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Original Research

Chromatographic analyses and in *vitro* screening of the antimicrobial activity of tannins extract of fenugreek seeds grown in the Taghit region (Southwest of Algeria)

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herbaceous plant belonging to the *Fabaceae* family. The seeds of the fenugreek plant are known to have important medicinal, therapeutic and nutritional properties. The objective of this study was to characterize and evaluate the antimicrobial activity of *Trigonella foenum-graecum* L. seeds' tannins extract grown in Taghit town (Bechar-Algeria). After degreasing the plant material, the tannins were extracted by maceration using ethyl acetate, dichloromethane, and acetone and analyzed by two analytical techniques: Thin-Layer Chromatography (TLC) and High-Pressure Liquid Chromatography (HPLC). The antimicrobial effect was evaluated by the agar diffusion method, against 26 microbial strains, with the determination of the minimal inhibitory concentration by the broth macrodilution technique.

The TLC and HPLC results of the tannins extract of fenugreek seeds showed the presence of tannic acid, phloroglucinol, resorcinol, and catechol where tannic acid was the major constituent of this extract. From a concentration of 30 mg/mL, the antimicrobial effect results showed a proportional relationship between the concentration of the extract and the antimicrobial activity. A low to medium antimicrobial effect against the tested species was found due to the increased multidrug resistance of the tested strains. The uropathogenic strains, namely *S. aureus*, *Klebsiella sp*, *Candida albicans*, and *E. coli* (7), as well as *S. aureus* American Type Culture Collection (ATCC) 25923 and B. cereus ATCC 14579 – which were more susceptible compared to the other tested bacteria – with an activity index varying from 0.33 to 1.09 compared to gentamicin. This consolidates the place of fenugreek seeds within traditional herbal medicine. This species has great potential as a herbal antibacterial agent.

Keywords: *Trigonella foenum-graecum* L., antimicrobial activity, phytochemicals, tannins extract, Taghit (Bechar-Algeria)

Introduction

Since ancient times, people have used plants for food. But additional plant benefits were also discovered, particularly the power of healing [1]. Indeed, the plants used in traditional pharmacopeia are real factories that produce biologically active natural substances including secondary metabolites. The protective effects of medicinal and aromatic plants against various diseases have been attributed to the presence of phytochemicals, including polyphenols, which can be considered as the most abundant plant secondary metabolite. Phytochemicals are a very important class of molecules and are known for their numerous biological activities, such as antiviral, anti-inflammatory, antioxidant, anticancer and other beneficial effects [2, 3].

Fenugreek (*Trigonella foenum-graecum* L.) is an ancient medicinal and aromatic plant. The seeds are widely used in traditional medicine in many countries to fight various pathologies [4]. They have extraordinary and promising therapeutic properties (antitumoral, hypoglycemic, anti-inflammatory, hypocholesterolemia, and antioxidant) [5, 6]. To highlight the medicinal and aromatic qualities of this plant, the present study aimed to characterize tannins extracted from fenugreek seeds grown in Taghit, and to assess their antimicrobial power against some uropathogenic and bacterial reference strains.

Materials and methods

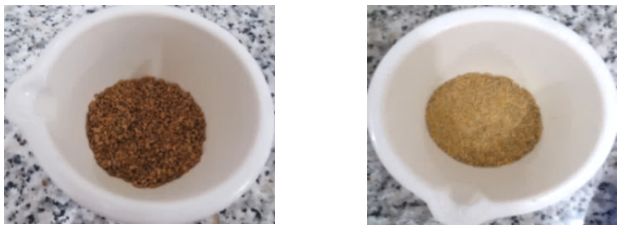
All chemicals used for the analytical procedures were of an analytical grade or the highest available purity. Chemicals and suppliers were from Liofilchem Diagnostici, Italy, Sigma-Aldrich, India, and Sigma-Aldrich, Mexico.

The bacterial reference strains ATCC were obtained from the Teaching Hospital Centre of Oran, Algeria. This study was carried out at Mohammed Tahri University of Bechar, Algeria.

Plant material

Preparation of plant material

T. foenum-graecum L. seeds were collected in autumn (2021) in the region of Taghit-Bechar (south-west of Algeria). The plant material was dried at room temperature to preserve the integrity of the molecules as much as possible. After drying, the seeds were ground into a powder and carefully stored in a dry place for subsequent analysis (**Fig. 1**).



(a) **(b)**

Figure 1 Seeds of *Trigonella foenum-graecum* L. (Original, 2021) **(a)** Intact fenugreek seeds; **(b)** Powdered fenugreek seeds

Defatting process of plant material

This process was carried out by refluxing the hexane in the Soxhlet apparatus to degrease the plant material according to the method described by El-Hela *et al* (2016) [7] and Benyagoub et al (2020a) [8].

Extraction of selective extracts

The extraction of tannins extract of *T. foenum-graecum* L. seeds was carried out by the methods described by Benyagoub *et al* (2020a) [8], and Lin *et al* (2006) [9].

