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## EXPERIMENTAL PAPER

# Evaluation of antimicrobial activity and phytochemical qualitative analysis of *Ephedra foliata* Boiss. ex C.A. Mey.

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## Summary

**Introduction:** *Ephedra foliata* Boiss. ex C.A. Mey of the *Ephedraceae* family is an evergreen shrub distributed throughout North Africa and Southwest Asia. It has been a characteristic source of alkaloids like ephedrine, pseudoephedrine and other related mixes, which are of great importance for their biological and pharmacological potential.

**Objective:** This study is aimed to evaluate the antimicrobial potential and phytochemical constituents studies on stem, leaves and flowers extracts of *E. foliata*.

**Method:** The air-dried plant sample was powdered with an electric grinder, then extracted successively with solvents, namely petroleum ether, petroleum benzene, ethyl acetate, methanol, and aqueous using Soxhlet apparatus for 72 hours. The solid matter was separated by filtration and then solvents were evaporated with a vacuum rotary evaporator to obtain the crude extracts. Freshly prepared crude extracts were subjected to the standard procedures of preliminary phytochemical screening for the investigation of the presence or absence of different phytoconstituents. The result showed the presence of reducing sugars, flavonoids, and cardiac glycosides. Antimicrobial activity of the crude extract was determined by agar well diffusion method.

**Results:** Ethyl acetate extract showed the highest antimicrobial activity against all the tested pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Streptococcus pneumonia*, *Aspergillus terreus*, *Cladosporium herbarum* and *Candida tropicalis*). All five extracts inhibited the growth of *Y. enterocolitica*.

**Conclusion:** The antimicrobial properties of *E. foliata* extracts are of great interest in light of the ongoing threat of microbial resistance to conventional antibiotics. Phytoconstituents present in the *E. foliata* extracts might be a good alternative to modern antimicrobials as a natural compound.

**Key words:** *Ephedra foliata*, crude extract, phytochemical screening, antimicrobial activity

**Słowa kluczowe:** *Ephedra foliata*, surowy ekstrakt, badania fitochemiczne, aktywność antybakteryjna

## INTRODUCTION

Medicinal plants are edible or non-edible plants which at least accumulate substances in one of their organs that reflect health benefits in the treatment or prevention of mental illnesses [1]. It has been estimated that 20 to 85% of the world's population in the developed and developing countries use medicinal plants [2]. *Ephedra foliata*, ordinarily known as Somalata, is a gymnosperm plant belonging to family *Ephedraceae*, is an evergreen shrub developing from 0.15 to 1.0 m in height. It is distributed in North Africa and Southwest Asia. It is one of the most established medicinal plants in the world, notable in conventional Chinese drug, used to treat hypersensitivities, bronchial asthma, chills, colds, hack, fever, influenza, cerebral pains and nasal blockage. It is a source of alkaloids like ephedrine, pseudoephedrine and their mixes [3]. The stems of most individuals from this family contain ephedrine and are used in the treatment of asthma and numerous different contradictions of the respiratory framework. The activity of ephedrine is more delayed than that of adrenaline, being a functioning vasoconstrictor; alkaloids from *Ephedra* can be utilized to hoist circulatory strain and respiratory rate [4]. It possesses antimicrobial, antioxidant, antidiabetic, hepatoprotective and cardiovascular activities [5].

Secondary metabolite has a pharmacological or bioactivity used in drug discovery process. Based on the diverse uses of *E. foliata* in folk medicine, it is interesting to perform the antimicrobial potential and phytochemical qualitative analysis of different plant extracts (stem, leaves and flower) of *E. foliata*.

## MATERIALS AND METHODS

### Plant materials

*E. foliata* chosen for the present study has proficient medicinal significance. The plant material was collected from the Herbal Garden, Botany Department, Kurukshetra University, Kurukshetra.

