**ABSTRACT**

**Elaeagnus conferta** Roxb. (Elaeagnaceae), is being used in traditional herbal medicines; as the fruits, leaves and roots of this plant have been explored for the treatment of different diseases such as diabetes, ulcer, pain, rheumatism, diarrhea, inflammation and pulmonary disorders and also possess high nutritional value. Various extracts i.e. methanol, chloroform and acetone of *E. conferta* seeds were prepared by cold maceration and subjected to qualitative phytochemical screening (flavonoids, alkaloids, terpenoids, saponins, tannins, steroids, quinins, proteins, cardiac glycosides, fatty acid, carbohydrates and proteins) using standard procedures. All the extracts were further screened for antibacterial activity by disc diffusion method.

The three different extracts of seeds of *E. conferta* were found to contain various secondary metabolites like fatty acids and flavonoids and exhibited moderate to significant antibacterial activity against both Gram-positive and Gram-negative bacteria. The potency of methanolic extract at conc. i.e. 100 µg/ml was found to be > 19 mm in all the strains of bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*), which is comparable to standard drug ciprofloxacin. The various extracts of *E. conferta* exhibited variable antimicrobial activity, hence may be a natural potentially effective antimicrobial agent. Therefore, the above findings are important to strive medicinal role of *E. conferta* in treatment of various diseases including microbial infection.

**Keywords:** *Elaeagnus conferta*; Phytochemical; Ascorbic acid; Flavonoids; Fatty acids; Antimicrobial agent

**Introduction**

*Elaeagnus conferta* Roxb (Nerli), commonly known as bastard oleaster; is an edible herb mainly found in India, Vietnam, Malaysia, and South China. It is a dense thorny shrub or small bushy deciduous tree, found in the lower temperate zone and belongs to family Elaeagnaceae. The various parts of plant have been used in different regions such as Tibetan, Mongolia and Uygur for treatment of indigestion (Devachandra et al., 2018). The fruit of *E. conferta* possess high nutritional value as the plant contains high amount of macroelements such as nitrogen, phosphorus, potassium, calcium, magnesium, sodium and also contains microelements which includes ferric, zinc, copper and manganese (Deshmukh and Waghmode, 2011; Uprety et al., 2016; Valvi et al., 2014). It also contains carbohydrate, vitamin, phytic acid, oxalate, peroxidase, catalase and superoxide dismutase (Valvi et al., 2014). Plant is rich in phenolic and flavanoidal content which is responsible for its antioxidant potential (Dandge et al., 2011). The various parts of plant such as fruit, pulp and seeds have been reported to possess different pharmacological activities, hence can be a potential source of biomedicine, and most of the edible parts of plant are underutilized (Sundriyal and Sundriyal, 2003). Moreover it is reported that silver nano particles of leaf extract of *E. conferta* has been prepared and has wide application in field of pharmacology and industries (Gowtham Prasanth E, 2017; Phanjom et al., 2012). In traditional herbal medicine, the fruit, leaves and roots have been explored for the ailment of multiple diseases such as diabetes, ulcer, pain, rheumatism, diarrhea, inflammation and pulmonary disorders (Binu, 2011; Deshmukh and Waghmode, 2011; Gill and Gupta, 2018; Jin et al., 1999; Liu et al., 2019; Patil et al., 2012; Raghavendra et al., 2015; Rana and Samant, 2011). Fruits, either raw or in the form of juice, syrup, pickle, jelly etc, have been reported to be used as food supplement and is free from any side effects (Deshmukh and Waghmode, 2011). Although, several lines of corroborate support beneficial effects of *E. conferta*, however, as per best of our knowledge, its phytochemical screening and antimicrobial activity of seeds have not been explored till date. Therefore, the present study was aimed to investigate presence of phytoconstituents and antimicrobial potential of different extracts seeds of *E. conferta*.

**Materials and Methods**

**Chemicals and drugs**

Seeds of *E. conferta* Roxb. were procured from local market of Manali, Himachal Pradesh, India during the month of September-October and authenticated at G.B. Pant National Institute of Himalayan Environment and Sustainable
Development, Himachal Pradesh, India (GBPNIHESD/ SIC/358). Hexane, methanol, gelatin, sodium chloride (NaCl), wagner reagent, hager reagent, mayer reagent, dragendorff reagent, ferric chloride (FeCl₃), chloroform (CHCl₃), acetic anhydride, sulphuric acid (H₂SO₄), sodium hydroxide (NaOH), hydrochloric acid (HCl), ammonia (NH₃), molisch reagent, benedict reagent, fehling reagent, ninhydrin reagent, biuret reagent, potassium hydroxide (KOH) were purchased from Loba Chemie Pvt. Ltd., Mumbai, India.

