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Bactericidal potential of seed, leaf and stem bark sequential extracts of *Elaeagnus conferta* Roxb

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Abstract
Plants are utilised to treat a variety of infectious disorders, and their therapeutic efficacy is attributed to the existence of bioactive secondary metabolites with antibacterial capabilities. The present study reports on the bactericidal properties of Eleagnus conferta Roxb. an endangered and indigenous tree of the Western Ghats.
The presence of phenolics, alkaloids, and terpenoids in the seed, leaf, and stem bark sequential extracts has been examined qualitatively and quantitatively. All of the extracts have been tested for their bactericidal properties.against human pathogenic bacterial strains *viz*. Staphylococcus aureus, Streptococcus pneumoniae, Vibrio cholera, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumoniae using agar well diffusion method.
The MIC value was obtained using the agar well diffusion method and the Resazurin Micro Titer Plate Method. In leaf ethanol extract, the bactericidal property was well displayed against Streptococcus pneumoniae, with a considerable zone of inhibition, followed by Klebsiella pneumoniae. Seed ethanol extract had a considerable zone of inhibition against Vibrio cholera, and stem bark ethanol extract had bactericidal activity against Pseudomonas aeruginosa. The findings of this study back up the traditional applications of Eleagnus conferta leaves extract to treat pneumonia and bronchial diseases, seed extract to treat diarrhea, and stem bark extract to treat diabetic pus sores.

Keywords: *Eleagnus conferta;* ethanol extracts; pneumonia; jaundice

Introduction
People all around the world are making herbal medicine with a variety of medicinal plants, which has a lot of potential. Like Alkaloids, flavonoids, terpenoids, tannins, and phenolic substances are secondary metabolites with diverse pharmacological effects (Sen *et al.*, 2012) [1, 10, 19]. According to the WHO, almost 80% of the world's population uses traditional medicine made from plant extracts. Microorganisms produce enormous amounts of waste, Antibiotic resistance has developed as a result of the indiscriminate use of antibiotics. and have become resistant to antibiotics. Many antibiotics have negative consequences for patients, such as allergic reactions, hypersensitivity, gastrointestinal weakness, and so forth. As a result, many are looking for alternative medicine. to combat bacterial infectious diseases. One of these methods is to look for antibacterial medicines in medicinal plants (Ahmad *et al*., 1998) [2]. Clinical trials had demonstrated potentially useful activity in nearly 16 percent of herbal medicines, but there was limited *in vitro* or *in vivo* evidence for roughly half of the medicines, and there was only phytochemical evidence for around 20 percent of the medicines were allergenic or toxic, and nearly 12 percent had not yet been studied scientifically (Bussmann, 2013). The current study focuses on the bactericidal characteristics of *Eleagnus conferta* based on traditional claims.
Eleagnus conferta Roxb. Is an endemic and endangered medicinal plant of the Western Ghats belongs to family Elaeagnaceae. It is found to be distributed in Vietnam, Malaysia, India and South China. Fruit is sweetish sour in taste, edible and have been used in traditional Indian, Tibetan, Mongolian and Uygur medicine for the treatment of indigestion (Palani *et al*., 2010). The native people in Yunnan province (South China) have been using its dried fruits to relieve the after effects of alcohol for hundreds of years. Effect of *Elaeagnus Conferta* Roxb. (Elaeagnaceae) dry fruit on the activities of hepatic alcohol dehydrogenase and aldehyde dehydrogenase activities. The traditional practitioners residing in the vicinity of Bhadra Wild Life Sanctuary of the Western Ghats, Karnataka, India are using the seed extract to cure diarrhea and liver chirrosis leaf extract is used to control cough branchial disorders and the stem bark paste is used to cure diabetic pus wounds. Reports are very scare on the phytoconstituents and the antibacterial properties. So, present study reports the phytochemical analysis and the antibacterial potential of *Elaeagnus conferta* Roxb.

