

Full Length Research Paper

Antibacterial activity of extracts of diploid and induced autotetraploid Tunisian populations of *Trigonella foenum-graecum* L.

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Accepted 12 April, 2012

Trigonella foenum-graecum L. is a medicinal plant known for its various pharmacological properties, including the anti-bacterial and the anti-microbial effects. This study aimed to determine and compare the antibacterial activities of extracts of two Tunisian *T. foenum-graecum* populations, diploid ($2n=16$) and its induced autotetraploid ($4n=32$). Aqueous and organic extracts (petroleum ether, ethyl acetate and methanolic fractions) prepared from seeds and leaves were assayed to determine their antibacterial potential against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli* and *Salmonella Typhimurium* using agar disk diffusion method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. The results showed that the organic extracts had no antibacterial potential against the tested bacteria contrary to the aqueous extracts. The extracts prepared from the seeds were more active compared to those prepared from the leaves against all tested strains. And the induced autotetraploid population presented an antibacterial activity higher than that of the diploid population.

Key words: *Trigonella foenum-graecum*, diploid, autotetraploid, antibacterial activity, aqueous extracts, organic extracts, seeds, leaves.

INTRODUCTION

The appearance of resistant pathogens paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents. Bacterial resistance to antimicrobial agents is a medical problem with public health and socio-economic implications. The change in the resistance patterns will continue to menace the developed and developing countries (Abdou et al., 2011). The world is nowadays witnessing an emergence of several multi-drug resistant organisms rendering the treatment options more and more limited. The emergence of resistant Gram negative (Abdel-Massih et al., 2010) and Gram positive bacteria (Akins et al., 2005) presents a major challenge for the antimicrobial therapy and

significantly narrows the treatment options of human infections. In view of the negligible development of antibiotics in the past few years, there is an urgent need for new antibacterial compounds in order to fight the emergence of these new resistant pathogens.

For centuries, plants have been used as remedies and treatments of diseases. The Mediterranean region is rich in plant species many of which are considered to have medicinal effects. However, there is relatively limited research on medicinal plants in this region (Saad et al., 2005). *Trigonella foenum-graecum* L., or fenugreek, is one of the oldest medicinal plants, found in nature and cultivated in North Africa, the Middle East and India (Petropolous, 2002). It is an annual herb of *Fabaceae* which is used as human food and forage. Their seeds are described in the Greek and Latin Pharmacopoeias for their various pharmacological properties, including the

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anti-bacterial (Bhatti et al., 1996) and the anti-microbial effects (Alkofahi et al., 1996).

T. foenum-graecum is a diploid plant with $2n = 16$ (Ahmad et al., 2000) and the aneuploid forms of this species have not yet been found (Petropoulos, 2002). The induced polyploidy can increase variability within species. The polyploidy plays a very important role in evolution and constitutes an important mechanism of diversification and creation of genetic variability. Although the first polyploid was discovered over a century ago, the genetic and evolutionary implications of polyploidy are not clearly elucidated (Bennett, 2004; Soltis et al., 2003) and artificial polyploids are still used as a valuable tool in plant breeding programs (Ranney, 2006). The importance of polyploidy in plants breeding arouses a considerable interest to induced polyploids development when the mitotic inhibitors were discovered for the first time in 1930s. At this time, the artificial polyploidy was induced using the colchicine to inhibit the formation of spindle fibers which results in a temporal arrest of mitosis at the anaphase stage (Blakeslee and Avery, 1937). At this point, the chromosomes have replicated, but cell division has not yet taken place resulting in polyploid cells (Ranney, 2006). In later year, a number of other mitotic inhibitors including oryzalin, trifluralin, amiprofos-methyl and N_2O gas had been identified and used as doubling agents (Bouvier et al., 1994; van Tuyl et al., 1992; Taylor et al., 1976), but colchicine was preferred as polyploidization agent and was by far the substance with which Beji (1991), Madon et al. (2005) and Wei et al. (2007) found the best results in their experiments of polyploidy induction.