### Preparation of extracts

The aerial portions, stem, leaves and flowers of *E. foliata* were collected and washed with tap water pursued by distilled water to take out the dust and dirt on the surface of the plant. The plant material was dried at room temperature for 15 days. The dried plant material was powdered with an electric grinder. The different extracts were prepared by taking 50 g of plant powder extracted by immersing with 200 ml of five solvents namely petroleum ether, petroleum benzene, ethyl acetate, methanol, and aqueous using Soxhlet apparatus for 72 hours. The extracts were filtered with filter paper (Whatman No. 1) and solvents were evaporated with a vacuum rotary evaporator to obtain the crude extracts, residue stored at 4°C until further use. The final concentration of 200 mg/ml was made to test antimicrobial activity by dissolving extracts into DMSO (dimethyl sulfoxide) with the concentration of 1 mg/ml being evaluated for biological potential.

### Test microorganisms

The microbial strains used in the research experiment namely, *Escherichia coli* MTCC 1570, *Staphylococcus aureus* (NCIM 5345), *Pseudomonas aeruginosa* MTCC 1034, *Yersinia enterocolitica* (MTCC 3235), *Streptococcus pneumoniae* (MTCC 2672), *Aspergillus terreus* (NCIM 1325), *Cladosporium herbarum* (NCIM 1112) and *Candida tropicalis* (MTCC 184) were collected from the IMTECH Chandigarh and NCIM, Pune.

### Preparation of inoculums

Stock cultures were sustained at 4°C on slants of nutrient agar test tubes. Active cultures were readied by moving cells from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacterial strains, incubated for 24 h at 37°C and Sabouraud Dextrose broth (SDB) for fungal strains that were incubated for 7 days at 30°C.

## Antimicrobial assay

The extracts were assessed against pathogens by agar well diffusion method. The turbidity of the inoculum was adjusted to  $1.5 \times 10^8$  CFU/ml (corresponding to 0.5 McFarland standards). The antibacterial viability of plant extracts were compared by standard antibiotics streptomycin and antifungal activity by fluconazole. The final concentrations for antimicrobial drugs were streptomycin (15  $\mu$ g/ml) and fluconazole (130  $\mu$ g/ml) according to CLSI AST norms. Streptomycin was dissolved in distilled water and fluconazole in DMSO to make the final concentration for antimicrobial testing. Distilled water and DMSO act as a negative control. A 6 mm diameter cork borer was used to make well in the medium and filled with 50  $\mu$ l of final concentrations of control and plant extracts. The zone of inhibition was measured in millimetre (mm) as clear zones seen around the wells.

## Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of crude extracts was decided by broth microdilution

method. Stock solutions were set up with the concentration of 200 mg of crude plant extracts in 1 ml of DMSO and further diluted using two-fold serial dilution in sterile broth. A series of test concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.13 were given by serial dilution. Afterward, 100  $\mu$ l volumes of samples were poured into the well of 96-well microtiter plate followed by 100  $\mu$ l volume of test strain broths. After incubation, the minimum concentration of the test sample which inhibited the growth of the test organism was considered as MIC of the plant extract.

## Preliminary phytochemical screening

Freshly prepared crude extracts of *E. foliata* were subjected to the standard methodologies (Table 1) of preliminary phytochemical screening for the investigation of the presence or absence of different phytoconstituents [6, 7]. The qualitative results of the analysis were expressed as + for the presence and - for the absence of phytochemical.

*Ethical approval: The conducted research is not related to either human or animal use.*

**Table 1.**  
Preliminary phytochemical screening methods

Phytochemical	Test/ Reagent	Method	Observation	Inference
Alkaloids	Wagner's reagent	Extract + 3–5 drops of Wagner's reagent	reddish brown precipitate or coloration	alkaloids present
Reducing sugars	Fehling's Test	1 ml of extract + few drops of Fehling's reagent and stirring	rusty red precipitate	reducing sugars present
Tannins	Braymer's test	2 ml of extract + 10% alcoholic ferric chloride	blue or greenish coloration	tannins present
Phenols	Ferric chloride test	Extracts + aqueous 5% ferric chloride	deep blue or black coloration	phenols present
Flavonoids	Alkaline reagent test	2 ml of extract + few drops of 20% sodium hydroxide + dil. HCl	intense yellow color, turns colorless on addition of acid	flavonoids present
Saponins	Foam test	2 ml of extract + 6 ml of water and shaken well	formation of persistent foam	saponins present
Amino acids	Ninhydrin test	2 ml of extract + 2–5 drops of 1% ninhydrin solution in acetone and boiled for 1–2 min.	purple coloration	amino acids and protein present
Cardiac Glycosides	Keller Kelliani's test	5 ml extract + 2 ml glacial acetic acid + 1 drop of ferric chloride + 1 ml conc. H <sub>2</sub> SO <sub>4</sub>	brown to blue color ring at the interface	cardiac glycosides present
Steroids	Salkowki's test	2 ml of extract + 1 ml chloroform + few drops of conc. H <sub>2</sub> SO <sub>4</sub>	red color on the upper layer and yellow color on lower layer	steroids present