Extraction yield

The dry extract yield was determined according to the following formula [10]:

$$Y(\%) = \frac{W}{W_0} \times 100$$

Where:

Y (%): Extraction yield expressed in (%)

W: Weight in (g) of the obtained dry extract

$\mathbf{W_0}$: Weight in (g) of the plant material (test sample)

Chromatographic analysis of fenugreek seeds' tannins extract

Qualitative analysis by TLC

Thin-layer chromatography (TLC) is a valuable tool for phytochemistry analysis. In this study, we used silica gel TLC plates of size 20×20 cm (Merck) to characterize qualitatively the constituents of tannins' extract (**Fig. 2**) [11].

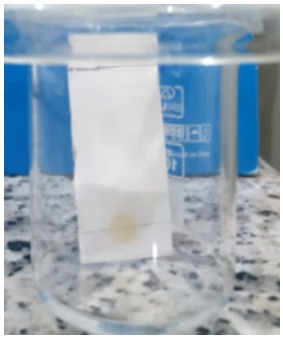


Figure 2 Qualitative analysis of tannins' extract by TLC technique (Original, 2021)

The choice of mobile phase was as follows:
1. Toluene/ethyl acetate/formic acid (5/4/1)
2. Acetic acid/distilled water (0.44/6.56)
3. Chloroform/methanol/acetic acid (9/1/0.1)
4. Acetone/toluene/formic acid (3/3/1)

The TLC plate was sprayed with the methanolic solution of aluminum chloride ($AlCl_3$) at 2%. The fluorescent spots under the UV light (254 nm) made it possible to calculate the frontal ratio. However, the revelation of polyphenols was based on the spot colouration, the chromatographic behavior 'retention factors' (R_f), and fluorescence under UV light [12, 13].

Quantitative analysis by HPLC

High-pressure liquid chromatography (HPLC) was carried out using Shimadzu LC 20-A system type HPLC chromatograph equipped with a vacuum degasser, PerkinElmer (Norwalk, CT, USA), a 200 LC Shimadzu® LC 20 AD pump (Kyoto, Japan), and an injector with a 20 µL Rheodyne 1907 sample loop. A Shimadzu SPD-20 A UV-visible detector (Kyoto, Japan). The tannins extract was dissolved in methanol at a concentration of 600 µg/mL. The operating conditions of the column are given in **Table 1**.

Table 1 HPLC experimental conditions

Parameter	Value
Colomn type	C 18
Quantity injected	20 µL
Flow	1 mL/min
Elution solvent	Methanol/Acetic acid 1% (in gradient mode)
UV/Vis detector	λ = 280 nm
Analysis time	25 min

Biological material

Origin of bacterial strains

A total of 26 microbial strains were tested, five of them (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Enterococcus faecalis* ATCC 29212) were reference bacterial strains. The other microbial strains were uropathogenic ones from urine samples, isolated using the following culture media: Hektoen agar, and Salmonella Shigella agar for Gram-negative bacteria (*E. coli*, *Pseudomonas sp*, *Klebsiella sp*, *Proteus sp*, *Shigella sp* and *Provedencia sp*), and Chapman agar for Gram-positive bacteria (*Staphylococcus sp*), and Sabouraud-chloramphenicol agar for the fungal species *Candida albicans*. The pure isolates were stored in a suitable storage medium based on 10% glycerol as a cryo-protective agent in Eppendorf tubes at -20°C. The uropathogenic isolates were non-repetitive (single sample per patient).

Microbial identification

Pure uropathogenic isolates underwent a series of identification tests according to standard microbiological methods [14, 15].

Antimicrobial test

Preparation of the bacterial inoculum

A colony of a pure and young culture of 18 to 24 hours on nutrient agar was taken and placed in a tube containing sterile physiological water,

where the density of the inoculum was adjusted to 0.5 MacFarland.

Antibiotic susceptibility test

The multidrug resistance profile of the isolated strains was determined by the diffusion method on agar medium (disc method). The Antibiotic Susceptibility Test (AST) consisted of using different antibiotic discs placed at equal distances on the surface of Mueller-Hinton agar that had been previously inoculated by the tested strain. After incubation of Petri dishes at 37°C for 18 to 24 hours, the diameter of the zone of inhibition was measured and then interpreted into susceptible (S), intermediate (I), or resistant (R) according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI) [16].

Antibacterial effect of tannins extract

A series of tannins extract concentrations were prepared with 1/10 DMSO diluent (Dimethyl sulphoxide) as follows: 10, 30, 40, 50, 60, 70, 90, and 100 mg/mL. The antibacterial effect was carried out using the wells' diffusion method, which consists of pouring a volume of 10 µL of the tannins extract in a well of a 6 mm diameter confined using a sterile Pasteur pipette.

The result was expressed by measuring the diameter of the zone of inhibition produced around the wells after incubation of Petri dishes at 37°C for 18 to 24 hours [17, 18].

