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PRELIMINARY PHYTOCHEMICAL SCREENING OF *ELAEAGNUS CONFERTA* ROXB. SEEDS AND BIOLOGICAL EVALUATION

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ABSTRACT

Elaeagnus conferta Roxb. (Elaeaganaceae), 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, quinons, 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, dragndroff reagent, ferric chloride ($FeCl_3$), chloroform ($CHCl_3$), acetic anhydride, sulphuric acid (H_2SO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), ammonia (NH_3), 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.

Liberman 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 flanonoids

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%$CuSO_4$ 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% $CuSO_4$ and heated at 120 ^{0}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 sports 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 *Pseudomonas aeruginosa*, *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

Phytochemical test	Methanolic extract 1	Acetone extract	Chloroform extract
Carbohydrates			
Fehling test	++	+	+
Benedict test	+++	+	+
Proteins			
Biuret Test	+	+	+
Millon test	+++	+	+
Alkaloids			
Dragndroff test	-	-	-
Mayer test	-	-	-
Flavanoids			
Sulphuric acid test	++	+	+
Lead acetate test	+++	+	+
Alkali test	+++	+	+
Cardiac glycosides			
Legal test	-	-	-
Baljet test	-	-	-
Steroids/terpenoids			
Salkowski test	-	-	-
Libermann Burchard test	-	-	-
Liberman test	-	-	-
Anthraquinone glycosides			
Borntragger test	-	-	-
Saponins			
Foam test	-	-	-
Tannins			
Ferric chloride test	-	-	-
Lead acetate test	-	-	-
Acetic acid test	-	-	-
Potassium permanganate test	-	-	-
Coumarin			
Alkaline test	-	-	-
Fatty acid			
Spot test	+++	+	+
Sulphuric acid test	+++	+	+
Ascorbic acid			
Ferrous sulphate test	+++	+	+
Potassium permanganate test	+++	+	+

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 (Ardhany and Novaryatiin, 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.

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

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

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 contains 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 to strive medicinal role of *E. conferta* in treatment of various diseases including microbial infection.

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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.

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