## RESULTS AND DISCUSSION

### Antimicrobial Activity

*In vitro* antimicrobial activity of different solvent extracts of *E. foliata* was assessed by investigating the presence or absence of zones of inhibition. The data of the antimicrobial activity of the extracts of *E. foliata* and reference standard antimicrobials are shown in Table 2. The stem extracts of *E. foliata* in different solvents have potent antimicrobial efficacy against many bacterial strains and fungal strains carried out by agar well diffusion method. The antimicrobial activity of all the crude extracts could be explained by the presence of several phytochemicals such as reducing sugars, alkaloids, phenols, flavonoids, and cardiac glycosides etc., which could be responsible for the observed biological activities [8]. Results showed some variation among different extracts (petroleum ether, petroleum benzene, ethyl acetate, methanol and aqueous) of *E. foliata* against most of the tested strains (Gram-positive and Gram-negative) in a dose-dependent manner. Indeed, the bacterial strain *Y. enterocolitica* was observed to be the most sensitive pathogen among all the test microorganisms. All the five extracts inhibited the growth of *Y. enterocolitica* and showed the zone of inhibitions ranging from  $28\pm 0.66$  to  $34\pm 0.15$  mm and the ethyl acetate extract was observed to be the most effective extract followed by aqueous, petroleum benzene, methanol and petroleum ether. Ethyl acetate extract gave the highest antimicrobial activity against all the tested pathogens with the zone of inhibition  $34\pm 0.15$  mm against *Y. enterocolitica* followed by *S. pneumoniae* ( $32\pm 0.03$  mm), *S. aureus* ( $31\pm 0.34$  mm), *E. coli* ( $30\pm 0.38$  mm), *P. aeruginosa*

( $29\pm 0.26$  mm), *C. herbarum* ( $20\pm 0.54$  mm), *A. terreus* ( $16\pm 0.20$  mm), and *C. tropicalis* ( $10\pm 0.07$  mm). Streptomycin was used to compare the sensitivity of the bacterial strains and *S. pneumoniae* as well as *Y. enterocolitica* were found the most sensitive with inhibition zone  $22\pm 0.63$  mm and  $22\pm 0.58$  mm respectively. Fluconazole was used as a reference antifungal drug and was found to be most effective against *A. terreus* and *C. tropicalis* with the zone of inhibition  $21\pm 0.90$  mm and  $20\pm 0.25$  mm respectively. Among the different solvent tested, hydrophobic fractions were petroleum ether and petroleum benzene while hydrophilic fractions were ethyl acetate, methanol and aqueous. Among all these the hydrophilic fraction, ethyl acetate dissolve more compounds and gave best inhibition activity. Besides this, methanol extracts of *E. foliata* have been well observed for their antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*. [3]. Mathur *et al.* [9] reported the antimicrobial efficacy of *E. foliata* against human pathogenic bacteria. Bissa [10], evaluated the antimicrobial potential of different plant part (stem and leaves) extracts (aqueous, alcoholic, chloroform and petroleum ether) of *E. foliata* against human pathogenic (*E. coli*, *S. typhi*, *K. pneumoniae*, and *Enterobacter aerogenes*) as well as plant pathogenic (*Agrobacterium tumefaciens*) bacteria and reported that all the plant parts exhibited antimicrobial activity that is comparable to the present research work.

