

EVALUATION OF ANTIBACTERIAL POTENTIAL OF *EPHEDRA FOLIATA* BOISS. EX. C. A. MEY

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KEYWORDS

Ephedra foliata
Gymnosperms
Disc Diffusion
Klebsiella pneumoniae
E.coli

Received on :
13.05.2015

Accepted on :
25.08.2015

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ABSTRACT

The present study is an attempt to evaluate the antimicrobial potential of different plant part extracts (aqueous, alcoholic, chloroform and petroleum ether) of a gymnospermous plant *Ephedra foliata* Boiss. ex C.A.Mey. against human pathogenic (*E.coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*) as well as plant pathogenic (*Agrobacterium tumefaciens*) bacteria using disc diffusion method. All the plant parts exhibited antibacterial activity. The maximum antibacterial activity in *Ephedra foliata*, was presented by petroleum ether extract of dried stem against *Klebsiella pneumoniae* and dried leaves against *Enterobacter aerogenes*. Phytochemical analysis (Stem part) was also done. Thin Layer Chromatography (TLC) identity test was also performed which corresponded to the ephedrine present in the plant.

INTRODUCTION

With increase in resistance of bacterial pathogens to antibiotics, there is an urgent need to develop novel antibacterial agents. Plants have long been investigated as potential source of innovative antimicrobial agents as they contain many bioactive compounds and presents low toxicity (Djeussi *et al.*, 2013; Ram *et al.*, 2012; Chandekar and Madhugiri, 2011).

Indian subcontinent is considered to be a vast treasure of medicinal plant wealth and the first information about the medicinal uses of plants is found in the Rig Veda, perhaps the oldest repository to human knowledge, written between 4500 and 1600 B.C. (Chopra *et al.*, 1958). Plants have served as a source of new pharmaceutical products. Besides angiosperms, gymnosperms are forming the important medicinal flora of Indian forests (Mourya *et al.*, 2011). Naked seeded plants exhibit wide spectrum antimicrobial activity against many human pathogenic bacteria (Bissa *et al.*, 2008). Gymnosperms have proved to be an important source of antimicrobial agents as revealed by many researchers (Johnston *et al.*, 2001; Ozcan, 2001; Hafez and Abdel, 2004; Torras *et al.*, 2005, Smith *et al.*, 2005; Santi-Gadelha *et al.*, 2006).

Ephedra foliata, a gymnospermous plant, belonging to family Ephedraceae, is an evergreen Shrub growing to 0.15m by 1m. The flowers are dioecious. The plant prefers acid, neutral and basic (alkaline) soils. Ephedra is considered to be one of the oldest medicinal herb known to mankind and is well known plant in traditional Chinese medicine, used to treat allergies, bronchial asthma, chills, colds, cough, fever, flu, headaches and nasal congestion. It has been a natural product source of

alkaloids such as ephedrine, pseudoephedrine and other related compounds (Parsaeimehr *et al.*, 2010). The stems of most members of this genus contain the alkaloid ephedrine and are valuable in the treatment of asthma and many other complaints of the respiratory system. Dehkordi *et al* (2015) studied the antioxidant and antibacterial activity of extracts of *Ephedra procera*. In the present investigation antimicrobial activity of different plant parts extract was tested against human pathogenic as well as plant pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant material

Fresh plant parts of *Ephedra foliata* were collected from Mandalnath area of jodhpur district and its identity was confirmed from literature available in Department of Botany, J.N.V,University, Jodhpur. The voucher specimens were deposited in herbaria of Department of Botany, J.N.V.University, Jodhpur(Raj.), India.

Preparation of plant extracts

From fresh plant parts

25 g of fresh plant parts, viz. stem and leaves were washed 3-4 times with tap water and distilled water and then surface sterilized with 90% alcohol. Subsequently, the plant materials were grounded in 100 ml of distilled water, ethanol, chloroform and petroleum ether separately for aqueous, alcoholic extracts, chloroform extracts and petroleum ether extracts, respectively. The macerates were kept for 24 hours at room temperature to evaporate the solvents. The macerates

were squeezed through double layered muslin cloth and filtered through filter paper. After filtration, aliquot was centrifuged at 10,000 rpm for 20 minutes. The supernatants were filtered through Whatman No. 1 filter paper and then sterilized by passing through 0.2 micron disposable filters. The extracts were diluted to get a concentration of 50 mg per ml and were used for the *in vitro* studies.

From dried plant parts

The plants were thoroughly washed and then dried under shade at $28 \pm 2^\circ\text{C}$ for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50-150mm. The plant powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried plant powder was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100mL of solvent. Solvents used for extraction were Petroleum ether (60°C - 80°C), Chloroform (61°C), Ethanol (78.5°C) and Aqueous (80°C) as solvents (Fong, 1973) and the extracts were dried. The dried extracts were stored in a refrigerator at 4°C . Finally, concentration of 5 mg per disc was loaded on each disc.

Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of Nutrient Agar Medium and were incubated without agitation for 24 hrs at 37°C . The cultures were diluted with fresh Nutrient Agar broth to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/mL) for bacteria.

Antimicrobial susceptibility test

All the plant extracts were screened against five pathogenic bacterial strains. The tested organisms were *E.coli* (MTCC No. 729), *Salmonella typhi* (MTCC No.734), *Klebsiella pneumoniae* (MTCC No.109), *Enterobacter aerogenes* (MTCC No. 111) and *Agrobacterium tumefaciens* (MTCC No. 431), obtained from IMTECH, Chandigarh, India. The disc diffusion method (Bauer *et al.*, 1966) was used to test the antimicrobial activity of the plant extracts. 20ml of sterilized nutrient agar medium for *E.coli*, *S.typhi*, *K.pneumoniae*, *E.aerogenes* and *A.tumefaciens* were poured into each sterile petridish. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) soaked in .1 ml of the plant extract (In case of fresh extract) or loaded with 5 mg/disc, of dry extract and were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at 37°C for 24h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Phytochemical analysis

Phytochemical screening of plant extract was carried out qualitatively for the presence of terpenoids, tannin, flavonoids, saponin, glycosides and steroids (Harborne, 1998).

TLC identity test for *Ephedra foliata* (Mukherjee, 2002; Wagner and Bladt, 2004) test solution

1g of powdered plant part is mixed thoroughly with 1ml 10% ammonia solution or 10% Sodium carbonate solution and then extracted for 10 min with 5ml methanol under reflux.

Development of plates

20 μL of test solution was applied on the activated plate of uniform thickness (0.2 mm). The spots were allowed to dry and plates were developed in previously saturated chamber containing mobile phase (Toluene, chloroform, ethanol). The solvent was allowed to run a sufficient distance on the plate and the plate was removed from chamber, marked the solvent front and dried in air.

Visualization

The plates were visualized after spraying with Dragendorff reagent. The plates were heated for 5 min, the chromatogram was recorded and R_f value was determined.

RESULTS

All the plant parts exhibited significant antimicrobial activity against bacterial pathogens. Table 1 illustrates antibacterial activity of fresh plant part extracts (Stem and Leaves) and Table 2 illustrates antibacterial activity of dried plant part extracts (Stem and Leaves). The plant extracts responded to pathogenic bacteria as follows:

E. coli

Fresh stem extract showed significant antibacterial activity against *E.coli* with maximum inhibition zone of 10mm and 12mm in chloroform and petroleum ether respectively. Fresh leaves extract also inhibited growth of bacteria. Dried stem extract exhibited high antibacterial activity with inhibition zones of 6mm, 9mm, 8mm and 12mm in aqueous, alcoholic, chloroform and petroleum ether respectively. Dried leaves extract inhibited growth of bacteria with maximum inhibition zone of 10mm in petroleum ether extract.

S. typhi

In case of fresh stem extract, again petroleum ether extract showed highest antibacterial activity. Fresh leaves extract exhibited inhibition zones of 10mm in alcoholic extract and 11mm in petroleum ether extract. Again *S.typhi* was inhibited by all the dried plant part extracts. Chloroform and petroleum ether extract of dried stem gave inhibition zones of 9mm and 11mm respectively. Similar results were observed in the antibacterial potential of dried leaves extracts.

K. pneumoniae

Except for aqueous stem extract all the other fresh plant part extracts were found effective against the tested bacteria. Dried stem extracts exhibited one of the highest inhibition zones i.e. 12mm, 8mm, 9mm and 18mm in aqueous, alcoholic, chloroform and petroleum ether extracts respectively. Similarly dried leaves also showed significant antibacterial potential against *K. pneumoniae*.

E. aerogenes

All the fresh extracts exhibited high antibacterial potential with highest activity in petroleum ether extracts. Dried leaves extracts inhibited growth of bacteria to a large extent with

Table 1: Antibacterial activity of fresh plant part extracts of *Ephedra foliata*

Plant part	Plant extracts	Zone of inhibition (mm)				
		<i>E. coli</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	<i>Agrobacterium tumefaciens</i>
Stem	Aqueous	6	3	-	2	6
	Alcoholic	8	3	5	-	5
	Chloroform	10	7	7	8	9
	Pet. ether	12	9	6	12	8
Leaves	Aqueous	4	2	6	-	-
	Alcoholic	6	10	3	7	6
	Chloroform	5	9	7	8	6
	Petro. ether	9	11	8	10	9

Table 2: Antibacterial activity of dried plant part extracts of *Ephedra foliata*

Plant part	Plant extracts	Zone of inhibition (mm)				
		<i>E. coli</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	<i>Agrobacterium tumefaciens</i>
Stem	Aqueous	6	5	12	-	7
	Alcoholic	9	8	8	-	10
	Chloroform	8	9	9	8	12
	Pet. ether	12	11	18	10	15
Leaves	Aqueous	5	2	-	9	-
	Alcoholic	5	6	8	13	-
	Chloroform	8	10	10	10	9
	Pet. ether	10	12	11	18	11

maximum inhibition zones of 13mm and 18mm in alcoholic and petroleum ether extracts respectively. In case of dried stem extracts chloroform and petroleum ether extracts were highly inhibitive.