We found that the treatment using a 0.5% colchicine solution on the shoot meristem of *T. foenum-graecum* gave a higher survival percentage of polyploid plants than on germinated seeds (Marzougui et al., 2009). A comparative study between diploid and autotetraploid fenugreek ($4n=32$) populations was conducted to determine differences of morphology and minerals contents (Marzougui et al., 2009), vitamins and protein reserves (Marzougui et al., 2010a), the physiological behavior (Marzougui et al., 2010b), salt stress tolerance (Marzougui et al., 2010c) and molecular profile (Marzougui et al., 2010a) between them. This study is aimed to determine and compare the antibacterial activities of extracts of two Tunisian *T. foenum-graecum* populations, diploid and its induced autotetraploid.

MATERIALS AND METHODS

Plant material

The seeds of a *T. foenum-graecum* diploid population were collected from the north-east of Tunisia in the region of Menzel Temim characterized by an argillaceous soil. Autotetraploid population was induced by colchicine as described in previous works (Marzougui et al., 2009; Marzougui et al., 2011). The two populations were cultivated in pots of 20 cm in diameter in a conditioned room at 25°C, 70% humidity and photoperiod of 16 h.

The pots were filled with sandy-loam calcareous soil, characteristic of the region of Elfejh in south-east of Tunisia. The establishment of the pots was conducted in randomized block with four replications. Plant leaves and seeds were collected, well washed with water, disinfected by immersion in a 2% sodium hypochlorite solution for 30 min and rinsed with distilled water to eliminate residual hypochlorite (Rodríguez et al., 2005). The material should be dried, stored at 4°C and ground into powder when it was used.

Preparation of aqueous extracts

Aqueous decoctions of ground seeds and leaves at 10% concentration were prepared by boiling 10 g in 100 ml sterile distilled water for 15 min. The flasks were then plugged and removed from heat and allowed to cool. After cooling the contents of flasks were filtered through a Whatman disk and then through a nitro-cellulose paper ($\varnothing = 0.45 \mu\text{m}$) to reduce the risk of interference by micro-organisms. Preparation of the aqueous precipitates consisted in boiling 10 g in 100 ml sterile distilled water for 15 min. After cooling, centrifugation at 12000 rpm/20 min allowed to separate the extract from the supernatant for seeds and leaves. Aqueous extracts pH was measured, but only minor differences were registered (from pH 5.8 to pH 6.1). The precipitate and decocted extracts were stored at 4°C.

Preparation of organic extracts

Ten grams of the powdered fenugreek seeds and leaves were subjected to Soxhlet extraction successively with 100 ml of petroleum ether, chloroform, ethyl acetate and methanol to obtain respective fractions at 10% concentration (Haouala et al., 2008; Hayder et al., 2004). Solvents were evaporated under reduced pressure using Rotavapour R-114 (Buchi, France) and concentrated fractions were stored at 4°C until use.

Antibacterial activity

Bacterial strains

The antibacterial activities of seed and leaf extracts of fenugreek were tested on five human pathogenic bacteria, including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25963, *Staphylococcus epidermidis* CIP 106510 and *Enterococcus faecalis* ATCC 29212) and Gram-negative bacteria (*Escherichia coli* ATCC 35218 and *Salmonella* Typhimurium ATCC 1408). All these strains were obtained from the culture collection of the Laboratory of Analysis, Treatment and Valorization of Environmental Pollutants and Products (LATVPEP), Faculty of Pharmacy of Monastir, Tunisia. They were stored on Mueller-Hinton Agar (Bio-Rad) at 4°C. The Mueller-Hinton agar and the nutrient broth (Bio-Rad) were used, respectively, for growing and diluting the microorganism suspensions for the antimicrobial assays.

Agar disk diffusion method

In vitro antibacterial activities of *T. foenum-graecum* extracts were determined by the agar disk diffusion method according to Ghalem and Mohamed (2009). Disk assays were found to be a simple, cheap and reproducible practical method (Maidment et al., 2006). The inocula grown in the nutrient broth at 37°C for 24 h were diluted to approximately 10^6 CFU/ml in nutrient broth. The concentration of the suspension used for inoculation was standardized by adjusting the optical density to 0.5 at 570 nm wavelength (spectrophotometer UV/visible, Jenway 6405). Absorbent disks (Whatman disk of 6 mm

Table 1. Antibiogram results of studied strains with tested antibiotics.

