INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 13, No. 1, p. 44-51, 2018
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Evaluation of the antibacterial potential of *Eruca sativa* Mill. (Brassicaceae) *in vitro*

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Article published on July 27, 2018

Key words: Antibacterial, Well diffusion method, *E. sativa*, Medicinal plants, Crude extract

Abstract

The alarming increase in antibiotic resistance is a threat to primary healthcare worldwide. Thus, it is very important to investigate the antimicrobial properties of medicinally important plants for the discovery of natural, non-antibiotic based alternative therapies. Leaves and stem crude extracts (chloroform, ethyl acetate and aqueous) of *E. sativa* Mill. Were screened at four concentrations (0.5, 1.0, 1.5, 2.0 mg/ml) against four pathogenic bacterial strains using standard well diffusion method. Results reveal that *E. sativa*ethyl acetate stem extract (2mg/ml) had shown maximum inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* with 20.47mm, 16.44mmand 15.24mm zone of inhibition respectively whereas; against *Klebsiella pneumoniae* both ethyl acetate stem extract and chloroform leaves extract had shown maximum inhibition with 19.24 and 18.24mm zone of inhibition respectively. Considerable inhibition had been shown by *E. sativa* chloroform and aqueous stem extract at a high concentration of 2mg/ml against pathogenic bacterial strains. The overall inhibition was concentration dependent and showing a progressive decrease in inhibition with a decrease in concentration from 2mg/ml to 0.5mg/ml. The present work determined the medicinal and antibacterial importance of *E. sativa*.

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Introduction

Medicinal plants are the main source of natural medicines for primary health care in many nations. They treat and prevent various human as well as livestock illnesses. A medicinal plant is any plant, which contains active ingredients that are used directly for healing purposes or can be used for the preparation of new herbal drugs (Sofowara, 1993). Plant-based folk medicine plays a key role in natural drug discovery (Wright, 2005). Ethno-pharmaceutical studies are the methods for identifying new sources of herbal drugs. Currently, more than 80% population of the world still depends on plant-derived health care products for daily regime due to their effectiveness, no side effect, easily accessible and affordable prices (Hassan *et al.*, 2009; Gangadhar *et al.*, 2012). Generally, from the bulk of 258,650 species of higher plants more than 10 percent are used medicinally (Choudhary *et al.*, 2005). *E. sativa* Mill.is an edible, annual herb (Brassicaceae-mustard family) with erect, simple or branched stem usually covered with hairs (Jafri, 1973). Locally it is known as Rocket salad; *G*arden salad (Rani *et al.*, 2010) or Tarmira (Rani *et al.*, 2010; Gulfraz *et al.*, 2011). It is mostly cultivated for its oil and edible leaves. It is native to Mediterranean region and Central Asia (Pimpini and Enzo, 1996; Uğur *et al.*, 2010). It is also found in India and Pakistan (Gulfraz *et al.*, 2011).The therapeutic potentials of medicinally important plants are usually attributed to the presence of natural products also known as secondary metabolites (Westh *et al.*, 2004).The common secondary metabolites present in leaves and seed of *E. sativa* including flavonoids (Michael *et al.*, 2011), alkaloids, cardiac glycosides, saponins, tannins, phenolic compounds (Gulfraz *et al.*, 2011; Hussein *et al.*, 2013) and essential oil (Miyazawa *et al.*, 2002). Fresh *E. sativa*also contains glucosinolates (Bennett *et al.,* 2006;Antuono*et al*, 2008), erucin and erysolin (Lamy *et al.*, 2008).These secondary metabolites have excellent therapeutic values due to their tremendous biological activities (Dionisi, 2012)such as antifungal, antibacterial, antioxidants, antidiabetic and anticarcinogenic (Missiry *et al.,* 2000; Alam *et al.,* 2007).