Instruments
Rotary evaporator (RV-8, IKA, Bengaluru, India) were used.

Preparation of extracts of E. conferta
Seeds of E. conferta were shade dried, cleaned and grounded to coarse powered using mortar and pestle. Further defatting was performed by macerating with n-hexane for 7 h. The solvent was removed by filtration and formed marc was pressed. The dried marc was extracted with different solvents such as methanol, acetone and chloroform for 48 h by cold maceration method and evaporated to dryness by rotary evaporator (RV-8, IKA, Bengaluru, India) under reduced pressure to obtain different extract of E. conferta (Girma et al., 2015).

Phytochemical qualitative analysis
The plant extracts prepared were accessed for presence of phytochemical analysis as per the protocol given by standard procedures (Njoku and Obi, 2009; Wadood et al., 2013).

Detection of alkaloids
Mayer test: 1 ml of extract was treated with Mayer’s reagent (saturated solution of mercuric iodide). Appearance of creamy yellowish precipitates indicates presence of alkaloids (Mir et al., 2016).

Wagner’s test: Few ml of filtrate was treated with Wagner’s reagent (saturated solution of iodine in potassium iodide). Formation of reddish brown precipitates shows presence of alkaloids.

Dragendorff’s reagent: To plant extracts, Dragendorff’s reagent (saturated solution of potassium bismuth iodide) was added. Red precipitates detect alkaloids in sample (Zohra et al., 2012).

Detection of carbohydrates
Extracts (2 ml) were mixed with small quantity of water and filtered. The filtrate was used to check existence of carbohydrates in the sample

Benedict’s test: 0.5 ml of filtrate was mixed with 0.5 ml of Benedict’s reagent and heated on water bath for 2 min. Characteristics orange red precipitates indicates presence of reducing sugar (Ismail et al., 2016; Mir et al., 2016).

Fehling’s test: Dil. hydrochloric acid was added to hydrolyze extract followed by neutralization with sodium hydroxide. The above solutions were heated with Fehling’s A and B solutions. Appearance of intense red precipitates indicates reducing sugar.

Detection of glycosides
Extracts were hydrolyzed with acid and subjected to various identification tests of glycosides.

Borntrager’s test: Ferric chloride solution was treated with few ml of extract and kept in water bath for 5 min. The mixture was cooled and partitioned with equal volume of benzene. Organic layer was separated and treated with ammonia solution. Appearance of pink color in ammonical solution specifies presence of glycosides (Auwal et al., 2014).

Legal’s test: Extract was treated with methanolic sodium hydroxide, pyridine followed by treatment of sodium nitropruside. Precipitate of blood red color shows presence of glycosides.

Lieberman test: Plant extract was mixed with acetic acid (2 ml) and 2 ml chloroform, cooled and treated with concentrated sulfuric acid. Green color reflects steroidal aglycone in structure (Gul et al., 2017).

Detection of saponins
Froth test: Extract was mixed with water and was rigorously shaken for 10 min. Appearance of foam shows saponins (Senguttuvan et al., 2014).

Detection of phytosterol
Salkowski’s test: 2.5 ml of extract was mixed with 1ml of chloroform followed by 2 ml of conc. sulphuric acid. Reddish brown coloration at interface indicates presence of triterpene (Makkar et al., 2007).

Detection of tannins
Ferric chloride test: 2 ml extract was mixed with water (5 ml), filtered and was shaken with ferric chloride reagent. Bluish black precipitate is evidence of presence of tannin (Ukoha et al., 2011).

Lead acetate test: 1 ml of extract was mixed with lead acetate (3 ml) solution. Yellowish gelatinous coloration shows occurrence of tannins (Ukoha et al., 2011).

Test for flavonoids
Sulphuric acid test: Few drops of concentrated sulphuric acid was added to plant extract. Development of red color indicated presence of flavonoids in sample (Ismail et al., 2016).

Lead acetate test: To 1 ml of plant extract, 1 ml of lead acetate (10 %) was added. Appearance of yellow precipitate is indication of flavonoids (Njoku and Obi, 2009).

