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Antibacterial Activity of Some Fruits; Berries and Medicinal Herb Extracts Against Poultry Strains of *Salmonella*

[1]A. Ayachi, [1]N. Alloui, [2]O. Bennoune, [3]G. Yakhlef, [3]S Daas Amiour, [3]W. Bouzid, [3]S. Djemai Zoughlache, [3]K. Boudjellal and [3]H. Abdessemed

[1]Laboratory of Microbiology (LESPA), Veterinary Department, University of Batna, Algeria
[2]Laboratory of Histology (LESPA), Veterinary Department, University of Batna, Algeria
[3]Laboratory of Biochemistry, Biological Department, University of Batna, Algeria

Abstract: *Thymus vulgaris*; three variety of dates fruits widely consummated *(Phoenix dactylifera*) and four local wild berries (*Crataegus azarolus* L; *Crataegus monogyna* L; *Ziziphus lotus* L; *Eleagnus angustifolia* L) were tested for antibacterial activity against seven strains of *Salmonella typhimurium* isolated from poultry chain and characterized by their antibiotics resistance to ticarcillin amoxicillin chloramphenicol and sulfamids trimethoprim Candidates were primarily screened using the disk-agar method for antibacterial activity against *E. coli* ATCC 25922 and showed a high antibacterial activity of the medicinal herbs tested. Methanolic; dichloro-methalonic and etheric extracts of *(Thymus vulgaris*) exhibited inhibition zones against *E. coli* (ATCC 25922) with (19.9, 28.34 and 33 mm) respectively. The three dates variety extracts showed a lower antibacterial activity against *E. coli* (ATCC 25922) with (7.5, 8 and 9.5 mm vs. 19.12, 24.08 and 32.2 mm) against *Salmonella typhimurium* respectively. While extracts of the wild local berries did not showed any activity against all *Salmonella typhimurium* serotypes but presented a moderate activity against *E. coli* (ATCC 25922) with (12, 8 and 15 mm of inhibiting diameter). In general only *Thymus vulgaris* has the potential to provide an effective treatment for salmonellosis.

Key words: Antimicrobial activity · *Thymus vulgaris* · Wild berries · Date fruits · *Salmonella typhimurium*

INTRODUCTION

In recent years food safety concerns have been focused on pathogens, such as *Salmonella* which is recognized as a primary cause of food poisoning worldwide and massive outbreaks have been occurred in several parts of the world [1]. *Salmonella typhi* and *S. paratyphi*, are considered as one of the major diseases resulting in considerable morbidity and usually cause severe diseases such as enteric fever in humans and lead to death [2]. Similarly, serotypes that are highly adapted to animal hosts, such as *S. Gallinarum* causes economic losses in poultry production. These major *Salmonella* remains a public health and economic problem in developing countries [3] In opposite; human salmonellosis following consumption of contaminated foods has increased worldwide but is less widespread in developing countries [4]: Nontyphoidal *Salmonella* spp. is estimated to account for 2.4 million cases of human gastroenteritis annually in the United States [5] *Salmonella. enteritidis* or *Salmonella. typhimurium,* are among the major bacterial pathogens of poultry in the all world and most of their infections in humans result from the ingestion of contaminated poultry [6]. The administration of antimicrobial agents in chickens creates selection pressure that favors the survival of antibiotic resistant pathogens. Resistance of *Salmonella* to commonly used antimicrobials is increasing both in the veterinary and public health sectors and has emerged as a global problem The emergence of strains of *Salmonella* enteric pathogens with multiple drug resistance (MDR) is a great concern worldwide. Increasing rates of antibiotic resistance have been reported in various regions throughout the world and antibiotic resistant clones; such as *S.typhimurium* DT104; seems to have spread in areas rather distant from each other. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to that these medicinal herbs are safe and more