Determination of the activity index

The zones of inhibition were measured and compared to the standard reference antibiotic where the Activity Index (AI) of tannins extract was calculated as follows [19, 4]:

$$AI = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard reference antibiotic}}$$

Statistical analysis of data

The different analyzes were carried out three times to confirm the results obtained, where the mean value was used for each parameter, on which the graphical presentation in the form of a histogram was plotted using the Origin Lab software (2018).

Results

Yield of tannins extraction

The extraction process of tannins of *T. foenum-graecum* L. seeds showed an average yield extraction equal to 0.113±0.0094%. **Fig. 3** shows the result of three tannins extraction trials.

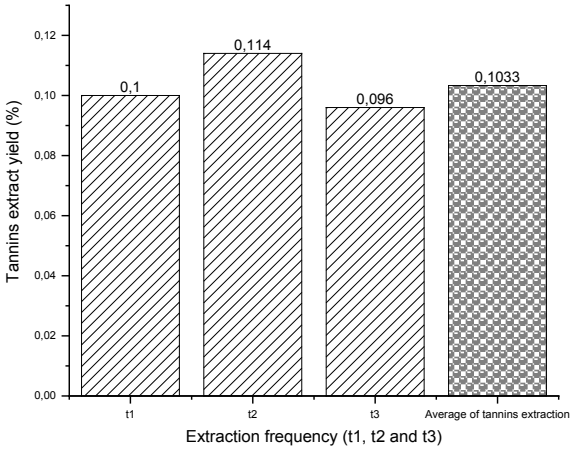


Figure 3 Yield extraction of tannins of *T. foenum-graecum* L. seeds

Chromatographic analysis of tannins extract

Qualitative analysis by TLC

The qualitative analysis of the tannins extract using different elution systems made it possible to calculate the frontal ratio (Rf) of the chemical species given in **Table 2**.

Four brown spots were obtained by different elution systems varying from 0.05 to 0.94. The colour changed from brown to pale yellow, to yellow, and then to green. These different colours confirm the nature of the tannic compounds. The comparison between the obtained frontal ratios of the tannic compounds and those of some references cited in the literature (which used the same elution systems) indicates the presence of tannic acid, phloroglucinol, resorcinol, and catechol.

Table 2 TLC analysis of *T. foenum-graecum* L. seeds' tannins extract

Elution system (v/v/v)	Number of spots	R_f	Compounds (standard)	References
Toluene/ethyl acetate/ formic acid (5/4/1)	05	0.44 0.5 0.56 0.62 0.94	Tannic acid	Sharma *et al* [20]
Acetic acid distilled water (0.44/6.56)	02	0.47 0.73	Tannic acid Catechol	Elgailani and Ishak [21]
Chloroform/ methanol/ acetic acid (9/1/0,1)	03	0.05 0.27 0.42	Phloroglucinol Resorcinol Catechol	Sharma *et al* [22]
Acetone/toluene/ formic acid (3/3/1)	04	0.52 0.66 0.74 0.76	Tannic acid Phloroglucinol Resorcinol Catechol	Kholkhal [23]

Quantitative analysis by HPLC

The HPLC chromatogram shown in **Fig. 4** and **Table 3** revealed compounds characterized by their peaks and retention times in the spectrum of the studied tannins extract. The chromatogram revealed three peaks between 2,471 and 3,635 min; the highest one described a retention time of 2,471 min.

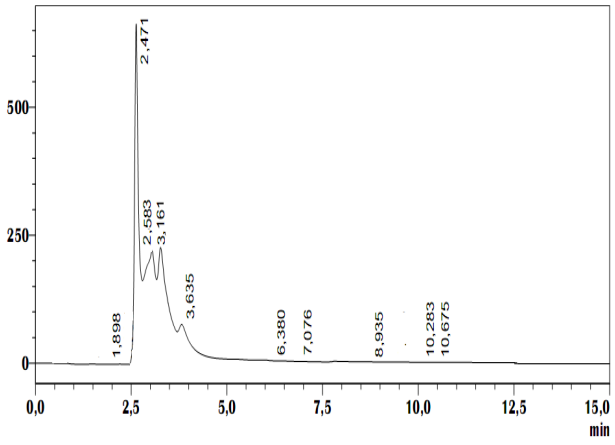


Figure 4 HPLC chromatogram of fenugreek seeds' tannins extract

Table 3 HPLC results of fenugreek seeds' tannins extract.

Peak	Retention time (min)	Capacity factor (k)	Percentage (%)	Compounds [24]	Retention time (min) [24]
1	2,583	2,369	54,299	Tannic acid	2,477
2	2,743	3,187	18,033	Phloroglucinol	2,607
3	3,161	4,119	18,570	Resorcinol	3,105
4	3,635	4,719	6,265	Catechol	3,600

Isolation and identification of microbial strains

We were able to isolate 21 microbial uropathogenic strains: the species *E. coli*, *Staphylococcus aureus*, *Klebsiella sp*, Proteus mirabilis, *Provedencia sp*, *Shigella sp*, *Pseudomonas aeruginosa*, and the fungal species *Candida albicans*.