Traditionally *E. sativa* is used as a stringent, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient, stimulant (Perry *et al.,* 1978; Yaniv *et al.,* 1998), aphrodisiac and eyes infection (Yaniv *et al.,* 1998).The present study was aimed to evaluate the inhibition potential of *E. sativa* Mill. against four common pathogenic bacterial strains.

Materials and methods

Collection and processing of plant materials

Healthy leaves and stem of *E. sativa* were collected from Village Rajjar, District Charsadda, Pakistan, (34-03' and 34-38' North latitudes and 71-28' and 71-53' East longitude). The plant parts were washed properly with sterilized water and shad dried for 4 weeks at room temperature. The complete dried plant parts were grounded into powder form using homogenizer. Powders were stored in plastic bags for experimental purposes.

Preparation of crude extract

About 50g powder of leaves and stem were soaked in 200 ml ethyl acetate, chloroform, and water using clean and sterilized beakers. They were incubated for 2 weeks at room temperature 25°C). The mixture was filtered twice after 14 days of extraction using Whatman-41 filter paper and the extract was reduced till dryness via rotary evaporator. The material was then stored in small bottles.

Bacterial strains used

In the present study, four pathogenic bacterial strains were used. Among the selected pathogenic strains *S. aureus* and *B. subtilis* were gram-positive whereas; *K. pneumoniae* and *P. aeruginosa* were gram negatives. The bacteria were maintained on nutrient agar at 4°C.

Standard drug (Positive control)

Chloramphenicol (1.0 mg/ml) was used as a positive control against the selected pathogenic bacterial strains.

Preparation of seeded agar plates

Nutrient agar medium was prepared by adding nutrient agar (MERCK) 2.3g in 100ml of distilled water; pH was adjusted at 7.0 and sterilized autoclave at 121°C.

It was allowed to cool up to 45°C. Petri plates were prepared by pouring 75ml of seeded nutrient agar and allowed to solidify. Four wells per plate were made with a sterile cork borer (5mm).

Pouring of test solutions, incubation, and measurement of inhibition zones

Using micropipette, 100µl of test solutions was poured into respective wells. These plates were incubated at 37°C. After 24 hours of incubation, the diameter of the clear zones of inhibitions was measured by a ruler. Antibacterial activity of 4 dilutions of each plant part extract was determined against four bacterial strains.

Statistics

The clear zones of inhibition were measured in millimeter. All the data values are the means and standard deviations of 3 replicates which were tubulated as a Mean ± standard deviation.

Results

Inhibition potential against S. aureus

The result revealed that *E. sativa* ethyl acetate stem extract showed maximum inhibition zone as 20.46mm against *S. aureus* at 2mg/ml concentration. Considerable inhibition effect has been shown by *E. sativa* chloroform and aqueous stem extract (2mg/ml) with 18.13 and 17.7mm inhibition zone respectively.

Table 1. Inhibition potential of *E. sativa* Mill. against *S. aureus.*

Solvent extracts	2.0 mg/ml	1.5 mg/ml	1.0 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Standard drug 1.0mg/ml	26.47 ± 0.16	24.06 ± 0.28	23.92 ± 0.25	22.22 ± 0.02
Chloroform leaves extract	19.47 ± 0.19	16.06 ± 0.11	12.92 ± 0.04	11.89 ± 0.09
Ethyl acetate leaves extract	14.13 ± 0.23	8.39 ± 0.21	8.25 ± 0.03	8.22 ± 0.07
Aqueous leaves extract	7.80 ± 0.05	6.06 ± 0.10	6.25 ± 0.07	6.22 ± 0.15
Chloroform stem extract	18.13 ± 0.07	12.06 ± 0.14	10.25 ± 0.01	8.89 ± 0.04
Ethyl acetate stem extract	20.47 ± 0.05	14.39 ± 0.14	12.25 ± 0.19	10.56 ± 0.04
Aqueous stem extract	17.80 ± 0.06	15.06 ± 0.33	12.25 ± 0.15	10.56 ± 0.10

Keys; Standard drug= Chloramphenicol, Mean ± SD= Mean Zone of inhibition (mm) and standard deviation.

