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Supercritical carbon dioxide extraction of *Trigonella foenum graecum* Linn seeds: Determination of bioactive compounds and pharmacological analysis

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ABSTRACT

Objective: To investigate the effect of temperature and pressure on supercritical CO_2 extraction of *Trigonella foenum graecum* Linn seeds, to determine the optimal condition which leads to highest percentage of the accumulative yield and revealing the chemical composition of supercritical CO_2 extract.

Methods: Temperatures in the range of 40–60 °C and pressures in the range of 10–25 MPa were used. FTIR and GC–MS analysis were used to detect the bioactive compounds present in the extract. The broth dilution method and slope method were used to evaluate the anti-microbial and anti-tuberculosis activities and the *in vitro* anti-malarial assay was carried out according to the micro assay protocol of Rieckmann and his co-workers.

Results: The temperature was more affected than the pressure on the extraction performance and the highest yield of the extract (3.111%) was attained at 60 °C and 10 MPa. FTIR and GC–MS showed that the chemical composition of the extract included conjugated linoleic acid methyl ester as the major active principle (with concentration of 72.28%), followed by saturated fatty acid methyl esters (16.03%), steroids (8.09%) and organic siloxane compound (3.61%). The extract showed moderate anti-bacterial activity with MIC values 100, 250, 125 μg/mL towards *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenus* respectively. It exhibited high inhibition effect towards the fungi *Candida albican* with MFC value (250 μg/mL). The extract had low anti-tuberculosis activity with MIC value (100 μg/mL) and comparable MIC value (0.29 μg/mL) towards *Plasmodium flaciparum*.

Conclusions: Supercritical CO_2 extraction as alternate and green technology is performed successfully to extract the bioactive compounds from the seeds of *T. foenum graecum* Linn and it is concluded that this extract can be used as an alternate source of synthetic anti-biotic drugs.

1. Introduction

Natural products have been considered as an essential source of drugs and pharmaceutical products. The practice of herbal medicine is established and documented in Asia and most medicinal plants that have been known come from China and India. A huge number of new anti-biotics over the last three decades have been synthesized by the pharmaceutical industry. However, the resistance to them by microorganism has increased [1]. Recently, the studies turned towards the plants to extract a convenient and an effective anti-microbial drug. The use of plant extract has achieved a considerable significance in therapeutic treatments and may help to cure the problems of these multi-drug resistant organisms. *Triognella foenum graecum* Linn (*T. foenum graecum* L.) belongs to Fabaceae family with the height of 30–60 cm. It has compound pinnate, trifoliate leaves, axillary white to yellow flowers with 13–15 cm long. The

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seed highlighted in this study is golden-yellow in color, small in size, hard, and has four-faced stone such as structure. It is 3–6 mm long, 2–5 mm wide and 2 mm thick. The name of *Trigonella* varies from one country to another. It is called Hulba in Arabic countries, Methi in India, Fenugrec in France, Tsimeni in Greece and Alforva in Brazil [2,3]. Locally, fenugreek seeds have been traditionally applied to cure cough, congestion, bronchitis, fever, high blood pressure, headache, migraines, diarrhea, flatulence, anemia, irregular menstrual cycles and arthritis. Also it is utilized to ease labor pains and menstruation pain, and as an appetite stimulant [4]. Nowadays fenugreek seeds are regarded as a low cost source of good quality protein as they include a high proportion of protein ranging from 20% to 30% as well as amino 4-hydroxyisoleucine that has high potential for insulin-stimulating activity. They also contain saponins, hemicelluloses and lipoprotein cholesterol in blood by inhibiting the salts re-absorption in the colon [5]. Over the last two decades, supercritical fluid extraction has been considered as a clean and environmentally friendly technique [6] and often as a suitable substitute to organic solvent-based extraction of natural products. Supercritical fluid solvents are of great interest chemically for their involvement in chemical reactions and their solvent effects that are influenced by pressure and temperature. Supercritical fluid solvents like carbon dioxide are intermediates between liquid and gases [7] and play a significant role in the process of separation based on the physico-chemical properties such as density, diffusivity, viscosity and dielectric constant which are manipulated easily by pressure and temperature. Separation and detection of residual drug affected significantly on human health [8]. Carbon dioxide has the ability to penetrate through the solid matrix and dissolve the desired extract due to its dual gaseous and liquid like characteristic [9]. It can dissolve non polar to slightly polar compounds; and to improve its solvent power, a small quantity of polar organic solvent like methanol or ethanol is added, which help to increase the solubility of the polar analyte in CO_2 [10,11].

2. Materials and methods

2.1. Materials

T. foenum graecum L. dried seeds were purchased from the local market of Aurangabad, India. CO_2 with purity of 99.95% and methanol of analytical grade were brought from SDFCL-SD fine chemicals Co. (Maharashtra, India).

2.2. Preparation of the sample

The seeds of *T. foenum graecum* L. were ground properly before extraction in a grinder to increase the extraction efficiency and then were stored in air tightened bottle.

2.3. Supercritical fluid extraction

Supercritical fluid extraction (SFE) instrument and methods were described properly in our prior work [12]. The extraction of *T. foenum graecum* L. seeds was conducted at 5 different temperatures in the range of 40–60 °C. Every extraction process was performed by keeping the required temperature constant and raising the pressure in the range of 10–25 MPa; four different portions of the extracts were collected separately in a previous weighted test tube at every pressure used within 10 min. The extract was allowed for drying at room temperature until it gained a constant weight. Hence, the yield % was calculated as Y% = (Mass of extract/Mass of dried seeds) × 100. The details about Fourier transform infrared spectrophotometer (FTIR) and gas chromatography–mass spectrometry (GC–MS) methods were mentioned in details in our previous work [13].