Corresponding Author: A. Ayachi Laboratory of Microbiology (LESPA), Veterinary Department, University of Batna, Algeria

dependable than the costly synthetic drugs, many of which have adverse side effects. Antimicrobial agents can also be derived from herbs, and over 1000 plants exhibit antimicrobial effects [7]. Traditionally, these herbs are said to provide safe and effective treatments against many diseases. A large number of diverse types of plants grow wild in different parts of our country. In Algeria the use of different parts of several medicinal plants to cure specific diseases has been in vogue from ancient times. The antibacterial activity of one medicinal herb species traditionally used in Algeria to treat gastrointestinal infections *(Thymus vulgaris)* was used in our experiment; three variety of dates fruits widely consummated *(Phoenix dactylifera)* and four local wild berries *(Crataegus azarolus L*; *Crataegus monogyna L; Ziziphus lotus L; Eleagnus angustifolia L)* are also tested for their extract antibacterial activity.

MATERIALS AND METHODS

Plant Material: The plant materials used in the study consisted of Thyme (*Thymus vulgaris)* three varieties of dates fruits *(Phoenix dactylifera)* Deglet-Nour; Ghars, Mech-Degla and four local wild berries (*Crataegus azarolus* L; *Crataegus monogyna* L; *Ziziphus lotus* L; *Eleagnus angustifolia* L)

Bacterial Strains and Culture Medium: Salmonella typhimurium, isolated from the poultry chain in the Microbiology and Immunology laboratory of the Veterinary Department. Bacterial strain was suspended in Tryptic Soy Broth (TSB, Difco, USA) and incubated at 37 °C for 20 h. Mueller Hinton Agar (MHA, Difco, USA) was used for the agar diffusion method [8]. For the bacterial strain reference *E. coli* (ATCC 25922) it was used like a strain control in order to assess the salmonella susceptibility to plant extracts

Preparation of Herbal Extracts: The medicinal herbs; fruits and berries used in this study were air-dried in the dark at room temperature and then ground to powders using a mechanical grinder. Powders were extracted by maceration in water. All our specimens were also extracted in methanol. Approximately 50 g of the powdered materials were boiled in 200 ml of water for 3 × 60 min. For methanol extraction 50 g of the powders were soaked in 200 ml of methanol for 31 X 8 h under mantle-reflux. The solvent was then removed under reduced pressure in a rotary evaporator (IKA WERCK). Extracts were first filtered using Whatman No. 1 filter papers, filtrates were evaporated to dryness at 25°C in a steady air current. All dried crude extracts were made from one lot of each herb and were stored at-20°C until required for testing. The extracts were dissolved in water or 50% DMSO before use. [9]

Table 1: Sensitivity of 7 strains of *Salmonella typhimurium* to antibiotics

Antibiotics	Diam (mm)	Interpretation
Ampicillin	18	S
Ticarcillin	<6	R
AMX+Ac Clavulanic	<6	R
Imipenem	31	S
Cefalotin	20	S
Cefoxtin	26	S
Cefotaxim	32	S
Amykacin	23	S
Isepamycin	24	S
Chloramphénicol	<6	R
TMP+Sulfamides	<6	R
Pefloxacin	30	S
Colistin	16	S

Antimicrobial Resistance Testing: The resistances of the *Salmonella* strains to different antimicrobial agents were determined using the disk-agar method standardized by the National Committee for Clinical Laboratory Standards [10]. The quality control strains were *E. coli* ATCC25922. (Table 1).

Screening of Antibacterial Activity by the Agar Diffusion Method: The antibacterial activities of isolates to the different extracts were tested using the disk-agar method standardized by the National Committee for Clinical Laboratory Standards [8,10] Seven serotypes, *S. typhimurium*, were used in this assay. Six mm-diameter paper discs were bored in the agar plates and 20 µl of each herbal extract reconstituted in water or 50% DMSO to a concentration of 500 µg/ml was dispensed into the discs. Antibacterial activity was evaluated by measuring inhibition zone diameters. Amikacin and gentamycin, at 8-32 µg/ml, were included as positive controls and water or 50% DMSO served as negative controls.