### Minimum inhibitory concentration (MIC)

The MIC of the extracts of *E. foliata* is shown in Figure 1. The MIC of the four different extracts

**Table 2.**

Zone of inhibition of different solvent extracts of *E. foliata* against MTCC microbial strains

Microbial strains	Zone of inhibition [mm]							
	Petroleum ether	Petroleum benzene	Ethyl acetate	Methanol	Aqueous	DMSO	Streptomycin	Fluconazole
<i>E. coli</i>	$17\pm 0.62$	$29\pm 0.54$	$30\pm 0.38$	$25\pm 0.86$	$22\pm 0.32$	-	$20\pm 0.16$	NT
<i>P. aeruginosa</i>	$30\pm 0.59$	$27\pm 0.19$	$29\pm 0.26$	$31\pm 0.98$	$31\pm 1.01$	-	$18\pm 0.47$	NT
<i>S. aureus</i>	$28\pm 0.98$	$28\pm 0.25$	$31\pm 0.34$	$27\pm 0.15$	$30\pm 1.11$	-	$08\pm 0.07$	NT
<i>Y. enterocolitica</i>	$28\pm 1.06$	$30\pm 0.68$	$34\pm 0.15$	$29\pm 0.66$	$33\pm 1.34$	-	$22\pm 0.58$	NT
<i>S. pneumoniae</i>	$26\pm 0.82$	$27\pm 0.63$	$32\pm 0.03$	$26\pm 0.63$	$33\pm 1.10$	-	$22\pm 0.63$	NT
<i>A. terreus</i>	$02\pm 0.01$	$03\pm 0.07$	$16\pm 0.20$	-	-	-	NT	$21\pm 0.90$
<i>C. tropicalis</i>	$02\pm 0.04$	-	$10\pm 0.07$	-	-	-	NT	$20\pm 0.25$
<i>C. herbarum</i>	$04\pm 0.00$	$06\pm 0.06$	$20\pm 0.54$	-	-	-	NT	$10\pm 0.04$

Values are the mean of three replicates; significant at  $p\leq 0.05$  level of analysis of variance; diameter of well: 6 mm; MTCC – Microbial Type Culture Collection; DMSO – dimethyl sulfoxide; NT – not tested

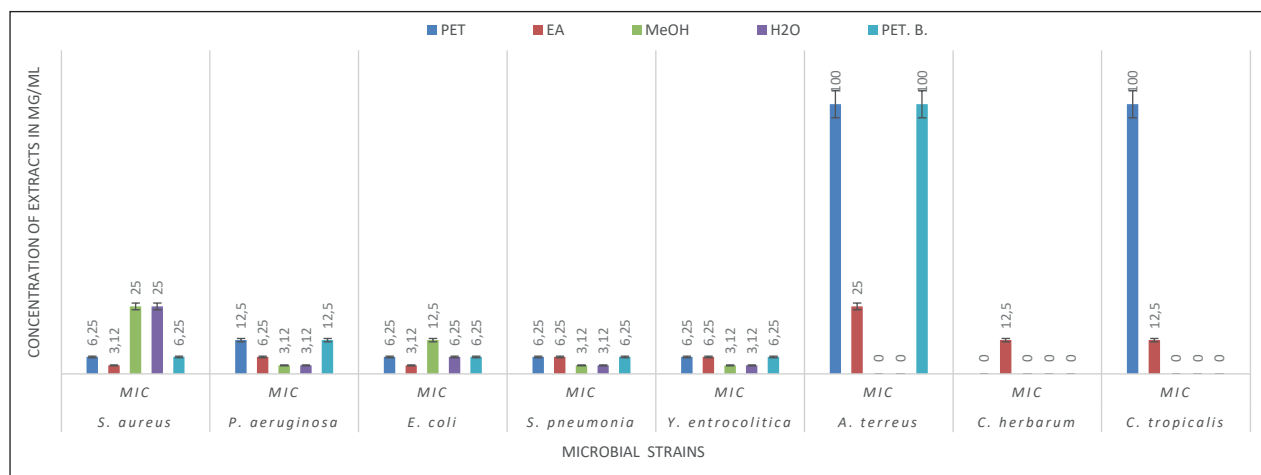


Figure 1.

