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Assessment of antimicrobial activity of flavonoids extract from *Ephedra alata*

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ABSTRACT

The study of the antibacterial activity of flavonoid extracts of Ephedra alata was carried out on Gram positive and Gram negative pathogenic bacteria. The results exhibited variable susceptibilities of microorganisms for different concentrations of flavonoid extracts. The activity was associated with high concentration. Using plate methods, the extracts of Ephedra alata displayed relatively important effects with a variable diameter of growth inhibition zones in most types of bacteria. However no effect was recorded against Serratia marcescens ATCC 13880 with butanol extracts of flowers and leaves and ethyl acetate and dichloromethane extracts of leaves as well as with butanol, ethyl acetate, and dichloromethane extracts of leaves against Enterococcus faecalis ATCC 29212.

Keywords: *Ephedra alata*, Flavonoids, Ephedraceae, antibacterial activity.

INTRODUCTION

Plants constituted the basis of traditional medicine systems that have been in existence for thousands of years. Such extensive dependence of human being on "Mother Nature" has invoked tremendous interest in the scientific world, which ultimately led to the isolation of a vast number of chemical agents with potentials for multipurpose uses [1]. Plants are sources of bioactive phyto-compounds and they are used directly as therapeutic agents, as well as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. There is a growing interest in the use of natural substances, generally known as bioactive phyto-compounds and antimicrobial agents. This need is prompted by various reasons including inadequate access to drugs, incidents of epidemic drug resistant microorganisms and emergence of hitherto unknown diseases. Antimicrobial resistance is one of the biggest challenges facing global public health [2]. Even though pharmacological industries have produced a number of new antibiotics in the last decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is a cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality [3].

Flavonoids are a large group of compounds naturally occurring in lower and higher plants. Flavonoids have been shown to be able to affect various biological functions: capillary permeability, cellular secretary processes involved in the inflammatory response and inhibition of enzymes, receptors and carriers [2]. The inhibitory activities of flavonoids against bacteria and yeast have been investigated by a number of researchers, especially in Latin

America. More than four hundred flavonoids have been identified in plants. Flavonoids are beneficial nutrients for health and a diet for optimal immunity. These nutrients enhance the activity of white blood cells and boost the body's defenses against a broad range of bacterial and viral infections, from urinary tract infections to HIV [3]. Studies have found that the flavonoids in these foods protect against heart disease and cancer [4].

Ephedra, a medicinal plant belonging to the Ephedraceae family, is a genus of non-flowering seed plants belonging to the Gnetales, the closest living relative of the Angiosperms. *Ephedra* has been used for more than 5000 years in Traditional Chinese Medicine to treat allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, headaches, common cold and nasal congestion and has been a natural product [5,6].

Ephedra species are characterized by alkaloids of the ephedrine series. Their flavonoid constituents include di-glycosylflavones, flavonol-3-O-glycosidesand proanthocyanidins [2,3]. Among these species, *Ephedra alata* grows wild in the desert and provides extracts used in folk medicine as depurative, hypotensive, Antiasthmatic, sympathomimetic and astringent agents. The branches are chewed for cephalalgia, used in miscarriage and as a bronchodilator [7], antifungal, and antimicrobial [8,9].

Ephedra alata (Flowers and leaves) were collected in Mars, 2011 from Ouargla Algeria.

MATERIALS AND METHODS

Flowers and leaves of *Ephedra alata* (1400 g) were macerated four times with 70% EtOH solution by replacing the solvent every day with fresh solvent. The hydro-alcoholic solutions were concentrated under reduced pressure to dryness and the residue was dissolved in water and kept in cold overnight. After filtration, the aqueous solution was successively extracted with CH_2Cl_2, EtOAc and *n*-BuOH for three times for each solvent, then the CH_2Cl_2, EtOAc and *n*-BuOH extracts were concentrated to dryness [10].