Antibiotics	Disk load (µg)	Tested bacteria									
		<i>S. aureus</i>		<i>S. epidermidis</i>		<i>E. faecalis</i>		<i>E. coli</i>		<i>S. Typhimurium</i>	
		IZ	Result	IZ	Result	IZ	Result	IZ	Result	IZ	Result
Ampicillin	10	19±0	I	15±0	R	18±0.5	I	nd	nd	15±0	R
Penicillin G	6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Nalidixic Acid	30	21±0.5	S	nd	nd	23±1	S	19±0	I	23±1.2	S
Kanamycin	30	10±0	R	nd	nd	11±1.5	R	21±0.5	S	15±0	I
Chloramphenicol	30	25±0.2	S	nd	nd	30±1	S	26±1	S	19±0.2	I
Streptomycin	10	nd	nd	nd	nd	nd	nd	15±0	S	nd	nd

Source: Wei et al. (2007).

IZ: Inhibition zone in diameter (mm±SD). R: resistant, S: sensitive, I: intermediate, nd: not detected.

in diameter) were impregnated with 20 µl of different extracts dilutions and then placed on the surface of inoculated plates (90 mm) and incubated at 37°C for 24 h. Antimicrobial activity was assessed by measuring the inhibition zone in millimeters (Tepe et al., 2004). This was the diameter of the zone visibly showing the absence of growth, including the 6 mm disk, where there was no inhibition. Ampicillin, penicillin G, nalidixic acid, kanamycin, chloramphenicol and streptomycin were used in this study as positive controls for the tested strains. The antibiotic susceptibility was determined by using the Kirby–Bauer method and Mueller–Hinton agar plates supplemented with 1% NaCl as described by Hajlaoui et al. (2010). After incubation at 37°C for 18–24 h, the diameters of the inhibition zones were measured and interpreted according to the Comité de la Société Française de l'Antibiogramme (Bonnet et al., 2010). All the tests were performed in triplicate.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a micro-organism after overnight incubation. MICs are important in diagnostic laboratories to confirm resistance of micro-organisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrews, 2001). The inocula of the bacterial strains were prepared from 12 h broth cultures. A range of concentrations was prepared with sterile distilled water for each extract. A volume of 1 ml of each concentration was added to 1 ml of bacterial inoculum in hemolysis tube. Positive and negative controls were used. The negative control tube contained 1 ml of sterile distilled water and 1 ml of sterile broth; the positive one was constituted by 1 ml of sterile distilled water and 1 ml of inoculum. The inocula were spread over the surface of the Petri plate containing Mueller-Hinton agar medium. Four disks were placed on agar containing different extract dilutions. In the center of the Petri plate, a control disk was impregnated in parallel with 2 µl of the same solvent as that used to dissolve the plant extracts. The Petri plates were then incubated at 37°C for 24 h.

Determination of minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined by subculturing samples from the tubes with concentrations above the MIC on new plates of Mueller-Hinton Agar. The MBC corresponded to the lowest concentration of the extract associated with no bacterial culture (Bolou et al., 2011).

Statistical analysis

All tests were carried out in triplicate and the results were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare data of growth inhibition zones affected between the diploid and autotetraploid populations using SPSS 16.0 for Windows program. Differences were considered significant when $P < 0.05$.

RESULTS

Resistance profile of the tested strains

The antibiotic resistance and susceptibility pattern of the Gram-negative and positive bacteria was listed in Table 1. The most effective antibiotics against *S. aureus* and *E. faecalis* were nalidixic acid and chloramphenicol, but none of the tested antibiotics had activity against the third Gram-positive bacteria, *S. epidermidis*. While, the most effective antibiotics against the Gram-negative bacteria *E. coli* were kanamycin, chloramphenicol and streptomycin. The second Gram-negative bacteria *S. Typhimurium* was sensitive to nalidixic acid.