The crude aqueous extract of the stem was least effective even at high concentration (2mg/ml) with a minimum zone of inhibition recorded as 7.79mm. it is clear from Table 1 that the overall inhibition was maximum at maximum concentration and showing a progressively decrease in inhibition with a decrease in concentration from 2mg/ml to 0.5mg/ml.

Table 2. Inhibition potential of *E. sativa* Mill. against *P. aeruginosa.*

Solvent extracts	2.0 mg/ml	1.5 mg/ml	1.0 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Standard drug 1.0mg/ml	22.44 ± 0.07	21.37 ± 0.06	20.36 ± 0.25	19.77 ± 0.04
Chloroform leaves extract	15.44 ± 0.02	13.37 ± 0.07	9.36 ± 0.13	9.44 ± 0.04
Ethyl acetate leaves extract	10.11 ± 0.33	5.70 ± 0.88	4.70 ± 0.33	5.77 ± 0.33
Aqueous leaves extract	3.77 ± 0.29	3.37 ± 0.58	2.70 ± 0.58	3.77 ± 0.33
Chloroform stem extract	14.11 ± 0.04	9.37 ± 0.10	6.70 ± 0.10	6.44 ± 0.33
Ethyl acetate stem extract	16.44 ± 0.05	11.70 ± 0.07	8.70 ± 0.88	8.11 ± 0.29
Aqueous stem extract	13.77 ± 0.08	12.37 ± 0.10	8.70 ± 0.16	8.11 ± 0.06

Keys; Standard drug= Chloramphenicol, Mean ± SD= Mean Zone of inhibition (mm) and standard deviation.

Inhibition potential against P. aeruginosa

The *P. aeruginosa* was most susceptible to ethyl acetate stem extract at high concentration. As shown in Table 2 the maximum inhibition has been recorded for ethyl acetate stem extract with inhibition zone recorded as 16.44mm at 2mg/ml.

The chloroform and aqueous stem extracts shown considerable inhibition zone recorded as 13.77 and 14.10m respectively. The minimum inhibition zone recorded as 3.77mm, shown by aqueous leaves extract. The overall inhibition was concentration dependent.

Table 3. Inhibition potential of *E. sativa*Mill.against*B. subtilis.*

Solvent extracts	2.0 mg/ml	1.5 mg/ml	1.0 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Standard drug 1.0mg/ml	21.24 ± 0.49	20.12 ± 0.32	21.37 ± 0.35	18.88 ± 0.24
Chloroform leaves extract	14.24 ± 0.38	12.12 ± 0.32	10.37 ± 0.71	8.55 ± 0.41
Ethyl acetate leaves extract	8.90 ± 0.43	4.45 ± 0.40	5.71 ± 0.61	4.88 ± 0.38
Aqueous leaves extract	2.57 ± 0.64	2.12 ± 0.35	3.71 ± 0.56	2.88 ± 0.27
Chloroform stem extract	12.90 ± 0.32	8.12 ± 0.79	7.71 ± 0.36	5.55 ± 0.34
Ethyl acetate stem extract	15.24 ± 0.41	10.45 ± 0.35	9.71 ± 0.26	7.22 ± 0.29
Aqueous stem extract	12.57 ± 0.64	11.12 ± v	9.71 ± 0.30	7.22 ± 0.24

Keys; Standard drug= Chloramphenicol, Mean ± SD= Mean Zone of inhibition (mm) and standard deviation.

Inhibition potential against B. subtilis

As shown in Table 3 the ethyl acetate stem extract had tremendous effects on been *B. subtilis* with an inhibition zone of 15.23mm. The chloroform and aqueous stem extracts (2mg/ml) shown almost similar inhibition effects recorded as 12.90 and 12.56mm. The minimum inhibition has been shown by aqueous leaves extract (2mg/ml concentration) with an inhibition zone of 2.56mm. The inhibition potential of leaves and stem extracts (chloroform, ethyl acetate and aqueous) against *B. subtilis* were also concentration dependent.