Preparation of the bacterial culture media:
3.7% of Mueller Hinton agar was mixed with hot distilled water and autoclaved at (121°C, 15 Psi, 15 minutes). After autoclaving, it was allowed to cool to 45 -50 °C in a water bath. Then the medium was poured into sterilized petri dishes with a uniform depth of approximately 5 mm [11].

Preparations of plant extract impregnated discs:
Whatman N°1 filter paper was used to prepare discs of 5 mm in diameter. Discs were sterilized by autoclaving and then dried during the autoclaving cycle. They were then impregnated with the extract of the plants dissolved in DMSO [12].

Disc diffusion method:
Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of antimicrobial activities of plant extracts. A bacterial suspension adjusted to 0.5 McFarland standard (1.5×10^{8} CFU/ml) was used to inoculate Mueller Hinton agar plates evenly using a sterile swab. The discs impregnated with the plant extracts were placed individually on the Mueller Hinton agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. The discs were spaced far enough to avoid both reflection waves from the edges of the petri dishes and overlapping rings of inhibition.

The plate was then incubated at 37°c for 18 hours in inverted position to look for zones of inhibition. Zones of inhibitions produced by the sensitive organisms were demarcated by a circular area of clearing around the plant extract impregnated discs.

The diameter of the zone of inhibition through the center of the disc was measured to the nearest millimeter [13] [14].

RESULTS

Results of disc diffusion test (table 1-6) showed that the flavonoids extract of *Ephedra alata*, if properly processed, could be used to treat some stubborn *Bacillus subtilis ATCC 6051, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923* and *Bacillus cereus* ATCC 11778 infections with Butanol extract of flowers whereas it is ineffective against *Serratia marcescens ATCC 13880*. Ethyl acetate and dichloromethane extracts of flowers are effective in most types of bacteria. This is in agreement with results reported by of Al-Qarawi et al [15].

The Butanol extract of leaves are effective against most the studied species but ineffective against *Serratia*

marcescens ATCC 13880 and *Enterococcus faecalis ATCC 29212*. Ethyl Acetate and Dichloromethane extracts of leaves are effective only against *Bacillus subtilis ATCC 6051, Staphylococcus aureus ATCC 25923* and *Bacillus cereus ATCC 11778 [*15].

Table 1: Diameter of inhibition zone at different extract concentrations: butanol extract of flowers (µg/mL)

Name of cultured Bacteria	Diameter of Inhibition zone (cm)				
	1000	666	500	400	333
Serratia marcescens ATCC 13880	0	0	0	0	0
Pseudomonas aeruginosa ATCC 10145	1,3	0	0,8	0	0
Bacillus subtilis ATCC 6051	-	1	1.3	1,1	0,7
Escherichia coli ATCC 25922	1	0,9	0	0	0
Enterococcus faecalis ATCC 29212	-	0,7	0.9	0	0,6
Staphylococcus aureus ATCC 25923	1	0,9	1,3	0,9	1,5
Bacillus cereus ATCC 11778	1,3	0,8	1	-	1
Methicillin -resistant Staphylococcus aureus (MRSA)ATCC 013300	1,3 (double)	-	-	-	-
Staphylococcus aureus ATCC 29213	1	-	-	-	-

Table 2: Diameter of inhibition zone at different extract concentrations: Ethyl Acetate extract of flowers (µg/mL)

Name of cultured Bacteria	Diameter of Inhibition zone (cm)				
	1000	666	500	400	333
Serratia marcescens ATCC 13880	1	0,7	0,7	0	0
Pseudomonas aeruginosa ATCC 10145	0,9	0,8	0,7	0,8	0,7
Bacillus subtilis ATCC 6051	1,5	1,5	1.5	1,3	1
Escherichia coli ATCC 25922	1	0,9	0,8	0,6	0
Enterococcus faecalis ATCC 29212	1,5	1,2	1.2	1	1,1
Staphylococcus aureus ATCC 25923	1,5	1,3	1,5	1,3	1,3
Bacillus cereus ATCC 11778	1,4	1,2	-	-	-
Methicillin -resistant Staphylococcus aureus (MRSA)ATCC 013300	-	-	-	-	-
Staphylococcus aureus ATCC 29213	-	-	-	-	-

