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# Antibacterial activity of Ethiopian *Lepidium sativum* L. against pathogenic bacteria

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***Lepidium sativum*** **is commonly known as "fetto" in Ethiopia, and a popular herbal plant which is widely used in folk medicine. The objective of this study is to investigate the potential of three different crude solvent extracts from seed of *Lepidium sativum* (ethanol, methanol and chloroform) against human pathogenic bacterial strains: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC-27736), *Pseudomonas aeruginosa* (ATCC-27853), *Staphylococcus aureus* (ATCC-25923) and *Shigella sonnie* (ATCC-25931) using agar well diffusion assay, and the test results were compared with standard antibiotics. This study revealed that the ethanol and methanol extracts showed maximum antibacterial activity against *E. coli* (ATCC 25922) with zone of inhibition mean value of 22.63±0.7 mm. The methanol extract showed minimum antibacterial activity against *P. aeruginosa* (ATCC-27853) with zone of inhibition mean value of of 9±0.3 mm. Among the extracts, ethanol has a higher minimum inhibitory concentration (MIC) with inhibition value ranges of 6.25 to 12.5 mg/ml than other solvents extract. The results suggest that ethanolic and methanolic extracts of *L. sativum* could be used for treatment of infectious diseases caused by *E. coli* and *P. aeruginosa* strains. Hence, further investigation of biochemical elements of the ethanolic and methanolic extracts and understanding of the genetic mechanisms of resistance will be beneficial.**

**Key words:** Antibacterial activity, extract, *Lepidium sativum*, minimum inhibitory concentration (MIC), zone of inhibition.

## INTRODUCTION

Infectious diseases are major concern due to resistance of bacterial pathogens to the existing drugs or antibiotics. Medicinal plants with identified antimicrobial properties have a significant role in the treatment of infectious diseases (Cowan, 1999). The challenge of microbial resistance is growing, and in the future the use of antimicrobial drugs is uncertain. Hence, to overcome this problem, there is need to study the genetic mechanism of resistance and develop novel drugs (Nascimento et al., 2000).

Medicinal plants are the cheap and safe alternative sources for the prevention against antimicrobial infections (Pretorious and Watt, 2001; Sharif and Banik, 2006). *Lepidium sativum* L. belongs to the family, Brassicaceae;

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in English, it is called "Garden cress" which is commonly used in folk medicine for the treatment of hyperactive airways disorders, such as asthma, bronchitis and cough (Najeebur et al., 2012).

In Ethiopia, it is known as "fetto" which is cultivated for its medicinal value, edible oil is also gotten from its seed. *L. sativum* is commonly grown in Ethiopia as a garden plant and found in any market, though usually in small quantities (Amare, 1976).

Hence, it is very essential for its novel antibacterial sources which is a continued process. The treatment of infectious disease caused by bacterial strains has become challenging, and to discover effective drugs is ever increasing. Study on antimicrobials based on plant has arisen during the past years, but antimicrobials based on plant are poorly explored. The antimicrobial activity of plants extract especially higher plants has shown a potential source of novel drug compound (Press, 1996).

A study demonstrated the protective action of *L. sativum* against carcinogenic compounds and growth inhibition of *Pseudomonas aeruginosa*, a bacterial strain with effective antibiotic resistance (Gupta et al., 2010).

The *L. sativum* plant leaves is a stimulant and diuretic, used for the treatment of scorbutic disease and hepatic complaints (Maghrani et al., 2005; Ahmed et al., 2009; Raval and Pandya, 2009). Ethno-medicinally, leaves of *L. sativum* are used as vegetables, salad and curries (Kiple and Ornelas, 2000; Moser et al., 2009). The seeds of *L. sativum* contain different imidazole alkaloids, flavonoids, glycosides, sterols, coumarins, sulphur and triterpenes (Agarwal and Verma, 2011; Datta et al., 2011; Radwan et al., 2007).

Screening of ethno botanical have been found to offer traditionally used folk medicine in modern drug formulation, and also giving information on the importance of traditional medicine. Thus, this study showed the effect of traditionally used medicinal plant *L. sativum* which contributes to scientific evidence on studied pathogenic tested bacterial.

Despite its antimicrobial uses, there is limited study on antimicrobial activities of Ethiopian garden cress (*L. sativum*) on pathogenic bacterial strains. Hence, this study investigated antibacterial activity of *L. sativum* against bacterial strains including; *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella sonnie* and *Staphylococcus aureus*. This study aimed to evaluate the *L. sativum* seed extracts against antibacterial characteristics of pathogenic bacteria of human.