A. tumefaciens

Fresh stem extract exhibited significant antibacterial activity with highest inhibition zone of 9mm in chloroform extract. Similarly fresh leaves extract also showed significant antimicrobial activity. Dried stem extract proved to be highly toxic to *A. tumefaciens* as revealed by inhibition zones of 7mm, 10mm, 12mm and 15mm in aqueous, alcoholic, chloroform and petroleum ether extracts respectively. Dried leaves also inhibited growth of *A. tumefaciens* in chloroform and petroleum ether extracts.

Phytochemical analysis

The stem extract reveals the presence of all the tested phytochemicals (Glycosides, Tannins, Saponins, Flavanoids and Triterpenoids) with excess of alkaloids.

TLC identity test

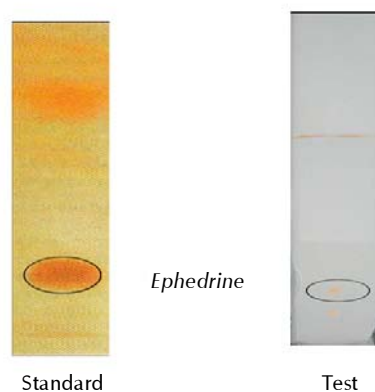
Thin layer chromatography of the plant extract was performed, after visualization with Dragendorff reagent, R_f value was recorded and found to be between ~ 0.2 which corresponds to ephedrine present in the plant. (TLC Plate 1.).

DISCUSSION

Discovery of new and potential drugs molecules can be focused on the production of bioactive compounds by plants. Caveney *et al.* (2001) studied the secondary chemistry and their antimicrobial activity in stems and seeds of many *Ephedra* species. Feresin *et al.* (2001) reported the antimicrobial activity of *Ephedra breana* against *Staphylococcus aureus*. In the present research work antimicrobial activity of fresh and dried stem and leaves of *Ephedra foliata* was taken under consideration. Both stem and leaves extracts were found

effective against tested bacteria. Fresh stem was found to inhibit growth of *E. coli* and *A. tumefaciens*. Fresh leaves extracts were most effective on *S. typhi* and *K. pneumoniae*. Dried stem extracts proved to be toxic to *A. tumefaciens* and *K. pneumoniae*. Similarly Bissa and Bohra (2011, 2012) studied the effect of different plant extracts on *E. coli*, *Salmonella typhi*, *K. pneumoniae* and *A. tumefaciens*. In present study dried leaves extract inhibited the growth of *E. aerogenes*, *E. coli* and *S. typhi*. Again petroleum ether extracts proved to be most effective. Similarly Mathur *et al.* (2010) reported antimicrobial potential of *E. foliata* against human pathogenic bacteria. Rustaiyan *et al.* (2011) investigated the antibacterial potential of *Ephedra sarcocarpa* against some Gram negative and Gram positive bacteria. Chebout *et al.* (2014) assessed the antimicrobial effect of flavanoid extracts of *Ephedra alata*.

In the present research study the stem extract of *E. foliata* exhibited the presence of all the phytochemicals tested with comparatively more concentration of alkaloids. Al-khalil *et al.* (1998) isolated transthorine, a new quinoline alkaloid from

**Plate 1: TLC profile of *Ephedra foliata* stem extract for Ephedrine**

Ephedra transitoria. Similarly Abourashed *et al.* (2003) reviewed the identity and composition of ephedrine and related alkaloids. Amakura *et al.* (2013) characterized the phenolic constituents of Ephedra herbs. TLC analysis confirmed the presence of ephedrine in given plant extract as compared by standard test.

In conclusion, maximum antibacterial activity in *Ephedra foliata*, was presented by petroleum ether extract of dried stem against *Klebsiella pneumoniae* and dried leaves against *Enterobacter aerogenes*. The above results showed that the *E. foliata* plant parts could be further exploited for chemotherapeutic agents that could be used against pathogenic bacteria. This plant could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

ACKNOWLEDGEMENT

We are thankful to University Grants Commission, New Delhi for providing financial facilities by granting Centre of Advanced Study in Botany.

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