Table 4. Inhibition potential of *E. sativa* Mill. against *K. pneumoniae.*

Solvent extracts	2.0 mg/ml	1.5 mg/ml	1.0 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Standard drug 1.0mg/ml	25.24 ± 0.55	21.89 ± 0.52	20.91 ± 0.41	17.77 ± 0.41
Chloroform leaves extract	18.24 ± 0.57	13.89 ± 0.67	9.91 ± 0.55	7.44 ± 0.55
Ethyl acetate leaves extract	12.91 ± 0.41	6.22 ± 0.55	5.25 ± 0.64	3.77 ± 0.64
Aqueous leaves extract	6.57 ± 0.30	3.89 ± 0.41	3.25 ± 0.88	1.77 ± 0.49
Chloroform stem extract	16.91 ± 0.60	9.89 ± 0.38	7.25 ± 0.44	4.44 ± 0.35
Ethyl acetate stem extract	19.24 ± 0.35	12.22 ± 0.46	9.25 ± 0.44	6.11 ± 0.38
Aqueous stem extract	16.57 ± 0.33	12.89 ± 0.33	9.25 ± 0.32	6.11 ± 0.38

Keys; Standard drug= Chloramphenicol, Mean ± SD= Mean Zone of inhibition (mm) and standard deviation.

Inhibition potential against K. pneumoniae

Table 4 shows that maximum inhibition has been shown by ethyl acetate stem extract and chloroform leaves extract with 19.24 and 18.24mm zone of inhibition at 2mg/ml concentration. The chloroform stem extract had considerable inhibition zone (16.90mm).

The minimum inhibition effect has been seen for aqueous leaves extract recorded 6.57mm. has been shown by *E. sativa* (2mg/ml concentration) against *Klebsiella pneumoniae*. The inhibition values progressively decrease with a decrease in inhibition, indicating that the inhibition potential was concentration dependent.

The overall findings reveal that ethyl acetate stem extract has tremendous potential of inhibiting the growth of all the four pathogenic bacterial strains as compared to other crude extract and ranked second to the standard drug in terms of inhibition potential (Fig. 1)

Discussion

Up to date a large number of therapeutic drugs have been obtained from the plant are used for different infectious disorders.

Antimicrobial studies of medicinally important plants are the key methods for identifying new sources of natural therapies, which may be further justified by phytochemical screenings.

In the present study, we screened the crude extracts (chloroform, ethyl acetate and aqueous) of leaves and stem of *E. sativa* Mill. for their possible antibacterial potential and reported that all extract showed considerable inhibition, which was concentration dependant.