Materials and Methods

Collection of Plant Material

Eleagnus conferta Roxb. leaves, seeds, and stem bark materials were obtained from the Bhadra Wild Life Sanctuary in the Western Ghats of Shivamogga District, Karnataka, India. A taxonomist, Dr. Y L Krishnamurthy, Professor, Post Graduate Studies and Research in Botany, Kuvempu University, recognised and authenticated the plant. After that, the plant materials were rinsed 2-3 times with running tap water before being treated with distilled water and dried in the shade.

Extraction of Crude Drug

Dried seeds, leaves and stem bark materials were separately crushed into powder form, subjected to successive solvent extraction from non-polar to polar solvents like petroleum ether, chloroform and ethanol respectively. The extraction has been carried out using the soxhlet apparatus. The sequential extracts of each material were pooled and the solvent was removed by using rotary flash evaporator. The concentrated extracts were dried in desiccator and used for phytochemical screening and antibacterial evaluation.

Phytochemical Screening

Qualitative Analysis

The petroleum ether, chloroform and ethanol extracts of leaves, seeds and stem bark of *Elaeagnus conferta* were subjected to qualitative phytochemical screening using standard methods (Arunodaya *et al.*, 2016) [5, 15]. Each extract was tested to detect the presence of alkaloids, flavonoids, terpenoids, saponins, tannins, phenolic compounds and glycosides using standard procedures (Sasidharan *et al.*, 2011; Mustapha *et al.*, 2016) [18].

Quantitative Analysis

Stem bark, seeds and Leaf extracts were allowed for quantitative estimation of alkaloids and total phenolic compounds.

Alkaloid estimation

1 gram of sample was mixed with 40 ml of 10% acetic acid, covered, and set aside for 4 hours. On a water bath, the filtrate was concentrated to 1/4th of its original volume. Drop by drop, concentrated ammonium hydroxide was added to the extract until it was completely precipitated. After allowing the entire solution to settle, the collected precipitate was washed with weak ammonium hydroxide and filtered. The residue was weighed after drying (Sen and Batra, 2012) [1, 10, 19].

Phenolic Compounds Estimation

The total phenolic content of *Elaeagnus conferta* extracts was measured using Singleton's spectrophotometric technique (Murray PR, Baron EJ 2003). 20 µl of extract (5 mg/ml) were combined with 0.75 ml of 20% sodium carbonate solution and 0.25 ml of Folin-Ciocalteau reagent. The reaction mixture was exposed to light for 3 minutes before being incubated in the dark for 2 hours. A UV-Visible Spectrophotometer was used to detect the absorbance at 765 nm. Total phenolics were determined using a calibration curve based on the absorbance of a known concentration of Gallic acid standard (0-100 µg/ml). All measurements were done in triplicates and the values were represented in µg of Gallic acid equivalents per ml.

Bacterial cultures (collection and maintenance)

The Institute of Medical Sciences in Shimoga, Karnataka, India provided the pure cultures of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumoniae* used in this study. Loop complete bacteria were inoculated into nutrient broth and incubated overnight at 37°C using these strains.

Agar Well Diffusion Method

According to conventional technique, this method was used to assess the antibacterial activity of plant extracts (Saleem *et al*., 2015; Sudhesh *et al.*, 2017) [11, 13] 20 ml Muller's Hilton agar media was placed into petri plates and allowed to solidify, then 100µl fresh culture (10^5 cells/ml) of each test organism was added and dispersed over the sterile agar plates. Then, using a sterile cork borer, wells were made in plates (6 mm diameter). Four wells were produced in each plate, three of which were induced with 500µg, 1000µg, and 1500µg/ml of dissolved extract in 10% DMSO, and the remaining one with positive control streptomycin (20µg/ml). These plates were incubated for 24 hours at 37° C in an upright position for 24 hours. The creation of a distinct zone of inhibition surrounding the well on the incubated plates was seen, indicating the induction of antibacterial activity (Sarker *et al*., 2007; Arunodaya *et al*., 2016) [14, 5, 15].