Comparisons of activities of aqueous and organic extracts from both seeds and leaves

The antibacterial activities of *T. foenum-graecum* L. extracts against bacteria examined in the present study and its potency were qualitatively and quantitatively assessed using the presence or the absence of inhibition zone diameter; MIC and MBC values. The results for both seed and leaf extracts for the diploid and the autotetraploid populations are given in Table 2 and presented an inhibitory activity comparable to that of the reference antibiotics (diameter of inhibition zone IZ between 19 and 25 mm in Table 1). The results showed that only the aqueous extracts from seeds of the diploid population had substantial antibacterial activity. The decocted showed activity against *S. Typhimurium* ATCC

Table 2. Comparison of antibacterial activities of seed and leaf extracts of diploid and autotetraploid populations of *Trigonella foenum-graecum* on the tested bacteria.

P	PM	Bacteria	Decocted			Precipitate			Petroleum ether			Chloroform			Ethyl acetate			Methanol		
			IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC
Diploid	Seeds	<i>S. aureus</i>	nd	nd	nd	19.97±0.95*	0.72	2.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>S. epidermidis</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>E. faecalis</i>	nd	nd	nd	20.35±0.58*	0.072	5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>E. coli</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>S. Typhimurium</i>	19.67±0.57*	0.79	2.5	21±1*	0.72	5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Leaves	<i>S. aureus</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>S. epidermidis</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>E. faecalis</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>E. coli</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>S. Typhimurium</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Autotetraploid	Seeds	<i>S. aureus</i>	nd	nd	nd	27.35±2.5*	0.074	>10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>S. epidermidis</i>	14.5±0.5*	0.82	10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>E. faecalis</i>	15±0.2*	0.082	10	23.7±1.5*	0.74	10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>E. coli</i>	14.77±0.25*	0.82	>10	5.35±2*	0.74	5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>S. Typhimurium</i>	20.2±0.72*	0.82	5	25±2*	0.74	5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Leaves	<i>S. aureus</i>	7.03±0.15*	0.56	>10	5.13±0.15*	0.68	5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>S. epidermidis</i>	7.07±0.11*	0.06	2.5	7.13±0.15*	0.68	>10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>E. faecalis</i>	5.27±0.25*	0.056	5	10.3±0.26*	0.34	>10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>E. coli</i>	4.97±0.15*	0.56	5	3.38±0.12*	0.68	10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		<i>S. Typhimurium</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

P: population, PM: Plant material, IZ: Inhibition zone in diameter (mm±SD), MIC: Minimum Inhibitory Concentration (mg/ml), MBC: Minimum Bactericidal Concentration (mg/ml), nd: not detected. *: significant at P<0.05.

1408 only (IZ=19.67 mm), while the precipitate had inhibitory activity against *S. aureus* ATCC 25963, *E. faecalis* ATCC 29212 and *S. Typhimurium* ATCC 1408 (IZ between 19.97 and 21 mm). Concerning the autotetraploid population, the organic extracts from leaves and seeds had not also an antibacterial effect against all tested bacteria whereas the aqueous extracts had antibacterial activity against the majority of them.

In fact the seed decocted inhibited the growth of all bacteria except for *S. aureus* ATCC 25963 (IZ from 14.5 to 20.2 mm) and the seed precipitate had no inhibitory effect against *S. epidermidis* CIP 106510 only (IZ between 5.35 and 25 mm). The aqueous extracts from leaves of the autotetraploid population had antibacterial potential against all tested bacteria except for *S. Typhimurium* ATCC 1408. Statistical analysis showed significant

variations (P<0.05) between the diameters of inhibition zones caused by the antibacterial activity of decocted extracts from seeds and from leaves of the diploid population against *S. Typhimurium* ATCC 1408 and for the precipitate variations were significant against *S. aureus* ATCC 25963, *E. faecalis* CIP 106510 and *S. Typhimurium* ATCC 1408. MIC and MBC values varied for aqueous extracts from seeds of the