## MATERIALS AND METHODS

### Experimental material

The seed sample of *L. sativum* was purchased from local market of Wolkite, Ethiopia during April, 2016. The sample was confirmed by a botanist, and brought to Microbiology Laboratory of Wolkite University.

### Preparation of seed extract

About 50 g of *L. sativum* seed sample was measured, cleaned with distilled water and ground by mortal and pestle. The seed extract was prepared with three different extraction solvents: chloroform, ethanol and methanol. About 50 g of seed powder *L. sativum* was mixed with 150 mL different solvent successively. The extraction of extracts was done by orbital shaker for three consecutive days. Then, the extract was filtered with Whatman No. 1 filter paper, and the filtrate evaporated in an oven at 60°C for 2 h, and powdered form obtained. Finally, the dry weight of each extract was measured using electronic balance. The stock solution of about 100 mg/mL concentration was prepared, autoclaved, vortexed and kept at 4°C in refrigerator (Huynh et al., 2001; Bhasin et al., 2012). However, the crude extract of chloroform was dissolved by microwave and distilled for 6 min with an interval of 3 min and continuous separation.

### Preparation of test organisms and sensitivity test

Standard culture of five strains namely, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. aureus* and *S. sonnie* were obtained from Ethiopian Public Health Research Institute, Addis Ababa, Ethiopia. Muller Hinton agar medium was prepared, and the test organism was grown at 37°C for 24 h. About 38 g of Muller Hinton Agar was dissolved in 1000 ml distilled water. Then, the solution was autoclaved under 121°C for 15 min. According to Andrews (2006), the standard 0.5 McFarland was prepared in saline solution.

### Evaluation of antimicrobial assay

A total of five strains namely, one Gram positive bacterial strain *S. aureus* (ATCC 25923) and four Gram negative bacterial strains namely, *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. sonnie* (ATCC 25931) and *K. pneumoniae* (ATCC 27736) were used for the susceptibility test.

The agar well diffusion method was used to determine the antibacterial activity against the seed extracts of ethanol, methanol and chloroform (Taye et al., 2011). These strains were maintained in suspension media at 4°C, and fresh inoculums were taken from the media to test the antimicrobial activities of seed extracts.

Then, inoculums were dispersed on solidified nutrient agar media consistently by using sterilized cotton swap. Three equidistant wells were prepared using sterilized cork borer with a 6 mm diameter. About 50 mg/ml concentration of plant extract was prepared, and from each extract 100 µl was aseptically transferred to a particular well. The positive controls were ampicillin (10 µg/ml) and vancomycin (30 µg/ml), and a negative control was distilled water.

Then, agar media was kept under laminar flow hood for 30 min then incubated at 37°C for 24 h. The formation of clear inhibition zone around the wells of about ≥ 7 mm diameters were taken as significant susceptibility measurement. The experimental study was laid down in a completely randomized design with three replications. The mean value and standard deviation value was used for analysis.

### Minimum inhibitory concentration determination

The determination of minimum inhibitory concentration (MIC) involves the lowest concentration of the extracts that showed inhibition zone of ≥ 7 mm diameter to inhibit the growth of tested microorganism (Guerin-Faublee et al., 1996). The double serial

**Table 1.** Means of inhibition growth diameter obtained by seed extract of *L. sativum* on selected bacterial strains.

| Test organism | Inhibition zone (mm) Mean+S.D | | | | Control | |
|---|---|---|---|---|---|---|
| | Water extract | Ethanol extract | Methanol extract | Chloroform extract | A30 | V30 |
| *Escherichia coli* (ATCC 25922) | 0.0 | 22.63±0.7 | 22.37±0.7 | 10.67±0.5 | NA | 18 |
| *Klebsiella pneumoniae* (ATCC 27736) | 0.0 | NA | NA | NA | 8 | 12.5 |
| *Pseudomonas aeruginosa* (ATCC 27853) | 0.0 | 10±0.5 | 9±0.3 | 11.33±0.5 | NA | NA |
| *Staphylococcus aureus* (ATCC 25923) | 0.0 | NA | NA | NA | 8 | 12 |
| *Shigella sonnie* (ATCC 25931) | 0.0 | NA | NA | NA | 9 | 20 |

*Statistically significant: P<0.05; (one way ANOVA). A30 = Ampicillin; V30 = Vancomycin; *****NA**-** not available.