Resazurin Micro Titre-Plate Assay

The stem bark and leaves' minimal inhibitory concentration (MIC) was determined using a modified resazurin microtitre plate assay (Akinyemi *et al*., 2005; Hasselmann, 2003) [16, 17]. 50 µl of nourishing broth were poured into each well. The first six wells of the first row of the titre plate were filled with 50 µl of test sample containing 250 µg of extract sample dissolved in 10% dimethyl sulfoxide. The standard streptomysin (1 mg/ml) solution in

10% DMSO was applied to the seventh well of the first row. Following that, eight of the first row's wells were used as a negative control. Using a multichannel pipette, a two-fold serial dilution was done, with each well containing 50 µl of the test substance (excluding the standard and control wells) in serially falling concentration. 10µl (0.015 %) resazurin indicator dye and 30µl of iso-sensitized broth were added to all the wells. To each other finally, 10 µl of bacterial suspension was added to each well, resulting in a concentration of roughly 5×10^6 CFU/ml. Streptomycin was utilised as a positive control and DMSO was used as a negative control. Color change was detected after 24 hours of incubation at 37°C. Color changes from blue to pink showed growth, and MIC was confirmed by ocular observation, where no change in colour was noticed at the lowest concentration of plant extract (Sasidharan *et al.,* 2011; Sen and Batra, 2012) [18, 1, 10, 19].

Statistical Analysis

The statistical analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software. The results are expressed as mean±sem.

Results

Phytochemical Screening

All the sequential extracts of leaf ethanol extract, seed ethanol extract showed the presence of Alkaloids, Flavonoids, Phenolics, Terpenoids, Glycosides, Tannins, and Saponins compounds, and Stem bark ethanol extract extracts showed the presence of Alkaloids, Phenolics, Terpenoids, Glycosides, Tannins, and Saponins compounds of *Elaeagnus conferta* as shown in the Table 1.

Table 2 shows the quantitative estimation of total phenolics, alkaloids, and terpenoids contents contained in *Elaeagnus conferta* extracts. The phenolic content of the stem bark ethanolic extract (24.02µg/mg) is higher than that of the seed ethanolic extract (14.87µg/mg), although the alkaloid content of the stem bark ethanolic extract is higher (19.32µg/mg). The terpenoid content of the leaves pet ether extract was higher (47.32 µg/mg), while the quantities of alkaloids, phenolics, and terpenoids in the chloroform extracts were relatively low.

Table 1: Qualitative phytochemical analysis of extracts of *Elaeagnus conferta* Roxb.

Tests	LPE	LCE	LEE	SBPE	SBCE	SBEE	SPE	SCE	SEE
Flavonoids	-	-	+	-	-	+	-	-	-
Alkaloids	+	+	+	+	+	+	+	+	+
Phenolic	+	+	+	+	+	+	+	+	+
Glycosides	-	+	+	-	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+
Saponins	-	-	+	-	-	+	-	-	+
Tannins	-	-	+	+	+	-	-	-	-

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract. (+) indicate the presence and (–) the absence of the respective phytochemicals.

Table 2: Quantitative determination of Phenolics, Alkaloids and Terpenoids (µg/mg)

Tests	LPE	LCE	LEE	SBPE	SBCE	SBEE	SPE	SCE	SEE
Phenolic	10.07	12.36	19.92	17.32	19.54	24.02	08.39	11.58	14.87
Alkaloids	11.87	13.84	14.58	12.11	9.25	19.32	15.21	18.74	21.58
Terpenoids	47.32	38.41	31.62	27.94	22.37	20.98	32.54	29.47	28.46

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

Antibacterial Activity

The antibacterial activity of *Elaeagnus conferta* seed, leaves, and stem bark sequential extracts against human pathogenic bacterial strains (PA) *Pseudomonas aeruginosa*, (KP) *Klebsiella pneumoniae*, (ST) *Salmonella typhi*, (SP) *Streptococcus pneumoniae*, (SA) *Staphylococcus aureus*, and (VC) *Vibrio cholera*. Depending on the susceptibility of the tested microorganism, the antibacterial activity of the seeds, leaves, and stem bark extracts varied at different magnitudes of inhibitory patterns and was compared to the standard positive control Table 3 displays the zone of inhibition.

