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In vitro evaluation of antibacterial potentiality of *Linum usitatissimum* L. (seed) against four important species of bacteria

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Abstract
In vitro evaluation of antibacterial activity of aqueous seed extract of *Linum usitatissimum* L. were tested against four bacterial species *viz*., *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus* at 10 to 100% concentration. Maximum inhibition was observed in *S. aureus* and recorded 34.0 mm inhibition at 100% concentration, followed by *P. vulgaris* and recorded 32.0 mm inhibition at 100% concentration. *B. subtilis* recorded 28.0 mm inhibition at 100% concentration and minimum inhibition was observed in *E. coli* and recorded 23.0 mm inhibition at 60.0% concentration. Compare to control tetracyclin and chloramphenicol at a recommended concentration of 25mg, *E. coli* recorded 30.0mm, *P. vulgaris* recorded 18.0 mm, *S. aureus* recorded 26.0 mm and *B. subtilis* recorded 23.0mm inhibition respectively. In chloramphenicol, maximum inhibition was observed in *P. vulgaris* (31.0 mm) followed by *E. coli* (30.0 mm) and *S. aureus* and *B. subtilis* recorded 28.0 mm inhibition respectively.

Keywords: *Linum usitatissimum*, bacteria, antibacterial activity, tetracyclin and chloramphenicol

Introduction
Plants are major sources of developing antimicrobial agent and they have been used for the treatment of humans and animals for many years (Mon *et al*., 2008) [2]. Infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Death from infectious diseases ranked 5th in 1981, has become the 3rd leading cause of death in 1992, with an increase 58% (Venkataswamy *et al*., 2010) [3]. Recently, strains of multiple drug resistant *S. aureus* have appeared and proven very difficult to treat medically. It also is a major cause of food poisoning. *S. aureus* is resistant to heat, drying and radiation. Approximately 62 – 80% of the world’s population still relies on traditional medicines for the treatment of common illness (WHO, 2002) [4]. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing (Rajesh *et al.*, 2007) [6]. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki *et al.*, 1999) [8]. For a long period of time, plants have been a valuable source of natural products for maintaining human health and their use as medicines could be traced as far back as the beginning of human civilization (Saroj, 2019) [21]. Natural products perform various functions, and many of them have interesting and useful biological activities (Galal *et al.*, 1991). Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led the discovery of novel drug candidates used against diverse diseases (Elhoussine *et al.*, 2010) [10]. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (Hamburger and Hostettmann, 1991) [11]. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Lee *et al.*, 1998) [12]. Plants are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments. Extreme interest in plants with antibacterial activity has revived as result of current problems such as resistance associated with the use of antibiotics obtained from microorganisms (Nagendra *et al.*, 2010; Kiran *et al.*, 2011) [13, 14, 18]. In the present study, aqueous extract of seed of *Linum usitatissimum* L. belongs to family Linaceae were evaluated for antibacterial activity against four different bacterial species *in vitro* condition.

Materials and Methods

Plant Material: Healthy seeds of L. *usitatissimum* free from diseases were collected from Mysore. The seeds were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, seed material was then air dried on a sterile blotter under shade and used for extraction.

Aqueous extraction: 50 grams of thoroughly washed seeds of *L. usitatissimum* were macerated with 50 ml of sterile distilled water in a Waring blender (Waring International, New Hartford, CT, USA) for 10min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120 ^{0}C for 15 minutes. The extract was preserved aseptically in a brown bottle at 5^{0}C until further use (Lalitha *et al.,* 2011) [8].

Test pathogens: Four bacterial species *viz*., *Escherichia coli (Gram Negative), Proteus vulgaris (Gram Negative), Bacillus subtilis (Gram Positive)* and *Staphylococcus aureus (Gram Positive)* were collected from research center, Pooja Bhagavat Memorial Mahajana P.G. Centre, K.R.S. Road, Metagalli, Mysore. The obtained cultures were sub cultured on nutrient agar medium and incubated at 37°C for 24 hours. After incubation, the cultures were preserved aseptically in lower temperature until further use.

Preparation of Inoculum

Preparation of standard culture inoculums of test organism: All the test bacterial species were inoculated into 2 ml nutrient broth and incubated at 37 ^{0}C for 24 hours till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO (Bole *et al.,* 2010) [19].

Antibacterial assay

Agar cup diffusion method: An overnight culture of *E. coli, Proteus vulgaris, Bacillus subtilis* and *S. aureus* were inoculated into petri plates containing nutrient agar medium. The culture medium was allowed to set. Thereafter, a sterile cork borer of 5.0 mm diameter was used to punch wells in the seeded nutrient agar. Five wells were made in the petriplate containing media (One in Centre and Four at the border), the agar plugs were removed with a flamed and cooled wire loop. For each well 50 µl of different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% concentration) of the aqueous extract was added. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured in millimeter. For each treatment ten replicates were maintained. The same procedure were followed for standard antibiotics Tetracycline (25mg) and Chloramphenicol (25mg) to compare the efficacy of aqueous extract against test organisms (Joshi *et al.,* 2009) [20]. The Minimum Inhibitory concentration (MIC) was also determined for all the test bacterial species.

Statistical Analysis

The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05).

