Evaluation of antibacterial activity of *Lavandula stoechas* L. essential oil

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Abstract

The antibacterial activity of crude and essential oil extract of *Lavandula* (*Lavandula stoechas*) against the infectious bacteria *viz.*, *Escherichia coli, Bacillus subtilis, Pseudomonas* spp. and *Staphylococcus aureus* were evaluated *in vitro*. It was found that *L. stoechas* oil exhibits high antibacterial activity against *S. aureus, Pseudomonas* spp, *B. subtilis* and *E. coli* when compared to crude extract. The *in vitro* Minimum Inhibitory Concentration (MIC) of *L. stoechas* oil was found to be 50 µg mL^{-1} for inhibiting the growth of *B. subtilis* and *S. aureus* whereas, it was found to be 25 µg mL^{-1} for *E. coli* and *Pseudomonas* spp. The statistical analysis for MIC of *L. stoechas* oil was found to be 42.82 µg mL^{-1} for inhibiting the growth of *B. subtilis* and *S. aureus* where it was found to be 22.25 µg m^{-1} for *E. coli* and *Pseudomonas* spp.

Keywords: antibacterial activity, *Bacillus subtilis*, essential oil, *Escherichia coli*, *Lavandula stoechas*, *Pseudomonas* spp, *Staphylococcus aureus*

Introduction

The lavenders are a genus of about 25-30 species of flowering plant in the mint family, *Lamiaceae*, native of Mediterranean region south to tropical Africa and many regions of Asia and it has been used for centuries as a herbal remedy for many ailments. Lavender yields high effective essential oil with very sweet overtones and can be used in chemical industries (Hui *et al*. 2010). *Lavendula* essential oil is believed to be of benefit for a multitude of problems including stress, anxiety, exhaustion, irritability, head ache, migraine, insomnia, depression, cold, indigestion, liver, gall bladder problems and cancer (Hudson 1996; Kim *et al*. 2007; Henley *et al*. 2007).

The disease causing bacteria that have become resistant to antibiotics are causing an increasing public health problem due to the continuous use of antibiotics. From time immemorial, essential oils and other plant extracts have evoked interest as source of natural products (Prabuseenivasa *et al*. 2006). They are screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe *et al*. 2004). Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds in drug production (Prabuseenivasa *et al*. 2006).

Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant

material. An estimated 3000 essential oils are known, of which 300 are commercially important in the fragrance market (Van de Braak & Leijten 1999). Essential oils are complex mixes comprising of many single compounds derived from terpenes. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Burt 2004; Kordali *et al.* 2005). The present study was undertaken with the intention of finding out the efficacy of *L. stoechas* crude and essential oil extract against infectious Gram positive and Gram negative bacteria.

Materials and methods

The aromatic and medicinal plant *Lavandula stoechas* L., was collected from Gandhi Krishi Vigyan Kendra, Bengaluru and the pure cultures of *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas* spp., were procured from the stock cultures of Department of Microbiology, Bangalore University, Bengaluru.

Preparation of plant aqueous extract and essential oil

An aqueous crude extract was prepared following the method of Ateyyat *et al.* (2009) by boiling 10% (w/w) of the air dried leaf powder in sterile distilled water for 10 min and cooling to room temperature over night. The aqueous extract was filtered using a millipore filter to remove particulate matter and the final volume was adjusted to 100 mL with distilled water with 0.2% Tween 80 to account for the evaporated water during boiling. The lavender oil was extracted from *L. stoechas* using the Bienvenu (1995) method. The different concentration of oil was obtained by diluting it with Triton × 100.

Antibacterial activity

Screening of crude and essential oil of *L. stoechas* for antibacterial activity was performed using the Agar well diffusion assay. The bacterial inoculum was swabbed on to the Muller Hinton Agar plates and 100 μL of undiluted, 1:1 and 1:2 dilutions of crude and essential oil were added on to 8 mm diameter well bored on

these plates and 5 mg mL^{-1} streptomycin was added as a positive control. The plates were incubated at 37°C for 18 h and then the zone of inhibition was measured.

The Minimum Inhibitory Concentration (MIC) was performed for essential oil following the procedure of Deutsches Institut für Normung (1998) and the concentrations of 100, 50, 25, 12.5, 6.25 and 3.124 μg mL^{-1} was done using the 2- fold serial dilution technique. The bacterial turbidity was matched with 0.5 Mc Farland's standard (10^6–10^7 cfu mL^{-1}) and 100 μl of this culture were added to 10 mL of the above mentioned dilutions. The tubes were mixed well and incubated at 37°C, 120 rpm for 18 h and turbidity was checked to determine MIC. Streptomycin was used as a positive control for both the Agar well diffusion assay and MIC.