RESULTS

***In vitro* Antimicrobial Effects of the Medicinal Herb:** Candidates were primarily screened using the disk-agar method for antibacterial activity against *E coli* ATCC 25922 and showed a high antibacterial activity of the medicinal herb tested, the methanolic; dichloro-methalonic

Table 2: *In vitro* antibacterial activity of *Salmonella* for medicinal herb

		Inhibition zone Diameter for the *Salmonella typhymurium* (mm)							
Medicinal Herb	Nature of the extract	1	2	3	4	5	6	7	*E.coli*
Thyme	Methanolic	19.58	18.26	16.03	21.75	16.05	20.24	21.96	19.9
	Dichloro-methanolic	25.09	24.80	26.09	24.81	26.08	23.20	22.84	28.34
	Etheric	32.10	33.50	28.90	30.70	34.30	34.45	31.84	33

Table 3: Results of antibacterial activity of *Salmonella typhymurium* for dates fruits

			Inhibition zone Diameter for the *Salmonella typhymurium* (mm)							
Variety of fruits	Nature of the extract		1	2	3	4	5	6	7	*E. coli*
Deglet Nour	Butanolic	25 %	09	07	7.5	-	07	-	10	7.5
		100 %	-	07	-	-	07	08	-	7.5
Ghars	Butanolic	25 %	07	-	-	-	07	-	-	9.5
Mech Degla	Methanolic	25 %	-	-	-	07	7.8	-	-	9.5
		100 %	7.5	-	8.5	07	-	-	-	8.8

and etheric extracts of *Thymus vulgaris*) exhibited antibacterial activity against *E. coli* ATCC 25922 with respective means (19.9; 28.34 and 33 mm of inhibiting diameter) and against *Salmonella typhimurium* (19.12; 24.08; and 32.2) (Table 2).

***In vitro* Antimicrobial Effects of Date Fruits and Berries:** The dates fruits extracts showed a lower antibacterial activity against *E. coli* ATCC 25922 with respective means (7.5; 8 and 9.5 mm of inhibiting diameter for the three variety of dates) and against ST (7.8; 7.83 and 7.6) (Table 3).

While the solvent extracts of the wild local berries haven't showed any antibacterial activity against all ST serotypes but presented a medial antibacterial activity against *E. coli* ATCC 25922 with (12; 8 and 15; mm of inhibiting diameter)

DISCUSSION

In order to identify herbs with antibiotic properties against salmonellosis, 01 medicinal herb species traditionally used for gastrointestinal infections in Algeria was tested against seven different serotypes of *Salmonella* using the disk-agar method Water or 50% DMSO as the negative controls did not show any inhibition zones of the test strains. On the eight specimens three kinds of chemical methods and two dilutions (25%; 100%) were used. However between the extracts performed and tested on these specimens 9 were found to have antibacterial activity against at least one of the *Salmonella* strains tested; inhibition zones ranged from 16.03 to 34.45 mm for the thyme. Moreover, both the etheric and dichloro-methanolic extracts of *Thymus vulgaris* exhibited antibacterial activity against all the seven *Salmonella*. The methanolic extract have a slightly lower antibacterial activity Since the aqueous and methanolic extracts of the four berries exhibited effective antibacterial activity against *E coli* ATCC 25922 with (12; 8 and 15; mm of inhibiting diameter) but not against all seven *Salmonella* by the disk-agar method,. All strains of *Salmonella* tested were found to be resistant to four antibiotic ticarcillin-amoxicillin-chloramphenicol-sulfamids trimethoprim Strains resistant to three or more antibacterial drugs were defined multiresistant (MDR). [11]. Analysis of patterns of antibiotic resistance was further performed by separating serotype Typhimurium and other serotypes because of peculiar features due to the prominent role of the 'DT104 complex' and its resistance pheno-genotype in the epidemiological environment of such a serotype [12].

The antimicrobial property of thyme has been shown to be attributable to the essential oil fraction Some researchers have demonstrated the antimicrobial activity of the most common terpene compounds, such as thymol, carvacrol, linalool, eugenol, α-pinene, and β-pinene in this medicinal herb against several microbial strains [13].

E. coli was more sensitive to most of the extract oils of the berries and fruits dates than other *Salmonella* serotypes tested.

We conclude that only *Thymus vulgaris* has the potentiel to provide an effective treatment for salmonellosis.

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