Table 3: Antibacterial Activity by Agar Well Plate Method

Extracts	Concentration 1600 µg, Zone of Inhibition(mm)					
	S. aureus	***K.pneumonia***	***V.cholerae***	***S.pneumonia***	***P.aeruginosa***	***S. typhi***
LPE	15.33±0.33	11.67±0.33	08.33±0.33	11.67±0.33	12.67±0.33	08.00±0.00
LCE	16.00±0.00	10.33±0.33	09.33±0.33	12.67±0.33	11.33±0.33	10.33±0.33

LEE	18.33±0.33	18.67±0.33	10.67±0.33	20.67±0.33	13.33±0.33	12.33±0.33
SBPE	13.00±0.00	09.33±0.33	08.33±0.33	12.67±0.33	09.33±0.33	08.33±0.33
SBCE	16.33±0.33	12.33±0.33	12.33±0.33	14.67±0.33	12.67±0.33	08.00±0.00
SBEE	19.33±0.33	15.67±0.33	11.00±0.00	17.67±0.33	21.33±0.33	11.67±0.33
SPE	14.67±0.33	16.67±0.33	08.33±0.33	19.67±0.33	19.67±0.33	08.00±0.00
SCE	17.67±0.33	14.67±0.33	07.67±0.33	17.67±0.33	16.67±0.33	08.33±0.33
SEE	20.33±0.33	12.33±0.33	14.33±0.33	14..33±0.33	15.33±0.33	12.33±0.33

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

The leaves ethanol extract hindered the growth of bronchial infecting bacterial strains *Streptococcus pneumonia* and *Klebsiella pneumoniae* significant zone of inhibitions of 20.67±0.33mm and 18.67±0.33mm respectively. The seed ethanol extract controls the growth of gastrointestinal infecting microbes *Staphylococcus aureus* with a zone of inhibition of 20.33±0.33mm**.** The antibacterial property was well expressed in stem bark ethanol extract against the secondary wound infectious bacteria *Pseudomonas aeruginosa* with a zone of inhibition of 21.33±0.33mm.

The Activity Index was performed for each of the extracts. The optimal concentration was measured on the basis of the percentage of inhibition of bacterial strains and the activity index values are tabulated in Table 4.

The percentage of an inhibitory concentration of the leaf ethanol extract (LEE) extract showed good Activity Index in (SP) *Streptococcus pneumonia* 0.664 followed by (SA) *Staphylococcus aureus* 0.515. Then stem bark ethanol extract (SBEE) showed good Activity Index in (PA) *Pseudomonas aeruginosa* 0.564 and followed by (VC) *Vibrio cholerae* 0.436, and seed ethanol extract showed good Activity Index in (VC) *Vibrio cholera* 0.679 and followed by (ST) *Salmonella typhi* 0.621. The zone of inhibition and activity index values of petroleum ether and chloroform extracts of seeds, leaves and stem bark extracts of *Elaeagnus conferta* was comparatively less than the ethanol extract.

Table 4: Antibacterial Activity Index of *Elaeagnus conferta* extracts against the pathogenic bacterial isolates

Extracts	***S. aureus***	***K.pneumonia***	***V.cholerae***	***S.pneumonia***	***P.aeruginosa***	***S.typhi***
LPE	0.516	0.421	0.29	0.305	0.347	0.289
LCE	0.539	0.373	0.325	0.236	0.329	0.373
LEE	0.515	0.437	0.372	0.664	0.364	0.435
SBPE	0.338	0.337	0.299	0.236	0.329	0.301
SBCE	0.326	0.345	0.332	0.305	0.347	0.289
SBEE	0.381	0.421	0.436	0.405	0.564	0.351
SPE	0.394	0.272	0.349	0.278	0.394	0.289
SCE	0.395	0.365	0.467	0.409	0.488	0.345
SEE	0.605	0.421	0.679	0.356	0.399	0.621

LPE-Leaves pet ether extract, LCE-Leaves chloroform extract, LEE-Leaves ethanol extract, SBPE- Stem Bark pet ether extract, SBCE- Stem Bark chloroform extract, SBEE- Stem Bark ethanol extract, SPE-Seed Pet ether extract, SCE-Seed chloroform extract, SEE-Seed ethanol extract.