**Table 2.** Minimum inhibitory concentration of ethanol extract of *L.sativum* against selected microbial strains.

| Test organism | Gram type | Minimum inhibitory concentration (mg/ml) |
|---|---|---|
| *Escherichia coli* (ATCC 25922) | - | 6.5 mg/ml |
| *Klebsiella pneumoniae* (ATCC 27736) | - | NA |
| *Pseudomonas aeruginosa* (ATCC 27853) | - | 12.5 mg/ml |
| *Staphylococcus aureus* (ATCC 25923) | + | NA |
| *Shigella sonnie* (ATCC 25931) | - | NA |

*****NA**-** Not available.

dilution was used to prepare 25, 12.5, 6.25, 3.125 and 1.56 mg/ml, respectively, using distilled water, and 100 µl of diluted solvent extract was transferred to well on prepared media as it was done for sensitivity test followed by identifying MIC level. The control was prepared without inoculation with the test microbial strains.

#### Statistical analysis

The antibacterial activity of the extract data was collected, and then analyzed by using statistical analysis software SPSS ver. 16.0. The differences and response among test bacterial strains was presented by mean ± standard deviation (SD), and one-way ANOVA. The statistical significant test was done at a level of p<0.05.

## RESULTS

The present study results revealed that *L. sativum* seed extracts has a potential antibacterial activity against certain tested organisms as indicated in Table 1. The water extract used as negative control did not exhibit antibacterial activity against all the tested organisms. The growth of Gram positive bacteria, *S. aureus* (ATCC 25923) and Gram negative bacteria including *S. sonnie* (ATCC 25931) and *K. pneumoniae* (ATCC 27736) growth was not inhibited by methanol, ethanol and chloroform solvent extracts.

However, the ethanol, methanol and chloroform showed higher (P<0.05) inhibition against Gram negative bacteria, *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) as shown in Table 1. Among the extracts, ethanol and methanol showed maximum zone of inhibition of 22.6 mm against *E, coli* (ATCC 25922), while the minimum zone of inhibition was obtained for *P. aeruginosa* (9 mm). The chloroform extract showed the maximum zone of inhibition (11.33 mm) against *P. aeruginosa*, while the minimum zone of inhibition value of 10.67 mm was observed for *E. coli*.

The minimum inhibitory concentration test result when compared with standard antibiotics, ampicillin 10 µg/ml and vancomycin 30 µg/ml, is shown in Table 1. In comparison with the standard antibiotics, extract of ethanol exhibited higher inhibition value than vancomycin 18 mm against *E. coli* and 12.5 mm inhibition zone on *P. aeruginosa*. Similarly, in the minimum inhibition zone of negative control with water, there was no inhibition; hence the data were not incorporated.

The MIC test was conducted only for higher inhibition value indicated by ethanol extract solvent that is used in this study (Figure 1). The MIC value of ethanol extracts range between 6.5 and 12.5 mg/ml on *E. coli* and *P. aeruginosa* as shown in Table 2.

## DISCUSSION

The extracts of *L. sativum* antimicrobial activity are affected predominantly by the type of bacterial strain and extraction solvent. The extracts of ethanolic, methanolic and chloroform inhibited the growth of certain Gram negative bacteria strains including *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) as indicated in Tables