Sodium hydroxide test
To test sample, add 1 ml of dilute sodium hydroxide solution. Yellow precipitate reflects flavonoids in sample (Singh and Bag, 2013).

Determination of protein
Biuret test: To plant extract 2 ml of 4% NaOH solution was added followed by addition of 1 ml of 1% CuSO₄ solution. Formation of violet pink color indicates presence of protein (Ismail et al., 2016; Manas et al., 2010; Wokes and Still, 1942).


*Millon test*: To 2ml of extract, add 2 drops of Million’s reagent. The solution was shaken vigorously and kept for 5 min. Appearance of yellow precipitates indicates presence of proteins in the sample (Asthana *et al*., 2019; Ukoha *et al*., 2011).

**Test for coumarins**

*Fluorescence test*: To the alcoholic extract, added 1 ml of dil. NaOH solution. Continuous exposure of alkali solution results in appearance of yellowish blue fluorescence (Feigl *et al*., 1955).

*Ferric chloride test*: To the alcoholic extract, 1 ml of ferric chloride solution was added. Appearance of green color which turns yellow on addition of nitric acid shows presence of coumarins (Patel and Patel, 2016; Roberts and Link, 1937).

**Test for fatty acids**

*Spot test*: A drop of extract was spotted on pre-coated silica gel plate. The spot was treated with one drop of 1% CuSO4 and heated at 120 °C for 15 min. If the spot turns black, then fatty acid is unsaturated or vice versa.

* Sulphuric acid test*: A drop of extract was spotted on pre-coated silica gel plate. The chromatogram was allowed to develop and spots were visualized by UV lamp or sulphuric acid: water (1:1). If the spot appears blue, then fatty acid is present (Schlierf and Wood, 1965).

**Test for ascorbic acid**

*Ferrous sulphate test*: To 2ml of extract in water, 0.1 g of sodium bicarbonate and 0.02 g of ferrous sulphate is added. Shaked well and allowed to stand. Added 5 ml of dil. sulphuric acid, appearance of violet color indicated presence of ascorbic acid.

*Potassium permanganate test*: To 1ml of extract added, 0.5 ml of potassium permanganate solution. The color of potassium permanganate is immediately discharged indicating presence of ascorbic acid.

**In vitro antimicrobial activity**: All extracts of *E. conferta* were screened for antimicrobial potential by disc diffusion method against Gram-positive bacteria such as *Streptococcus pyogenes*, *Staphylococcus aureus*, and Gram-negative bacteria like *Escherichia coli* at a concentration of 100 µg /ml (Gowtham Prasanth E, 2017; Srinivasan *et al*., 2019). The 5 ml of extracts were taken and incubated at 37 °C for 24 h. After incubation, the zone of inhibition was measured in mm and was compared with standard drug ciprofloxacin (10 µg /ml). The results have been summarized in Table 2.

**Results and discussion**

The analysis and characterization of bioactive compounds from plants is important to ascertain their medicinal value. This study showed that pharmacologically active compounds such as ascorbic acid, reducing sugar, proteins, fatty acids and flavonoids were present in seeds of *E. conferta*. However alkaloids, glycosides, steroids and tannins were absent in all extracts of Elaeagnus. An interesting aspect of this study is that the methanolic extract of the plant contained more active compounds than others. The Phytochemical screening of different extracts of *E. conferta* has been depicted in Table 1. These tests reveals presence of various secondary metabolites present in seed extracts of plant which is responsible for its various pharmacological activities.