The 3-way ANOVA statistical analysis for *in vitro* studies of Agar well diffusion assay was done using IBM–SPSS (Statistical Package for Social Science) version 20.

The *in vitro* value obtained for MIC was analyzed and the exact value was calculated by applying the Logistic Regression Model, which is given below.

$$\pi(x) = P\ (Y=1) = e^{\beta_o + \beta_1 x}/\ 1 + e^{\beta_o + \beta_1 x}$$

Where, Y=Response (Binary outcome- Turbidity/ No Turbidity); X=Concentration (Covariate); β_o and β_1=Regression Coefficients; β_o = -52.752 and β_1=2.817 (for *E. coli* and *Pseudomonas* spp); β_o = -68.745 and $â_1$=1.836 (for *B. subtilis* and *S. aureus*)

Here, first the Logistic Regression Model was fit and later we used this model to find the value of 'x' (concentration), for which, P[Y=1] H" 1, i.e., the probability where there will be no turbidity is approximately equal to one. The above analysis was carried out using R-software.

Results and discussion

The antibacterial activity of *L. stoechas* crude extract and essential oil against two Gram-positive and two Gram-negative bacterial species performed using Agar well diffusion

method is depicted in Table 1, Figs. 1, 2, 3 and 4. The analysis of the results revealed that the concentrated crude extract had less inhibitory effect on all the bacteria tested such as *E. coli*, *Pseudomonas* spp., *S. aureus* and *B. subtilis* (Fig. 5). It was found that the 1:1 and 1:2 dilutions of the crude extract had no effect on all the 4 bacteria tested (Fig. 7). This may be due to low concentration of active compounds in the crude extract.

The undiluted essential oil exhibited antibacterial activity against all the 4 bacteria. The maximum inhibition zone was found in *Pseudomonas* spp. (25 mm) followed by *B. subtilis* (23 mm), *S. aureus* (23 mm) and *E. coli* (22 mm)

Table 1. Antibacterial activity of the crude extract and essential oil of *Lavandula stoechas* by Agar well diffusion method

Bacteria	Diameter of zone of inhibition (mm)							
	Plant crude extract				Essential oil extract			
	S	A	B	C	S	A	B	C
E. coli	22	6	— —-	— —-	22	22	18	14
B. subtilis	24	10	— —-	— —-	24	23	20	19
Pseudomonas spp.	25	18	— —-	— —-	25	25	18	15
S. aureus	25	8	— —-	— —-	25	23	17	10

S= -+ve control (Streptomycin); a=undiluted sample; b=1:1 dilution; c=1:2 dilutions

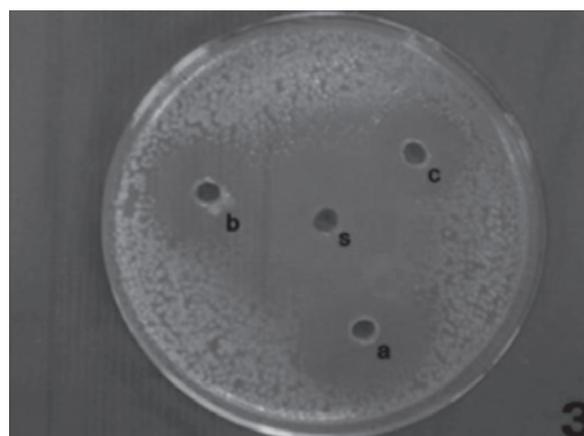**Fig. 1.** Agar well diffusion assay of lavender oil against *B. subtilis*

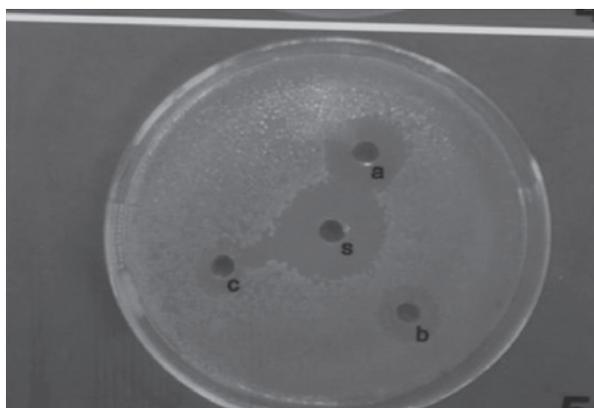**Fig. 2.** Agar well diffusion assay of lavender oil against *Pseudomonas* spp.