Result

Among the four bacterial species tested, maximum inhibition was recorded in *S. aureus* and recorded 34.0mm inhibition at 100%concentration, 31.0mm at 90.0% concentration, 29.0mm at 80.0% concentration, 26. 0mm in 70% concentration respectively. Least inhibition was observed in 10.0% concentration and recorded 13.0mm and 16.0mm inhibition at 20.0% concentration.

In *P. vulgaris* 32.0mm inhibition was recorded in 100.0% concentration, 28.0mm in 90.0% concentration, 23. 0mm in 80.0% and 22.0mm in 70.0% concentration respectively. Moderate inhibition was observed in 10 to 15% concentration of the aqueous extract.

Moderate activity was observed in B. *subtilis* and recorded 28.0mm inhibition at 100.0% concentration, 25.0mm at 90.0%, 21.0mm at 80.0% and 17.0mm inhibition in 70.0% concentration respectively.

Least activity was observed in E. *coli* and recorded 23.0mm inhibition at 50.0% to 100.0% concentration, at 10.0% concentration, it was recorded 13.0mm, at 20.0%, it was recorded 15.0mm, at 30.0% concentration, it was recorded 17.0mm and at 40.0% concentration, it was recorded 20.0mm inhibition respectively.

Compared to standard antibiotic, tetracyclin at a recommended concentration 25.0mg, E. *coli* recorded 30.0mm, P. *vulgaris* (21.0mm), S. *aureus* (26.0mm) and *B. subtilis* recorded 23.0mm inhibition respectively. In chloramphenicol, E. *coli* recorded 30.0mm inhibition, P. *vulgaris* recorded 31.0mm, *S. aureus* recorded 28.0mm and *B. subtilis* recorded 28.0mm inhibition respectively (Table -1). The Minimum Inhibitory Concentration (MIC) was 80.0% concentration for E. *coli,* 100.0% for P. *vulgaris,* S. *aureus* and B. *subtilis.*

Discussion

In the present time multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide (Peng *et al.,* 2006) [15]. It is aroused due to indiscriminate and repetitive use of antimicrobial drugs by inadequate disease treatment (Shariff, 2001) [16]. To acquire drug resistance microbes have developed new enzyme system to cleave the drug and make it useless for control of infection (Ritch *et al.,* 1999). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine (Abbas *et al.,* 2008). Various medicinal plants have been used for years in daily life to treat disease all over the world. The use of traditional plant extracts as well as other alternative forms of medical treatments have been getting momentum since the 1990s (Cowan, 1999) [7]. Recently scientific interests in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. Therefore, the search for new drugs from plants continue to be a major source of commercial drugs. Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease. hence, further exploration of plant antimicrobials need to occur. From the above observation, it can concluded that, the seeds of L. *usitatissimum* is a potent source as antibacterial agent. Many of the bioactive compounds were observed during the process of isolation procedure. Thus a further work is needed to isolate the bioactive compounds and evaluating its antibacterial activity against different human and plant pathogens.

Table 1: Antibacterial activity of aqueous extract of *Linum usitatissimum* L. (Seed) against four important species of bacteria

Bacteria	Inhibition(mm) Concentration of the Aqueous extract											Standard Antibiotics	
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	MIC	Tetracycline 25mg	Chloramphenicol 25mg
E. coli	13.0^{a} ±0.0	15.0^{b} ±0.0	17.0^{c} ±0.0	20.0^{d} ±0.0	23.0^{e} ±0.0	23.0^{e} ±0.0	23.0^{e} ±0.0	23.0^{e} ±0.0	23.0^{e} ±0.1	23.0^{e} ±0.0	50%	30.0^{f} ±0.0	30.0^{f} ±0.0
P. vulgaris	0.0^{a} ±0.0	6.0^{b} ±0.0	13.0^{c} ±0.0	15.0^{d} ±0.0	15.0^{d} ±0.0	17.0^{e} ±0.1	22.0^{f} ±0.0	23.0^{g} ±0.0	28.0^{h} ±0.1	32.0^{j} ±0.0	90%	28.0^{h} ±0.0	31.0^{i} ±0.0
S. aureus	13.0^{a} ±0.0	16.0^{b} ±0.0	18.0^{c} ±0.1	20.0^{d} ±0.0	20.0^{d} ±0.1	22.0^{e} ±0.1	26.0^{f} ±0.1	29.0^{h} ±0.0	31.0^{i} ±0.0	34.0^{j} ±0.1	100%	26.0^{f} ±0.0	28.0^{g} ±0.0
B. subtilis	0.0^{a} ±0.1	8.0^{b} ±0.0	11.0^{c} ±0.0	13.0^{d} ±0.0	13.0^{d} ±0.0	15.0^{e} ±0.0	17.0^{f} ±0.0	21.0^{g} ±0.1	25.0^{i} ±0.0	28.0^{j} ±0.1	100%	23.0^{h} ±0.0	28.0^{j} ±0.0

- Values are the mean of five replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

Conclusion

From the above observation, it was noted that seeds of L. *usitatissimum* showed a significant and moderate result against four bacterial species tested. In the present study, aqueous extract was evaluated and observed a maximum inhibition in all the test concentration tested. A further evaluation of solvent extracts is needed against different bacterial species and standardization of protocol for isolating the bioactive compound, its characterization and structural elucidation is needed.

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