diploid population from 0.072 to 0.79 mg/ml and from 2.5 to 5 mg/ml, respectively (Table 2). For the autotetraploid population, MIC and MBC values of aqueous extracts from seeds were from 0.082 to 0.82 mg/ml and from 5 to 10 mg/ml or higher, respectively. The aqueous extracts from leaves showed MIC and MBC values ranged from 0.056 to 0.56 mg/ml and from 2.5 to 10 mg/ml or higher, respectively. The report MIC/MBC reveals the bactericidal and bacteriostatic powers of the tested plant extracts. Extract has a bacteriostatic power when this report ≥ 4 and bactericidal when this report is ≤ 4 (Dramane et al., 2010). In our case all extracts of *T. foenum-graecum* showing an inhibitory effect had bactericidal power.

Comparisons of antibacterial activities between diploid and autotetraploid populations

The results comparing the antibacterial potential between the diploid and the autotetraploid populations of *T. foenum-graecum* were presented in Table 2. The data of zones of growth inhibition (IZ in mm) scored in Mueller–Hinton agar demonstrated that the seed precipitate of the diploid population had antibacterial activity against three of the five tested bacteria (*S. aureus* ATCC 25963, *S. epidermidis* CIP 106510 and *S. Typhimurium* ATCC 1408) with an IZ between 19.97 and 21 mm, while the precipitate prepared from the seeds of the autotetraploid population showed activity against *E. faecalis* ATCC 29212 and *E. coli* ATCC 35218 in addition to *S. aureus* ATCC 25963 and *S. Typhimurium* ATCC 1408 (IZ between 5.35 and 27.35 mm). The seed decocted of the diploid population had inhibitory activity against *S. Typhimurium* ATCC 1408 only with an IZ equal to 19.67 mm. In contrast the decocted of the autotetraploid population had antibacterial activity against *S. epidermidis* CIP 106510, *E. faecalis* ATCC 29212, *E. coli* ATCC 35218 and *S. Typhimurium* ATCC 1408 (IZ from 14.5 to 20.2 mm). For extracts prepared from leaves, all extracts of the diploid population had not inhibitory activities against the tested bacteria, whereas the aqueous extracts prepared from the autotetraploid population had antibacterial potential against *S. aureus* ATCC 25963, *S. epidermidis* CIP 106510, *E. faecalis* ATCC 29212 and *E. coli* ATCC 35218 (IZ between 3.38 and 10.3 mm). Statistical analysis showed significant variations between the diameters of inhibition zones caused by the antibacterial activities of aqueous extracts of diploid and autotetraploid populations ($P < 0.05$).

MIC and MBC values confirmed the fact that the fenugreek autotetraploid population had higher antibacterial activity than that of the diploid population. They were ranged for aqueous extracts from seeds of the autotetraploid population from 0.082 to 0.82 mg/ml and from 5 to 10 mg/ml or higher, respectively (Table 2). Values of aqueous extracts from leaves were comprised between 0.056 and 0.56 mg/ml and from 2.5 to 10 mg/ml

or higher, respectively.