MIC [mg/ml] of different solvent extracts of *E. foliata* against MTCC microbial strains

ranged from 3.12-25 mg ml<sup>-1</sup> for *S. aureus*, 3.12-12.5 mg ml<sup>-1</sup> for *P. aeruginosa* and *E. coli*, 3.12-6.25 mg ml<sup>-1</sup> for *S. pneumoniae* and *Y. enterocolitica*, 25-100 mg ml<sup>-1</sup> for *A. terreus*, 12.5 mg ml<sup>-1</sup> for *C. herbarum* and 12.5-100 mg ml<sup>-1</sup> for *C. tropicalis*. The lowest range of MIC (3.12-12.5 mg ml<sup>-1</sup> and 3.12-25 mg ml<sup>-1</sup>) were observed in the case of ethyl acetate and methanol extracts respectively of *E. foliata* against all the tested pathogens.

### Phytochemical Screening

The qualitative phytochemical screening of the fresh and dried shoot powder extracts of *E. foliata* in five different solvents showed the presence of several phytochemicals. Reducing sugars, flavonoids, and cardiac glycosides were found present in all the five extracts however, saponins and amino acids were found absent in all the extracts (Table 3). The existence of alkaloids, reducing sugars, flavonoids, phenols and cardiac glycosides has been reported in methanol, petroleum ether and petroleum benzene extract. In an aqueous extract, alkaloids, phenols, tannins, saponins, amino acids and steroids were not found. While high yields of phytochemicals with the presence of alkaloids, reducing sugars, flavonoids, phenols, tannins, cardiac glycosides and steroids were documented from ethyl acetate extract. According to previously conducted studies, phenolic compounds are the active ingredients of *Ephedra* plant [11, 12]. The antimicrobial activity of many species of *Ephedra* species like *E. major*, *E. monosperma*, *E. fragilis*, *E. distachya*, *E. foeminea*, *E. alata*,

*E. sinica* and *E. vulgaris* showed variations in their phytochemical constituents levels using different types of extracts [11, 12, 13, 14, 15, 16, 17].

*Ephedra* contains alkaloids (pseudoephedrine, norephedrine and methylephedrine), flavonoids (leucodelphinidin, leucoanthocyanidin, leucopelargonine, lucenine, vicenin-1, and vicenin-2), phenols (kaempferol 3-rhamnoside, quercetin 3-rhamnoside, herbacetin 8-methyl ether 3-O-glucoside-7-O-rutinoside and furanofuran) and tannins (proanthocyanidines) [18].

In the present research work, the stem extract of *E. foliata* exhibited the presence of several phytochemicals with comparatively more concentration of alkaloids that is comparable with Bissa [10]. This variation in concentration may be due to several factors such as soil characteristics, harvesting, storage conditions and analytical quantification techniques [18]. These different phytoconstituents interact with the membrane proteins of the bacteria through inter-helical hydrogen bonding, causing changes in membrane permeability and cell destruction, penetrating bacterial cells and coagulating the cell contents [19]. The cytoplasmic membrane of the bacteria also gets damaged through the perforation action of the flavonoid [20]. Flavonoid causes retardation in the growth of microorganisms by inhibiting their nucleic acid synthesis and energy metabolism [21]. Therefore, *E. foliata* species depicts the presence of various phytochemicals which may be acting independently or synergistically with other compounds to show medicinal as well as therapeutic efficacies. They showed potentials antimicrobial activity that can be exploited as the alternatives of several antibiotics in the treatment of microbial infections [22].

**Table 3.**Preliminary phytochemical screening of different solvent crude extracts of *E. foliata*

Phytochemical compounds	Petroleum ether	Petroleum benzene	Ethyl acetate	Methanol	Aqueous
Alkaloids	+	+	+	+	-
Reducing sugars	+	+	+	+	+
Tannins	-	-	+	-	-
Phenols	+	+	+	+	-
Flavonoids	+	+	+	+	+
Saponins	-	-	-	-	-
Amino acids	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+
Steroids	-	-	+	-	-

+: present; -: absent

## CONCLUSION

It has been well known the rate of microbial resistance towards antibiotics increasing day by day. Therefore, the medicinal plants and their phyto-compounds are creating a new interest as an antimicrobial agent. The background of the manuscript indicates that *E. foliata* phyto-complex extracts have an efficacious antimicrobial potential, as evidenced by the inhibitory effect on bacterial growth of different human pathogens. The antimicrobial properties of *E. foliata* extracts are of great interest in light of the ongoing threat of microbial resistance to conventional antibiotics. Phytoconstituents present in the *E. foliata* extracts might be a good alternative to modern antimicrobials as a natural compound.

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