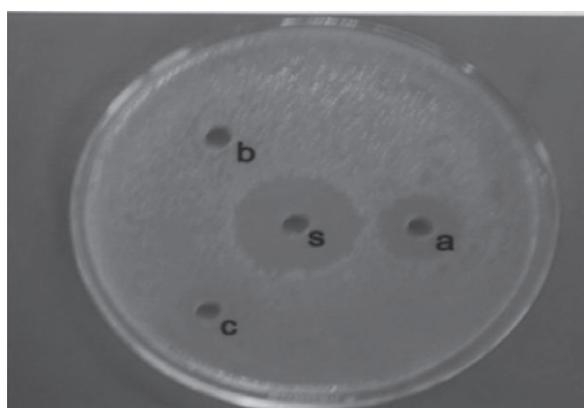**Fig. 3.** Agar well diffusion assay of Lavender oil against *S. aureus*

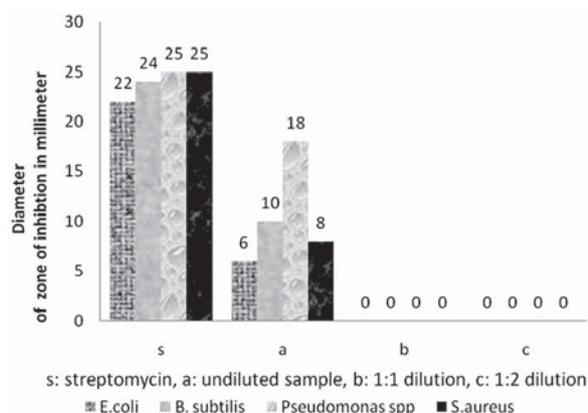**Fig. 4.** Agar well diffusion assay of crude extract against *E. coli*

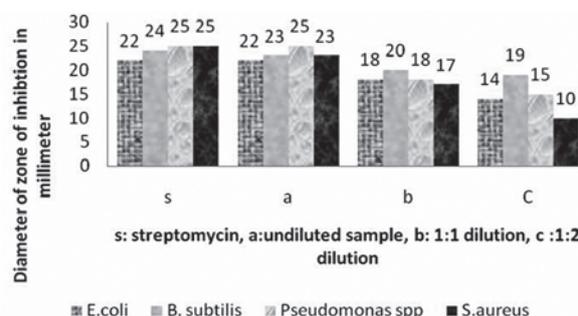**Fig. 5.** Antibacterial activity of crude extract of *L. stoechas*

Fig. 6. Antibacterial activity of essential oil extract of *L. stoechas*

(Figs. 6 and 8). This agreed with the findings of Sue *et al.* (2000) who reported inhibitory effects of 45 essential oils on 8 Gram positive and Gram negative bacteria. The inhibitory effect of undiluted essential oil is almost similar to antibiotic streptomycin as shown by the zone of inhibition (Table 1 and Fig. 6). All the 4 bacteria tested showed susceptibility to the essential oil but, decreased marginally with dilutions of 1:1 and 1:2 (Fig. 8). However, contrary to our study, according to Prabuseenivasa *et al.* (2006) reported that *E. coli* and *S. aureus* were resistant to Lavender oil. Sue *et al.* (2000) reported that Gram negative bacteria were generally more resistant than

Gram positive bacteria which are not in agreement with our results where both Gram positive and Gram negative bacteria showed sensitivity towards essential oil.

The 3-way ANOVA statistical analysis of the Agar well diffusion of crude and essential oil extract showed zero P value which is less than 0.05 for all the parameters such as bacteria versus extract, bacteria versus concentration, extract versus concentration and bacteria versus extract versus concentration. These values indicated that all the results obtained are unique and significant (Table 2).

The analysis of the results of Minimum Inhibitory Concentration (MIC) of different dilutions of essential oil is presented in Table 3 and Fig 9. The *in vitro* MIC value indicated that
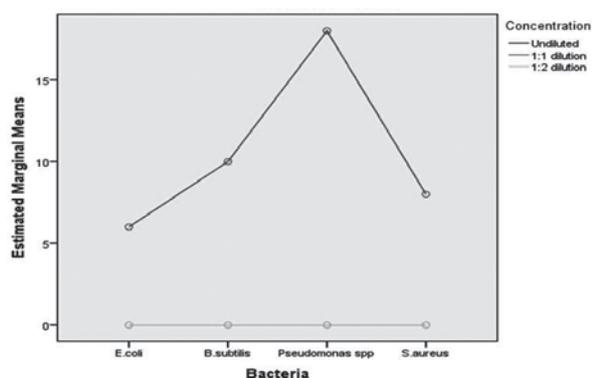
Table 2. Three way ANOVA analysis of Agar well diffusion assay of crude and essential oil extract from *L. stoechas* against bacteria