Antibiotic susceptibility test

The antibiotic susceptibility test (AST) results of the isolated strains showed multidrug resistance of *Escherichia coli* species against ampicillin, imipenem, ceftazidime, ciprofloxacin, and amoxicillin+clavulanic acid. While *S. aureus* (1) and *S. aureus* ATCC 25923 strains were resistant to penicillin, oxacillin, cefoxitin, tetracycline, and cotrimoxazole (**Table 4**). However, some antibiotics that belong to aminoglycosides, carbapenems, and phenolics retained an effective antibacterial action (**Fig. 5**).

Antimicrobial test of tannins extract

The obtained results are shown in **Table 5**, **Table 6** and **Fig. 6**. The antimicrobial effect was switched from low to average action where the tested bacterial reference strains were more susceptible compared to the isolated uropathogenic strains which were multidrug-resistant. However, the Minimal Inhibitory Concentration (MIC) value varied from 30 to 100 mg/mL depending on the tested strain (**Table 7**).

Table 4 Multidrug-resistant profiles of the tested uropathogenic agents and bacterial reference strains

Bacterial strains	Antibiotics
***E. coli* (1)**	IPM-CAZ-AMC-AM-CS-CIP and susceptible to SXT-C-CN-AK
***E. coli* (2)**	IPM-CAZ-AMC-AM-CS-CIP and susceptible to SXT-C-CN-AK
***E. coli* (3)**	IPM-CAZ-AMC-AM-SXT-CS and susceptible to C-CN-CIP-AK
***E. coli* (4)**	IPM-CAZ-AMC-AM-SXT-C-CS and susceptible to CN-CIP-AK
***E. coli* (5)**	IPM-CAZ-AMC-AM-CS and susceptible to SXT-C-CN-CIP-AK
***E. coli* (6)**	IPM-CAZ-AMC-AM-CS and susceptible to SXT-C-CN-CIP-AK
***E. coli* (7)**	IPM-CAZ-AMC-AM-SXT-CS and susceptible to C-CN-CIP-AK
***E. coli* (8)**	IPM-CAZ-AMC-AM-CS and susceptible to SXT-C-CN-CIP-AK
***E. coli* (9)**	IPM-CAZ-AMC-AM-SXT-CIP-CS and susceptible to C-CN-AK
ESBL-producing *E. coli* (10)	AMP-AMC-CZ-KF-FOX-CTX-CN-NA-CIP-CS-FOS-SXT-ATM-CAZ and susceptible to IPM-C-AK
ESBL-producing *E. coli* (11)	AMP-AMC-CZ-KF-FOX-CTX-CN-NA-CIP-CS-FOS-SXT-ATM-CAZ and susceptible to IPM-C-AK
Provedencia sp	IPM-AMC-AM-CIP-CS and susceptible to SXT-C- CN- AK
Shigella sp	IPM-CAZ-AMC-AM-SXT-C-CS and susceptible to CN-CIP-AK
***Klebsiella sp* (1)**	IPM-CAZ-AMC-AM-SXT-CS-CIP-AK and susceptible to C-CN
***Klebsiella sp* (2)**	IPM-CAZ-AMC-AM-SXT-CS-CIP and susceptible to C-CN-AK
***Klebsiella sp* (3)**	AMP-AMC-CZ-KF-FOX-CS-FOS-SXT and susceptible to CTX-CIP-ATM-IPM-C-CN-AK-NA
Proteus mirabilis	AMP-CS and susceptible to AMC-CTX-CAZ-CIP-ATM-KF- CZ-SXT-FF-C-CN-AK-NA
***Staphylococcus aureus* (1)**	Susceptible to C-RA-CN-VA-TE-OX-FOX-OFX-SXT
***Staphylococcus aureus* (2)**	OX-FOX-RA-TE and susceptible to C-CN-VA-OFX-SXT
P. aeruginosa	IPM-CAZ-CIP and susceptible to CN-AK
***E. coli* ATCC 25922**	IPM-AMP-AMC-CN-AK-CS and susceptible to SXT-C-FOS
***P. aeruginosa* ATCC 27853**	CAZ-CS and susceptible to IPM-CN-AK-OFX
***S. aureus ATCC* 25923**	P-SXT-OX-FOX and susceptible to TE-OFX-CN-C
***B. cereus ATCC* 14579**	IPM-AMP-AMC-AX-P and susceptible to AK-CN
***E. faecalis ATCC* 29212**	IPM-AMP-AMC-AX-P and susceptible to TE-FOS-VA-AK-CN

Amoxicillin-clavulanic acid (AMC 30µg), Cefoxitin (FOX 30µg), Cefotaxime (CTX 30µg), Ceftazidime (CAZ 30µg), Amikacin (AN 30µg), Gentamicin (CN 10µg), Imipenem (IPM 10µg), Nalidixic acid (NA 30µg), Ciprofloxacin (CIP 5µg), Trimethoprim/sulfamethoxazole (SXT 25µg), Aztreonam (ATM 30µg), Ampicillin (AMP 10µg), Cefazolin (CZ 30µg), Colistin (CS 10µg), Fosfomycin (FOS 50µg), Cephalothin (KF 30µg), Cefepime (FEP 30µg), Chloramphenicol (C 30µg), Penicillin (P 10µg), Oxacillin (OX 1µg), Tetracycline (TE 30µg), Ofloxacin (OFX 5µg), Rifampin (5µg), and Amoxicillin (AX 25µg).