**Table 1**: Phytochemical analysis of *E. conferta* extracts

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Methanolic extract</th>
<th>Acetone extract</th>
<th>Chloroform extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Fehling test</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Benedict test</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Proteins</td>
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<tr>
<td>Biuret Test</td>
<td>+</td>
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<tr>
<td>Millon test</td>
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<td>+</td>
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<tr>
<td>Alkaloids</td>
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<tr>
<td>Dragendorff test</td>
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<td>Mayer test</td>
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<tr>
<td>Flavanoids</td>
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<tr>
<td>Sulphuric acid test</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Lead acetate test</td>
<td>+++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Alkaline test</td>
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<tr>
<td>Cardiac glycosides</td>
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<tr>
<td>Legal test</td>
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<td>Baljet test</td>
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<tr>
<td>Steroids/terpenoids</td>
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<td>Salkowski test</td>
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<tr>
<td>Libermann Burchard test</td>
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<td>Liberman test</td>
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<tr>
<td>Anthraquinone glycosides</td>
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<tr>
<td>Borntraggert test</td>
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<td>Saponins</td>
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<td>Foam test</td>
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<tr>
<td><strong>Tannins</strong></td>
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<td>Ferric chloride test</td>
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<td>Lead acetate test</td>
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<td>Acetic acid test</td>
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<tr>
<td>Potassium permanganate test</td>
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<tr>
<td><strong>Coumarin</strong></td>
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<td>Alkaline test</td>
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<tr>
<td>Fatty acid</td>
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<tr>
<td>Spot test</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Sulphuric acid test</td>
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<td>+</td>
<td>+</td>
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<tr>
<td><strong>Ascorbic acid</strong></td>
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<td></td>
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<tr>
<td>Ferrous sulphate test</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Potassium permanganate test</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1: +++: Dark colour; ++: light colour; +: Very light colour; -: No colour

**Antimicrobial activity**

The Genus Elaeagnus is being used against microbial infection for years. The silver nano particles of leaves of *E. conferta* has also been reported to possess high potential against various species of micro-organism (Arthy and Novaryati, 2019; Kamath and Ramakrishna, 2016). All the extracts were evaluated for their *in vitro* antimicrobial potential against both Gram-positive and Gram-negative bacteria such as *S. pyogenes*, *S. aureus* *P. aeruginosa*, and *E. coli*. Both methanol and chloroform extracts exhibited excellent to moderate antimicrobial activity as compared to the test drug ciprofloxacin. The methanolic extract was found to be most potent against all test microorganisms, therefore can be used as a natural alternative for treatment of bacterial infections.
Preliminary phytochemical screening of Elaeagnus conferta Roxb. seeds and biological evaluation

Table 2: Antimicrobial activity of different extracts of E. conferta

<table>
<thead>
<tr>
<th>S. No</th>
<th>Extracts of E. conferta</th>
<th>S. pyogenes</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>20 ± 0.11</td>
<td>19 ± 0.9</td>
<td>24 ± 0.22</td>
<td>23 ± 0.18</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>18 ± 0.43</td>
<td>17 ± 0.41</td>
<td>22 ± 0.20</td>
<td>21.1 ± 0.34</td>
</tr>
<tr>
<td>3</td>
<td>Acetone</td>
<td>14 ± 0.17</td>
<td>10 ± 0.12</td>
<td>13 ± 0.80</td>
<td>15 ± 0.33</td>
</tr>
<tr>
<td>4</td>
<td>Ciprofloxacin</td>
<td>22 ± 0.51</td>
<td>23 ± 0.1</td>
<td>26 ± 0.54</td>
<td>27 ± 0.67</td>
</tr>
</tbody>
</table>

Zone of inhibition is expressed as mean ± standard deviation of triplicates.

S. pyogenes: Streptococcus pyogenes, S. aureus: Staphylococcus aureus, Pseudomonas aeruginosa: P. aeruginosa, E. coli: Escherichia coli

Conclusion

The phytochemical analysis showed that the E. conferta plant extract may contain a mixture of phytochemicals such as reducing sugars, proteins, fatty acids and flavonoids. E. conferta has been reported to possess diverse biological activities which may be attributed due to the presence of flavonoids. Along with the same, the seeds of E. conferta possess high nutritional value due to presence of high content of fatty acids (oleic acid, stearic acid, linoleic acid and palmitic acid) and minerals, and therefore can be used as nutritional supplement (Liu and Huang, 2007). All the extracts showed significant antimicrobial activity, however the maximum activity was observed with methanolic extract. Thus, the plant can be a promising natural source of antimicrobial drug. Therefore, the findings are important tot driver medicinal role of E. conferta in treatment of various diseases including microbial infection.

Acknowledgement

The authors express sincere gratitude to the management of I. K. Gujral Punjab Technical University, for providing essential requirements for carrying out this research work.

Authors's Contribution

Ms. Mukta Gupta has performed experimental work, whereas manuscript drafting and proof reading is done by Dr. Naresh Singh.

Conflicts of interest

None.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Binu, S. (2011). Medicinal plants used for treating body pain by the tribals in Pathanamthitta district, Kerala, India.