| Tests of Between-Subjects Effects				
Dependent Variable: Diameter of zone of inhibition (mm)				
Source	Type III Sum of Squares	df	Mean Square	F	Sig
Model	29770.667[a]	24	1240.444	1187.660	**
Bacteria	219.000	3	73.000	69.894	**
Extract	8402.778	1	8402.778	8045.213	**
Concentration	2499.556	2	1249.778	1196.596	**
Bacteria × Extract	99.000	3	33.000	31.596	**
Bacteria × Concentration	288.000	6	48.000	45.957	**
Extract × Concentration	203.556	2	101.778	97.447	**
Bacteria × Extract × Concentration	192.000	6	32.000	30.638	**
Error	125.333	120	1.044		
Total	29896.000	144			

** $P < 0.01$

Table 3. Minimum Inhibitory Concentration (MIC) of Lavender oil from *L. stoechas*

Bacteria	MIC for Streptomycin µg mL^{-1} (positive control)	*In vitro* MIC for lavender oil (µg mL^{-1})	Statistically calculated MIC for lavender oil (µg mL^{-1})
E. coli	12.5	25	22.25
B. subtilis	6.25	50	42.82
Pseudomonas spp.	3.125	25	22.25
S. aureus	6.25	50	42.82

Fig. 7. Agar well diffusion assay using different concentration of crude extract from *Lavendula stoechas* against the bacteria
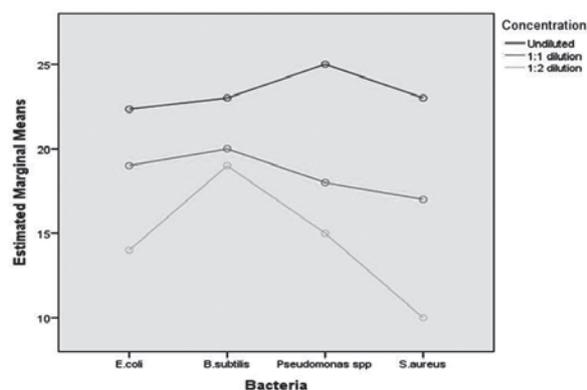
Fig. 8. Agar well diffusion assay using different concentration of essential oil from *L. stoechas* against the bacteria

Fig. 9. Minimum inhibitory concentration (MIC) of lavender oil

low concentration (25 µg mL^{-1}) of essential oil is very effective against Gram negative bacteria, *E. coli* and *Pseudomonas* sps. It was found that 50 µg mL^{-1} exhibited weak inhibitory activity against Gram positive bacteria, *S. aureus* and *B. subtilis*. The actual MIC values were calculated statistically by applying a Logistic Regression Model and was found to be 22.25 µg mL^{-1} for *E. coli* and *Pseudomonas* spp. and
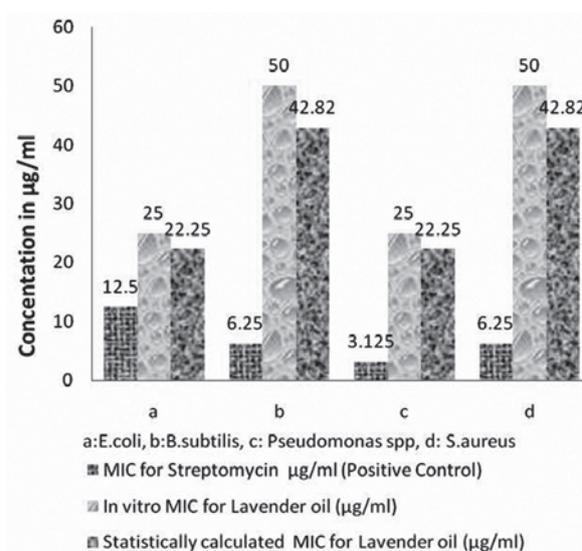
42.82 µg mL^{-1} for *S. aureus* and *B. subtilis*. The present findings agreed with the results of Zaika (1988) who have reported that Gram-positive bacteria were more resistant to the essential oils than Gram-negative bacteria. This is due to the fact that essential oils and their components exhibits hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering it more permeable (Knobloch *et al.* 1986; Sikkema *et al.* 1994). Probably, extensive leakage from bacterial cells or the exit of critical molecules and ions led to bacterial death (Denyer & Hugo 1991) in the present investigation.

The present investigation together with the previous studies provides support for the modulation of new therapeutic drugs. Further, additional *in vivo* studies and clinical trials would be needed to justify and evaluate the

potential of lavender oil as an antibacterial agent in topical or oral applications.

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