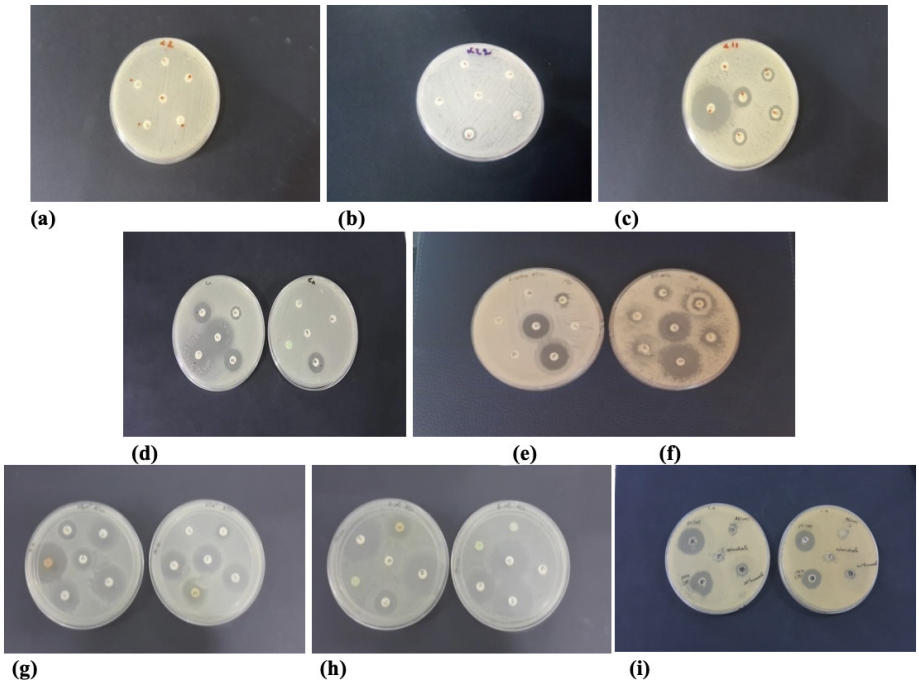


Figure 5 Antibiotic susceptibility test of the microbial strains by the disc diffusion method on MH agar (Original, 2021)
(a) ESBL-producing *E. coli* 10;
(b) ESBL-producing *E. coli* 11;
(c) *Klebsiella sp* 3;
(d) *Klebsiella sp* 1;
(e) B. cereus ATCC 14579;
(f) *E. faecalis* ATCC 29212 (Benyagoub *et al*, 2020a [8]);
(g): *S. aureus* ATCC 25923;
(h): *E. coli* ATCC 25922;
(i): *C. albicans* (Benyagoub *et al*, 2020a [8]).

Table 5 Antibacterial effect of *Trigonella foenum-graecum* L. seeds' tannins extract against some microbial uropathogenic strains, expressed as zones of inhibition (diameter in mm)

	Tannins extract (mg/mL)							
Microbial strains	**10**	**30**	**40**	**50**	**60**	**70**	**90**	**100**
***E. coli* (1)**	6	6	6	6	6	6	6	6
***E. coli* (2)**	6	8	8	8	9	9	**10**	**11**
***E. coli* (3)**	6	6	6	6	6	7	8	8
***E. coli* (4)**	6	6	6	6	6	6	6	6
***E. coli* (5)**	6	6	6	6	7	8	9	**10**
***E. coli* (6)**	6	7	7	9	9	9	**10**	**11**
***E. coli* (7)**	6	6	6	6	9	**10**	**10**	**11**
***E. coli* (8)**	6	6	6	6	6	6	7	8
***E. coli* (9)**	6	6	6	6	6	6	7	8
ESBL-producing *E. coli* (10)	6	6	6	6	6	6	6	6
ESBL-producing *E. coli* (11)	6	6	6	6	6	6	6	6
Provedencia sp	6	6	6	7	8	9	**10**	**11**
Shigella sp	6	7	7	7	8	8	8	**12**
***Klebsiella sp* (1)**	7	8	8	9	9	**10**	**10**	**11**
***Klebsiella sp* (2)**	6	6	7	8	8	**10**	**11**	**13**
***Klebsiella sp* (3)**	6	6	6	6	6	6	6	6
Proteus mirabilis	6	6	6	7	7	7	8	8
***Staphylococcus aureus* (1)**	6	9.25	**11**	**11**	**12**	**13**	**14**	**17**
***Staphylococcus aureus* (2)**	6	8	9	9	**10**	**11**	**12**	**14**
P. aeruginosa	6	6	6	6	6	6	6	8
Candida albicans	6	7	8	8	9	9	**10**	**12**
***E. coli* ATCC 25922**	6	8	9	**10**	**11**	**11**	**12**	**12**
***P. aeruginosa* ATCC 27853**	6	6	8	9	9	**10**	**10**	**11**
***S. aureus* ATCC 25923**	6	8.33	**10**	**11**	**12**	**12**	**13**	**15**
***B. cereus* ATCC 14579**	9	**11**	**12**	**13**	**13**	**14**	**14**	**15**
***E. faecalis* ATCC 29212**	6	6	6	6	7	**10**	**10**	**11**