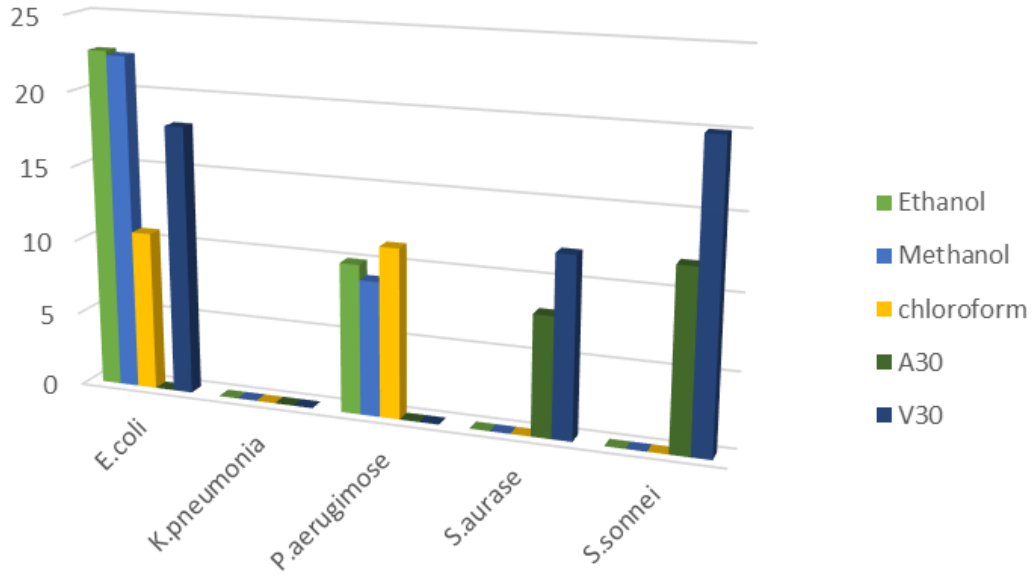


**Figure 1.** Mean inhibition zone (mm) of three solvent extracts and standard antibiotics against tested bacteria

1 and 2. Previously, Hammer et al. (1999) reported that essential oils and plant extract of various medicinal plants showed antibacterial activity on *E. coli* and *P. aeruginosa*.

Previously, Majhenic et al. (2007) reported that methanol and ethanol possessed better antimicrobial activity of medicinal plants. Also, El-Safey and Salah (2011) described a better antimicrobial agent from organic extraction solvent including methanol and ethanol. Therefore, the results indicated in this study are supported by that of previous studies. In correlation with Gupta et al. (2010), antimicrobial activity of *L. sativum* against food borne bacteria was evaluated by agar well diffusion method using chloroform, ethyl acetate, methanol and dichloromethane solvents. The result of the study indicated that the MIC values of the solvent methanol extract ranged from 1.56 to 25.0 mg/ml.

According to Vaghasiya and Chanda (2007), the antibacterial activity of plant extract against Gram negative bacteria might be due to the source of broad-spectrum antibiotic compounds in the plant. Similarly, Ahmet et al. (2004) studied another medicinal plant and revealed that the antibacterial action was comparable to positive control. The highest antibacterial action of solvent including methanol and ethanol extract against bacterial strain may be due to the ability of the solvent to extract some semi polar dissolved component of plants that have active properties.

Therefore, *L. sativum* seed extract antibacterial activity may be related to their ability to inactivate cell envelope transport proteins, enzymes, microbial adhesins, and may be complex with polysaccharides (Ya et al., 1998). Previously, Cowan (1999) reported that the highest antibacterial effect of ethanol and methanol extract were due to the presence of some active compounds of plants like phenolic compounds (flavonoids) and other secondary m etabolite.

Generally, crude plant extracts are a mixture of different active and non-active compounds against bacterial strains. Parekh and Chanda (2007) reported different antibacterial action, antifungal and inflammatory properties of medicinal plants based on various parameters to ensure their activity and efficacy. Among these properties of medicinal plants, some of them have facilitated in isolation and characterization of the active compounds for the development of drugs for therapeutics.

According to Muktanjali et al. (2005), human mortality rate was mainly due to infections caused by *S. aureus, P. aeruginosa, K. pneumonia, E. coli* and *S. sonnie* (Arya et al., 2005). Also, treatment of infections caused by these bacterial strains is challenging. Hence, the challenge to discover newer and effective drugs is increasing.

Therefore, this study was undertaken to test the extracts of *L. sativum* against these pathogens. Among different extract solvent, the highest antibacterial activity was observed for ethanol and methanol solvent extracts. However, ethanol and methanol solvent extracts did not show any activity on *P. aeruginosa*. Thus, this study ascertains value of *L. sativum* seed extracts which is used for treatment. Henceforth, this could be important for the development of drugs.

Future studies on antimicrobial compounds of *L. sativum* are required to characterize the active compounds. Furthermore, the antimicrobial property of *L. sativum* needs to be assessed and characterized, and

the detail of suitability for treatments of infectious diseases caused by bacterial pathogens needs to be investigated.

## Conclusion

Based on the result, ethanolic and methanolic extracts of *L. sativum* can be used in the treatment of infectious diseases caused by *P. aeruginosa* and *E. coli* strains. The anti-bacterial action of *L. sativum* may be attributed to various active compounds, and constituents presented either due to their separate or collective action. Thus, the study establishes the significance of *L. sativum*, and this is the preliminary work of antibacterial activity of *L. sativum*. Therefore, biochemical characterization of extract is needed to design the drug. Moreover, there is need to study the genetic mechanisms of resistance of antibiotics for the development of potent drug.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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