Table 3: Diameter of inhibition zone at different extract concentrations: Dichloromethane extract of flowers (µg/mL)

Name of cultured Bacteria	Diameter of Inhibition zone (cm)			
	1000	666	500	400
Serratia marcescens ATCC 13880	0,7	0,8	0,6	0,8
Pseudomonas aeruginosa ATCC 10145	0,7	0,7	0,7	0,8
Bacillus subtilis ATCC 6051	1,1	1	0.8	1
Escherichia coli ATCC 25922	0,7	0,8	0,6	0,9
Enterococcus faecalis ATCC 29212	0	0,7	0.7	0,7
Staphylococcus aureus ATCC 25923	1,2	0,8	0,9	0,7
Bacillus cereus ATCC 11778	1	-	-	-
Methicillin -resistant Staphylococcus aureus (MRSA)ATCC 013300	-	-	-	-
Staphylococcus aureus ATCC 29213	-	-	-	-

Table 4: Diameter of inhibition zone at different extract concentrations: butanol extract of leaves (µg/mL)

Name of cultured Bacteria	Diameter of Inhibition zone (cm)				
	1000	666	500	400	333
Serratia marcescens ATCC 13880	0	0	0	0	0
Pseudomonas aeruginosa ATCC 10145	0	0	0,7	0	0
Bacillus subtilis ATCC 6051	1,3	1	0,9	0,9	0
Escherichia coli ATCC 25922	0	0	0,8	0	0
Enterococcus faecalis ATCC 29212	0	0	0	0	0
Staphylococcus aureus ATCC 25923	0,7	0,8	0,8	0	0,8
Bacillus cereus ATCC 11778	0,8	-	-	-	-
Methicillin -resistant *Staphylococcus aureus* (MRSA)ATCC 013300	-	-	-	-	-
Staphylococcus aureus ATCC 29213	-	-	-	-	-

Table 5: Diameter of inhibition zone at different extract concentrations: Ethyl Acetate extract of leaves (µg/mL)

Name of cultured Bacteria	Diameter of Inhibition zone (cm)	
	1000	500
Serratia marcescens ATCC 13880	0	0
Pseudomonas aeruginosa ATCC 10145	0	0
Bacillus subtilis ATCC 6051	1,7	1.6
Escherichia coli ATCC 25922	0	0
Enterococcus faecalis ATCC 29212	0	0
Staphylococcus aureus ATCC 25923	1.2	1.4
Bacillus cereus ATCC 11778	1.2	-

Table 6: inhibition Diameter at different extract concentrations: dichloromethane extract of leaves (µg/mL)

Name of cultured Bacteria	Diameter of Inhibition zone (cm)	
	1000	500
Serratia marcescens ATCC 13880	0	0
Pseudomonas aeruginosa ATCC 10145	0.8	0
Bacillus subtilis ATCC 6051	1.2	0
Escherichia coli ATCC 25922	0	0
Enterococcus faecalis ATCC 29212	0	0
Staphylococcus aureus ATCC 25923	1.1	1.1
Bacillus cereus ATCC 11778	1.1	-

DISCUSSION

Many studies pertaining to the use of the plant as therapeutic agents were being carried out, especially those thought to have an effect against antibiotic resistant bacteria. In the present study crude flavonoids of *Ephedra alata* revealed the medical importance of this plant through the antimicrobial potential. The six crude flavonoids extracts averred effective against several bacteria strains because the sites and the number of hydroxyl groups are thought to be related to the toxicity against bacteria. It is thought that the increased increased hydroxylation results in increased toxicity. The antimicrobial properties of plant have been investigated by a number of researchers worldwide. It was documented that leaves extracts (butanol, ethyl acetate, dichloromethane) of this plant, inhibited the growth of *Bacillus subtilis ATCC 6051*, *Staphylococcus aureus ATCC 25923* and *Bacillus cereus* ATCC 11778. On the other hand the three flowers extracts were reported to be effective against most types of studied bacteria.

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