Table 6 AI of *Trigonella foenum-graecum* L. seeds' tannins extract against some microbial uropathogenic and bacterial reference strains

	Tannins extract (mg/mL)								Standard reference antibiotic
Microbial strains	**10**	**30**	**40**	**50**	**60**	**70**	**90**	**100**	**CN (ZI in mm)**
***E. coli* (1)**	-	-	-	-	-	-	-	-	16
***E. coli* (2)**	-	0.5	0.5	0.5	0.56	0.56	0.62	**0.67**	16
***E. coli* (3)**	-	-	-	-	-	0.41	0.47	0.47	17
***E. coli* (4)**	-	-	-	-	-	-	-	-	16
***E. coli* (5)**	-	-	-	-	0.44	0.5	0.56	0.62	16
***E. coli* (6)**	-	0.39	0.39	0.5	0.5	0.5	0.55	0.61	18
***E. coli* (7)**	-	-	-	-	0.56	0.62	0.62	**0.69**	16
***E. coli* (8)**	-	-	-	-	-	-	0.41	0.47	17
***E. coli* (9)**	-	-	-	-	-	-	0.37	0.42	19
ESBL-producing *E. coli* (10)	-	-	-	-	-	-	-	-	**6**
ESBL-producing *E. coli* (11)	-	-	-	-	-	-	-	-	**6**
Provedencia sp	-	-	-	0.30	0.34	0.39	0.43	0.48	23
Shigella sp	-	0.37	0.37	0.37	0.42	0.42	0.42	0.63	19
***Klebsiella sp* (1)**	0.44	0.5	0.5	0.56	0.56	0.62	0.62	**0.69**	16
***Klebsiella sp* (2)**	-	-	0.39	0.42	0.42	0.55	0.61	**0.72**	18
***Klebsiella sp* (3)**	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	18
Proteus mirabilis	-	-	-	0.33	0.33	0.33	0.38	0.38	21
***Staphylococcus aureus* (1)**	-	0.46	0.55	0.55	0.6	0.65	0.7	**0.85**	20
***Staphylococcus aureus* (2)**	-	0.4	0.45	0.45	0.5	0.55	0.6	**0.7**	20
P. aeruginosa	-	-	-	-	-	-	-	0.47	17
Candida albicans	-	-	-	-	-	-	-	-	-
***E. coli* ATCC 25922**	-	0.73	0.81	0.90	1.0	**1.0**	**1.09**	**1.09**	**11**
***P. aeruginosa* ATCC 27853**	-	-	0.4	0.45	0.45	0.5	0.5	0.55	20
***S. aureus* ATCC 25923**	-	0.49	0.59	0.65	**0.7**	**0.7**	**0.76**	**0.88**	**17**
***B. cereus* ATCC 14579**	0.47	0.58	0.63	**0.68**	**0.68**	**0.74**	**0.74**	**0.79**	**19**
***E. faecalis* ATCC 29212**	-	-	-	-	0.30	0.43	0.43	0.48	**23**

Gentamicin (**CN** 10µg); **ZI**: Zones of inhibition

Table 7 Minimum inhibitory concentrations of *T. foenum-graecum* L seeds' tannins extract

Microbial strains	MIC (mg/mL)
Uropathogenic strains of ***E. coli***	30–90
Provedencia sp	50
Shigella sp	30
Uropathogenic strains of ***Klebsiella sp***	10–40
Proteus mirabilis	50
Uropathogenic strains of ***S. aureus***	10–30
P. aeruginosa	100
C. albicans	30
E. coli ATCC 25922	30
P. aeruginosa ATCC 27853	40
S. aureus ATCC 25923	10–30
B. cereus ATCC 14579	10
E. faecalis ATCC 29212	60

