum bactericidal concentration (MBC) of ethanolic extract against *S. flexineri* (ATCC12022), methanolic extract against *S. pneumoniae* (clinical isolate), methanolic and chloroform extracts against *S. pneumoniae* (ATCC63) of *L. sativum* seed were 6.25%. But the MBC of ethanolic extract of *L. sativum* against both *K. pneumoniae* (ATCC13883) and *K. pneumoniae* (clinical isolate) were 3.125%.

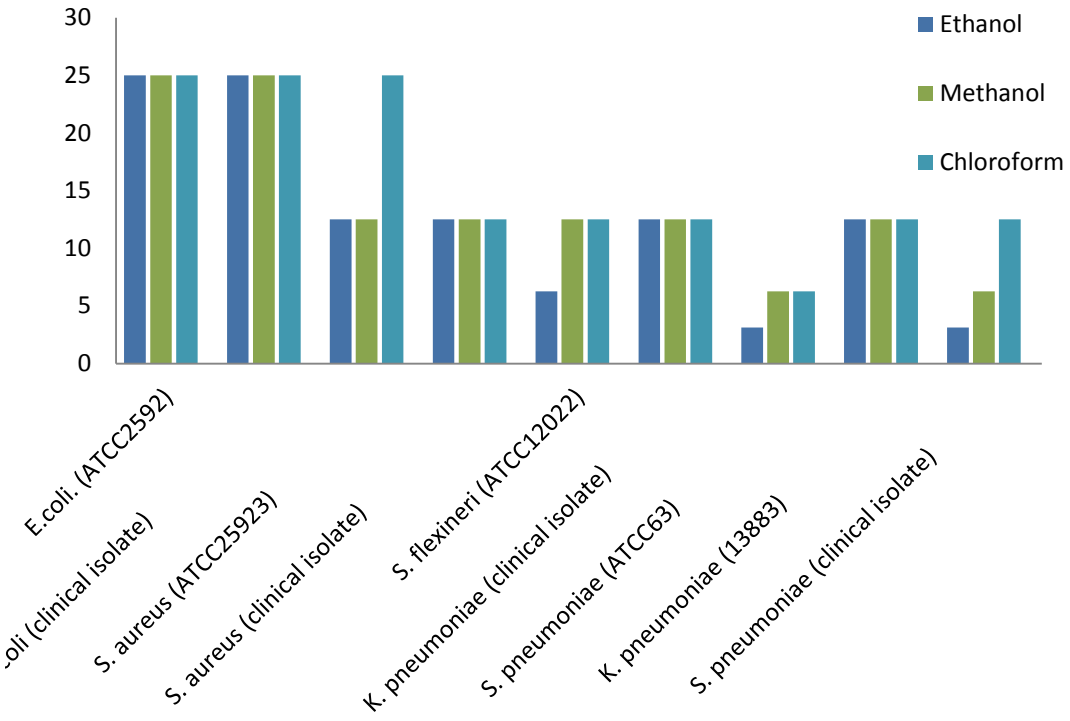

Fig. 2. MBC determination of ethanolic, methanolic and chloroform crude seed extracts of *L. sativum* against standard and drug resistant clinical isolated pathogenic bacteria.

DISCUSSION

A survey by UNCTAD (United Nations conference on trade and development) has shown that 33% of total drugs produced by the industrialized nations are plant derived, and 60% of medicinal products are of natural origin (UNCTAD, 1974). Rigveda mentions 67 plants having therapeutic effects, Yajurveda lists 81 plants and Atharveda 290 plants (Nabachandra and Manjula, 1992). The world health organization recently compiled an inventory of more than 20,000 species of medicinal plants. Medicinal plants and their products are used to control diverse disease such as catarrh, bronchitis, pneumoniae, ulcers and diarrhea. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Galal *et al.*, 1991; Hoffmann *et al.*, 1993). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not yet been adequately evaluated (Balandrin *et al.*, 1985).

The introduction of modern medicine to Ethiopia dates back to the 16th century during the regime of Emperor Libne Dingel (1508-1540). The first government run modern health care was established in 1906 with the opening of Menelik II Hospital in Addis Ababa. Since then the government has taken the formal responsibility of delivering health care to the population, and health institutions were established in the different regions of the country. However, the growth and development of modern

health care in Ethiopia as a whole has been very stunted and to date, its coverage is less than 50% of the population. The vast majority of the rural populations, therefore, still depend on traditional medicines and its practitioners (Yirga, 2010). Bacterial infection is one of the most serious global health issues in 21stcentury (Morris, and Masterton, 2002). A large number of these bacterial species have become resistant to antibacterial drugs and causing a number of infectious diseases. Therefore, it is critical to develop new antibiotics with novel mechanism of action to overcome these problems (Wang *et al.*, 2003). The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatments. *L. sativum* is one of the most commonly used natural antimicrobial agents and has been used traditionally for controlling many different pathogenic bacterial infections. This plant used in this study was based on the locally available prior information in treating different bacterial diseases. The three crude extracts viz. ethanolic, methanolic, and chloroform of *L. sativum* were tested against various Gram positive and Gram negative pathogenic bacteria. It can be inferred from the present study that the extracts of *L. sativum*, especially the ethanolic extract, had maximum antibacterial activity, which is consistent with the works of Parekh and Chanda (2008). The inhibition zone of crude seed extracts of *L. sativum* against most tested Gram positive pathogenic bacteria were significantly ($p \leq 0.05$) greater than the inhibition zone obtained by standard antibiotics. This may be explained, perhaps due to the development of antibiotic resistance adaptation of these clinically isolated bacteria. However, the antimicrobial effect of the extract indicated less antimicrobial effect against most Gram negative bacteria.

The mean inhibition zones of *L. sativum* crude seed extract against all tested pathogenic bacteria were significantly ($p \leq 0.05$) greater than the inhibition zones of the commercial antibiotic discs (Tetracycline 30µg and Vancomycin 30µg). And also the mean inhibition zones of commercial antibiotic discs against all clinical isolated pathogenic bacteria except Vancomycin for resistant *S. aureus* were 0.00 mm. This showed that they were resistant against used commercial antibiotics. Therefore, *L. sativum* crude seed extract has necessary secondary metabolites that have effect on the growth and multiplication of these pathogenic bacteria. However, to treat patients with this plant extract further optimization of the plant extract is essential.

There is shortage of published material concerning the effect of *L. sativum* crude extract against pathogenic bacteria elsewhere in the literature and thus it becomes very difficult to compare the results of this study with other researchers.

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