DISCUSSION

From all these results, it can be considered that the prepared organic extracts show no antibacterial potential against the tested bacteria contrary to the aqueous extracts. And concerning aqueous extracts, precipitate has an antibacterial potential higher than decocted. We can explain these results by the difference in composition of antibacterial substances between the tested extracts. In fact, antibacterial substances showed different solubility depending on the extract solvent used (Qstenvik et al, 1998). Water allows the extraction of very polar substances such as flavonoids, tannins, alkaloid, etc. (Snyder and Kirk, 1979). And these substances belong to the major classes of antimicrobial compounds in plants (Cowan, 1999). Organic solvents allow a better extraction of less polar compounds such as terpenic derivatives (Ali-Emmanuel et al, 2002). Qualitative and quantitative analyses of compounds of the different studied extracts are needed to justify our results. The work of Abdel-Massih et al. (2010) on antibacterial activity of the extracts obtained from the seeds of *T. foenum-graecum* on highly drug-resistant Gram negative bacilli confirmed our results. They have not detected an antibacterial effect with their fenugreek organic extracts (petroleum ether, dichloromethane and ethyl acetate) on *E. coli* and *K. pneumonia* and noted an antibacterial potential with their aqueous extract prepared from fresh plants. Their MIC and MBC values were from 10 to 20 $\mu\text{g}/\mu\text{l}$ and 10 $\mu\text{g}/\mu\text{l}$, respectively. The organic extracts of *T. foenum-graecum* could have activity against other microorganisms such as fungi. This was demonstrated by Haoula et al. (2008) which studied the antifungal potential of petroleum ether, ethyl acetate and methanolic fractions of the aerial fenugreek parts and showed that the antifungal activity resided mainly in the methanol fraction. MIC of methanol fraction which caused total inhibition of *Rhizoctinia solani* and *Alternaria* sp. was 60 $\mu\text{g}/\text{ml}$.

Furthermore, our findings showed that extracts prepared from the seeds of *T. foenum-graecum* are more active compared to those prepared from the leaves against the tested bacteria. Wagh et al. (2007) worked on fenugreek seeds and demonstrated that the oil extracted from them has high degree of antimycotic and antibacterial activity against *Aspergillus niger*, *A. fumigatus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, isolated from India. They concluded that oil of *T. foenum-graecum* can be used for developing plant-derived antimicrobial drugs. According to Bhatti et al. (1996) the antibacterial activity shown by fenugreek seed extracts may be due to its flavonoid content. Increasingly, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing

antifungal, antiviral and antibacterial activity (Tim Cushnie and Lamb, 2005). The seeds of *T. foenum-graecum* were found to contain luteolin (Varshney and Sharma, 1966), quercetin (Varshney and Sharma, 1966; Shang et al., 1998), vitexin (Adamska and Lutomski, 1971; Wagner et al., 1973; Huang and Liang, 2000), isovitexin (Wagner et al., 1973), orientin (Huang and Liang, 2000), isoorientin (Wagner et al., 1973), naringenin and tricetin (Shang et al., 1998). Moreover, numerous research groups have sought to elucidate the antibacterial mechanisms of action of selected flavonoids. The activity of quercetin, for example, has been at least partially attributed to inhibition of DNA gyrase (Tim Cushnie and Lamb, 2005).

To our knowledge, this work represents the first attempt to study the antibacterial activity of a polyploid fenugreek induced artificially. In this context, we found that the aqueous extracts prepared from the autotetraploid population of *T. foenum-graecum* have antibacterial potential greater than that of the diploid population against *S. aureus* ATCC 25963, *S. epidermidis* CIP 106510, *E. faecalis* ATCC 29212, *E. coli* ATCC 35218 and *S. Typhimurium* ATCC 1408. This can be justified by a probable difference in composition of antibacterial substances between diploid and autotetraploid populations. Polyploidy induction of *T. foenum-graecum* provoked an increase in the copy number of all chromosomes (Marzougui et al, 2009; 2011) and that affects all genes equally and should result in an increase in gene expression (Comai, 2005) through increase variation in dosage-regulated gene expression (Osborn et al, 2003). So may be expecting a doubling of the amount in antibacterial substance in the autotetraploid population of fenugreek. This can be proved by initiating a study on the composition of the studied fenugreek parts in antibacterial substances such as flavonoids and comparing the compositions in these substances between diploid and autotetraploid populations. And it is on this topic that we will focus our future research.

Conclusion

Our results showed that the organic extracts had no antibacterial potential against the tested bacteria contrary to the aqueous extracts; and for these, the precipitate had an antibacterial potential higher than the decocted. They indicated also that extracts prepared from the seeds of *T. foenum-graecum* were more active compared to those prepared from the leaves against the tested strains. We also noted that the autotetraploid population of *T. foenum-graecum* had antibacterial activity higher than that of the diploid population. Additional chemical studies of the autotetraploid fenugreek have to be performed if it is to be used for medicinal purposes.

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