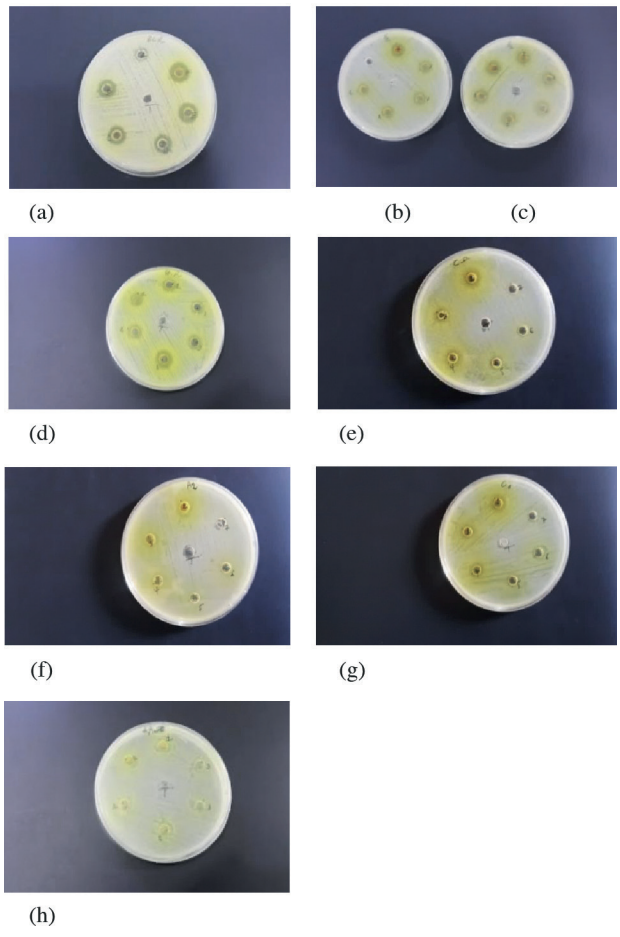


Figure 6 Antimicrobial effect of fenugreek seeds' tannins extract on some uropathogenic agents and bacterial reference strains (Original, 2021)
(a) *B. cereus* ATCC 14579; **(b)** *S. aureus* 2; **(c)** S. *aureus* 1; **(d)** *S. aureus* ATCC 25923; **(e)** *C. albicans*; **(f)** *E. coli* 2; **(g)** *Klebsiella sp* 1; **(h)** *P. aeruginosa*

Discussion

Fenugreek (*T. foenum-graecum* L.) is a very old aromatic and medicinal plant. The seeds of this plant are widely used in traditional medicine, in many countries, to fight against various pathologies. They have extraordinary and promising therapeutic properties (antitumoral, hypoglycemic, anti-inflammatory, hypocholesterolemia and antioxidant) and are also used in the treatment of diabetes and neurological diseases such as Alzheimer's [5, 25, 26, 27, 28, 29]. To this end, this study assessed the antimicrobial activity of fenugreek seeds' tannins extract grown in Taghit-Bechar (South-West of Algeria).

The results of a previous study by Benyagoub *et al* (2021) [4] on the phytochemical composition of fenugreek seeds, showed that tannins, flavonoids, terpenoids, and saponins were the dominant compounds of the studied species. This corroborates with the reported results of the studies by Benziane *et al* (2019) [30]; Ammar and Mawahib (2014) [31]; Chidambaram et al (2015) [32], where the tannins are one of the dominant phytochemical groups, but Rahmani et al (2015) [33] noticed the absence of tannins. The tannins extraction yield result was lower than that given by Chidambaram et al (2015) [32] (0.103±0.02% versus 0.81±0.05%). According to the results of the study by Benziane *et al* (2019) [30], the tannins extraction yield of the macerate of *T. foenum-graecum* was 8.69±0.02%. This was higher than the yield obtained by decoction (9.75±0.06%). The Indian species of *T. foenum-graecum* reveals tannins yields of 21.21±0.24; 11.43±0.54; 5.12±1.3; 2.32±1.32 and 1.43±0.56% for aqueous, ethanolic, methanolic, hexane and chloroform extracts, respectively [34].

Rahmani (2017) [35], who studied the physiology and valourization of fodder and medicinal plants in Sidi Bel-Abbés province: Case of Fenugreek, recorded heterogeneity in the av-

erage concentrations of condensed tannins varying from 0.73 to 1.051 mg CE/g dry weight. These results were higher than the obtained results (an average value of 0.103 mg/g).

The extract yield depends on the geographical origin, the phenological, and the physiological stage of the plant. Also, important factors that can affect the phytochemical composition of fenugreek seeds is the variety of the grown fenugreek plant as well as environmental factors such as temperature and soil quality [36]. Experimental conditions in the laboratory, particularly the extraction methods (maceration, decoction, infusion, etc.) and the organic solvents used (water, methanol, acetones, etc.) are also factors. Therefore, the biological activities of fenugreek seeds may vary from one region to another.

According to the geographical and pedoclimatic data of the study region, the commune of Taghit is located in the northwestern part of the Sahara at 1050 km southwest of Algiers. The climate of Taghit is marked by parameters that strongly limit the distribution of vegetation: low precipitation – which is due to the scarcity of humid air masses; high temperature; large thermal amplitude. The soil is characterized by its depletion in fine elements, driven by the wind on the surface. Its sandy-silt texture is often enriched with salts and limestone or gypsum [37].

The nutrients available in soil have a great influence on the production of phenols, particularly tannins, in many species. Nitrogen fertilization reduces the tannin content found in vegetables by one-fifth; and the reduction in soil nutrients leads to increased production of secondary compounds [38].

The TLC and HPLC results of *T. foenum-graecum* L. seeds' tannins extract showed the presence of tannic acid, phloroglucinol, resorcinol, and catechol; tannic acid was the main constituent. This validates the results obtained by Ammar and Mawahib (2014) [31], which found the tannin extract of *T. foenum-graecum* L. seeds to be rich in tannic acid.