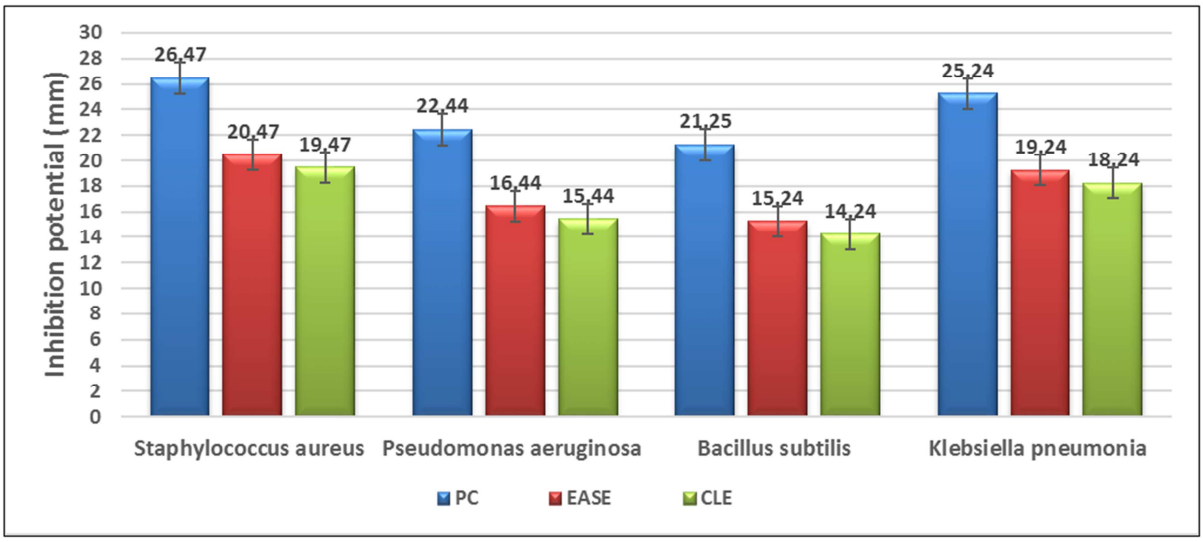

Fig. 1. Comparison of Mean± Standard deviation inhibition zone of positive control and crude extracts

Keys; PC= Positive control (Chloramphenicol), EASE = Ethyl acetate crude extract, CLE = Chloroform leaves extract.

The present findings agree with Koubaa *et al.* (2015) who evaluated the antioxidant and antibacterial activities of a various extract of rocket flowers against both gram-positive and gram-negative bacteria. Similarly, many other investigators (Rani *et al,* 2010; Gulfraz *et al,* 2011; Ali *et al,* 2014; Malik, 2015) also evaluated the antibacterial activity of *E. sativa* and therefore supported the use of *E. sativa* seeds in traditional medicine in various human disorders and all these are in support of the present experiment where *E. sativa* aqueous, chloroform and ethyl acetate extract showed inhibition against *S. aureus, B. subtilis, P. aeruginosa, K. pnemonie.* Comparison of the inhibitory action of *E. sativa* plant extracts changed with the change of solvent i.e. maximum inhibition has been shown by ethyl acetate stem extract against *S. aureus* while the weakest inhibitory activities were determined against *P. auregonosa*recorded as 3.77mm/mg in the aqueous stem extract recorded as 3.77mm/mg. The present study is also supported by the work of Khoobchandani *et al.* (2010) who investigated the antimicrobial activity of various solvent extracts of root and shoot of *E. sativa* and seed oil against *S. aureus*, *B. subtilis, E. coli* and *P. aeruginosa*. Previous findings show that E. *sativa* exhibit antibacterial activity against S. aureus, *Salmonella Enteritidis*, *Bacillus cereus,* B. *subtilis, E. coli P. aeruginosa* and *Vibrio parahaemolyticus* (Isshiki *et al*., 1992), *E. coli* and *Listeria monocytogenes* (Lin *et al*., 2000), Helicobacter pylori (Haristoy *et al*., 2005). It is suggested that the inhibition potential of *E.sativa* may be due to the presence of certain inhibitory compounds such as alkaloids, flavonoids, and

terpenoids (Cowan, 1999; Hussein, 2013). Similar inhibition potential of plant extracts against microorganisms has been previously reported (Tiedink *et al*., 1991; Vig *et al*., 2009; Doulgeraki *et al*., 2016).

Conclusion

It is concluded from our results that ethyl acetate stem extract had shown maximum inhibition against *S. aureus* (20.47mm), *P. aeruginosa* (16.44mm) and *B.* subtilis (15.24mm) at high concentration.*K. pneumoniae*was tremendously inhibited by both ethyl acetate stem extract and chloroform leaves extract with 19.24 and 18.24mm zone of inhibition respectively.

Acknowledgments

Special thanks to my dear friends Adan Farid, Irshad Ahmad and Mubashir Khan for helping me in plant materials collection. I am also grateful to Department of Botany, (BKUC) for providing the laboratory facilities.

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