Discussion

The presence of phytochemicals, whose composition is completely dependent on geographical and environmental conditions, is frequently attributed to the therapeutic value and biological activity of plants (Singh *et al.,* 2003). People favour medicinal plants for treating various disorders because they have few negative side effects. Synthetic pharmaceuticals and commercial antibiotics are known to have adverse effects including rashes and stomach ache, thus herbal treatments are becoming increasingly popular. According to the literature review, there are no reports on antibacterial activity from leaves, stem bark, or seed extracts, and this study confirms *Elaeagnus conferta*'s bactericidal properties.

Sequential phytochemical screening of *Elaeagnus conferta* leaves, stem bark, and seeds revealed the presence of alkaloids, terpenoids, phenolic compounds, and tannins in all extracts, however cardiac glycosides are lacking in LPE and SBPE. The principal secondary metabolites in these categories are alkaloids and terpenoids, which play an important role in bacterial disease control (Akula *et al*., 2011). Quantitative analysis of phenolics, alkaloids, and terpenoids revealed that stem bark ethanolic extract has higher phenolic content (22µg/mg). The terpenoid concentration of the leaf petroleum ether extract was higher (47.32gµg/mg). This revealed that the presence of phytocompunds varies in different areas of the plant, and that stress plays a significant role in secondary metabolite biosynthesis. Many researchers have noticed the distribution of therapeutic plant compounds in different parts of the plant (Karuppusamy, 2007; Ravishankar *et al.,* 2017).

The bactericidal of the sequential extracts also varies depending upon the localization of the secondary metabolites in the seed, leaf and stem bark extracts of *Elaeagnus conferta* against the bacterial strains selected. The traditional pratitioners of Malnad region of Karnataka especially in Thirthahalli and Shimoga District are using the seed extract of *Elaeagnus conferta* to cure diarrhoea, cholera and jaundice. In this investigation also,

the seed ethanol extract of *E. conferta* exhibited significant zone of inhibition and activity index percentages against the gastrointestinal infecting bacteria *V. cholera* and *S. typhi* which causes diarrhea / cholera and hindered metabolic activities of the liver. The leaf extract of *E. conferta* is being used to relieve cough and bronchial infections. In the present investigation also leaf ethanol extract is very potential to control the growth of gram negative bacteria *S. pneumonia* and *K. pneumonia* with significant zone of inhibition and activity index values. Traditionally stem bark paste is applied externally to cure diabetic old wounds noticed in the foot of affected persons. This may be due to secondary infections caused by *P. aeruginosa* and *S. aureus* and it is authenticated by study that stem bark ethanol extract is potential to control the growth of *P. aeruginosa* and *S. aureus.* The therapeutic efficacy of leaves, seed and stem bark of *Elaeagnus conferta* is due to the localization of different phytochemical compounds in different concentrations. Therefore, the presence of these groups in *Elaeagnus conferta* is considerable prospect of its significant role in bactericidal property against the pathogenic microorganisms.

Conclusion

Elaeagnus conferta Roxb. is an endangered medicinal plants of the Western Ghats known for the neutraceutical fruits. The bactericidal effect of seeds, leaf and stem bark sequential extracts is due to the presence of phytocomounds in different proportions and their bactericidal is at different magnitudes of growth hindrance against the pathogenic strains. From the present study it is clear that the plant extracts of *Elaeagnus conferta* contain considerable amounts of bioactive secondary metabolites which are authenticable for the pharmacological properties. This study supports the traditional claim of *Elaeagnus conferta* ethanol extracts to control bacterial infectious diseases.

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