The multidrug resistance pattern of the tested species corroborates the results given by Boukhemis and Boutersa (2015) [39] who reported the resistance of *E. coli* to amoxicillin, amoxicillin+clavulanic acid, *S. aureus* ticarcillin, and cefazolin, and agrees with data from Harrar (2012) [40] where *S. aureus* was susceptible to ciprofloxacin, fosfomycin, nalidixic acid and gentamicin. It also confirms our previous studies' results conducted in Bechar on the evolution of multidrug resistance of the uropathogenic strains [8, 10, 4, 41, 42].

The antimicrobial effect of the tannins extract varied from low to average action; *S. aureus* (1 and 2), *Klebisella sp*, *Candida albicans*, and *E. coli* (7) species, as well as the reference strains, *S. aureus* ATCC 25923 and *B. cereus* ATCC 14579 were the most susceptible to the effect of tannins extract among tested strains with zones of inhibition ranging from 9 mm to 15 mm, and an AI varying from 0.33 to 1.09 compared to gentamicin (Positive control).

These results are also in agreement with those given by Ben aissi (2018) [43] who highlighted the antibacterial activity of the aqueous extract of *T. foenum* against three bacterial strains and reported a very strong antibacterial effect against S*. aureus* and *Bacillus subtilis* with zones of inhibition ranging from 25 mm to 29.5 mm and 26 mm to 28.5 mm, respectively, but at high concentrations (200 mg/mL, 600 mg/mL and 900 mg/mL). However, no antibacterial effect was reported towards *E. coli*. This supports the present results where the antibacterial effect against *E. coli* species was qualified as weak in comparison to our previous studies' results on the aqueous extracts obtained by infusion and decoction, as well as the two fractions of flavonoids (n-butanolic and ethyl acetate) of fenugreek seeds grown in the same study region [4].

Our results showed high antibacterial activity against Gram-positive bacteria. The most susceptible strains to the tannins extract were

Bacillus cereus ATCC 14573, followed by the reference bacterial strains and the isolated uropathogenic strains: S. aureus, then C. albicans, and *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *Klebsiella sp*p, C. albicans, *Shigella sp*, *Provedencia sp*, *Proteus mirabilis*, and *P. aeruginosa*, in that order. However, ESBL-producing *E. coli* (10 and 11), as uropathogenic strains, were resistant to the tannins extract within the limits of the tested concentrations.

Most of the research supports the idea that the main site of action of phenolic substances is the bacterial cytoplasmic membrane they can disintegrate. The membrane is destructured to become more permeable to ions. Cell membrane injury can also promote a decrease in membrane potential [44].

The antimicrobial activity of the tested fraction showed medium to no effect; this implies that the antimicrobial activity demonstrated in our previous studies [4] with crude extracts was probably due to a synergistic action of two or more phytochemical compounds [45]. Also, the variation in the composition and the microbial activity of this extract could be linked to the used part of the plant: seeds, leaves, stems, or roots. Moreover, according to Settar and Takesrit (2017) [46], the method used for the assessment of antimicrobial activity influences the results, whereas the agar well diffusion method that was conducted in this study, gave better results compared to the disk diffusion method.

According to Gupta *et al* (2012) [24], most flavonoid compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant, and immunomodulatory properties. The biological activity of flavonoid compounds may be due to the presence of gallic acid, ellagic acid, quercetin, tannic acid, vanillin, resorcinol, catechin, etc. The structure of tannins within each group further varies with their degree of hydroxylation, conjugation with sugars or phenolic acids, or their degree of polymerization, which allows them to form insoluble complexes with carbohydrates, proteins, and metal ions [47, 48, 49]. This reaction with proteins is the source of many biological effects of tannins.

Conclusions

Medicinal plants remain a reliable source of bioactive compounds known for their therapeutic properties. This work focused on the assessment of the antimicrobial activity of fenugreek seeds' tannins extract; a plant widely used in traditional medicine in Algeria. A low tannins extraction yield is noted. The AST results show that most of the isolated uropathogenic species have a multidrug resistance character, and this is against at least three classes of antibiotics.

The qualitative study of the tannins extracts by TLC made it possible to isolate and identify four phytochemical compounds, of which tannic acid is the main compound. The antimicrobial activity of fenugreek seeds' tannins extract shows a relatively significant antimicrobial effect against the reference bacterial strains when compared to the isolated uropathogenic strains that were multidrug-resistant except *S. aureus* (1). The phenolic compounds present in the plant could be useful candidates for therapeutic purposes.

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Author contributions

Data gathering and idea for this study: EB; collection of fenugreek seeds: EB; extraction of tannins from fenugreek seeds: NN; FB; MNM; TLC and HPLC analysis: NN; FB; MN; isolation and identification of the uropathogenic strains:

EB; antibiotic susceptibility test and antimicrobial activity assay: EB; writing of original manuscript: EB; manuscript review: NN. All authors have read and approved the final manuscript.

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Conflict of interests

The authors declare that they have no conflict of interest.

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