2.4. Pharmacological analysis

Broth dilution method was used to evaluate the antimicrobial activity, the slope method was used to examine the anti-tuberculosis activity and the *in vitro* anti-malarial assay was carried out in 96 well microtitre plates according to the micro assay protocol of Rieckmann and co-workers with minor modifications. The minimal inhibition concentration (MIC) was determined by all the methods. The details about these methods were described in our previous work in [7].

3. Results

3.1. Yields of extracts

The effects of the two factors (pressure and temperature) were investigated in the ranges of 10–25 MPa and 40–60 °C respectively. The extraction yield was considered as the independent variable. SFE yields ranged from 0.053 2% to 3.111 0% as shown in Table 1. The highest SFE yield was achieved at SFE condition (60 °C, 10 MPa) whereas the lowest SFE yield was attained at 40 °C, 25 MPa and at 45 °C, 25 MPa.

3.2. Effect of pressure

The behavior of pressure at constant temperatures was analyzed. It was observed that increasing the pressure in the range of 10–25 MPa affected negatively on the extraction process as it led to decrease the percentage of the extraction yield for all the temperatures applied. This may investigate by increasing the mass transfer resistance due to decrease the effective diffusivity and mass transfer coefficient with higher pressures, and as a result of that, the rate of extraction decreased and the extraction efficiency became low.

3.3. Effect of temperature

The effect of temperature at constant pressures was investigated. At constant pressure of 10 MPa, it was observed that by raising the temperature in the range of 40–50 °C, the percentage of the accumulative extraction yield decreased, and then it increased by raising the temperature in the range of 50–60 °C.

Table 1
Extraction yield of Sc–CO_2 extract (%).

S. No.	Temp (°C)	Pressure (MPa)			
		10	15	20	25
1	40	2.993	0.710	0.680	0.532
2	45	2.117	1.207	0.804	0.532
3	50	2.496	1.419	1.112	0.591
4	55	2.567	1.088	0.852	0.615
5	60	3.111	0.828	0.745	0.732

Here the temperature worked diversely, since two parameters affected by the increase of temperature, the density of CO_2 represented the first parameter which diminished by the increase of temperature, resulting in minimize the solubility and extraction efficiency. On the other hand, raising the temperature led to increase the solutes volatility which represented the second parameter; and consequently the solubility of the solutes in CO_2 and their extraction increased. Hence, in the first case, the first parameter overcame the second parameter so the percentage of the extraction yield decreased but in the second case, the second parameter overcame the first parameter and led to increase the extraction efficiency.

At constant pressure of 15 MPa, by raising the temperature in the range of 40–50 °C, the percentage of the extraction yield increased, which means the volatility of the solutes increased largely so it can overcome the decrease in CO_2 density, hence the positive effect of temperature appeared. However, further increase in the temperature ranging from 55 °C to 60 °C, the percentage of the extraction yield decreased by minimizing the density of CO_2 to overcome the increase in the solutes solubility, which led to the negative effect of temperature. The same trend appeared at constant pressure of 20 MPa. At constant pressure of 25 MPa, it was observed that by raising the temperature in the range of 40–45 °C, the percentage of accumulative extraction yield remained constant, which may due to the balance between the decrease in CO_2 density and the increase in the volatility of solutes, exhibiting no effect on the extraction process. However, the raise in temperature in the range of 50–60 °C increased the volatility of solutes made it able to overcome the decrease in CO_2 density, and consequently improved the extraction efficiency by increasing the percentage of the extraction yield. The best percentage of the extraction yield was 3.111% which detected at 60 °C and 10 MPa.

3.4. Determination of bioactive compounds

3.4.1. FTIR analysis

The FTIR profile of supercritical CO_2 (Sc–CO_2) extract of *T. foenum graceum* L. seeds was studied as exhibited in Table 2. The peaks appeared at 2 823 cm^{-1} due to C−H stretching and 1 755 cm^{-1} due to C=O stretching may indicate the presence of saturated fatty acid methyl ester (FAMEs). The peaks presented at 3 626, 3 583, 3 379, 3 344, 3 286 and 3 224 cm^{-1} due to O−H stretching [14–17]; 667 and 621 cm^{-1} due to O−H bending; 1 616 cm^{-1} due to C=C stretching; 960, 899 and 795 cm^{-1} due to =C−H bending and 2 978 cm^{-1} due to C−H stretching, may indicate the presence of phytosterols. The peaks detected at 3 170, 3 136, 2 673, 2 596 and 2 542 cm^{-1} due to O−H stretching; 1 755 cm^{-1} due to C=O stretching, 1 616 cm^{-1} due to C=C stretching, 3 020 and 3 105 cm^{-1} due to =C−H stretching; 756 and 714 cm^{-1} due to =C−H bending and 2 870 cm^{-1} due to C−H stretching may refer to the presence of methyl ester of conjugated linoleic acid. The peak occurred at 1 165 cm^{-1} due to Si−O−C asymmetric stretching may refer to the presence of organic siloxane. The peak existed at 1 207 cm^{-1} due to C−O stretching may indicate the availability of ether compound.

3.4.2. GC–MS analysis

Investigation on mass spectrum of GC–MS was performed by applying the database of National Institute standard and Technology which includes more than 62 000 patterns. The chromatogram obtained in Figure 1 exhibited the availability of six active principles in Sc–CO_2 extract of the seeds. The name of expected compounds, retention time, molecular formula and percentage of area were tabulated in Table 3.

Table 2
Characteristic IR absorption frequencies of functional groups of Sc–CO_2 extract.

S. No	Characteristic absorptions (cm^{-1})	Intensity	Functional group	Type of vibration	Compound nature
1	3 626	Medium, narrow	O−H	Stretch (free)	Alcohol
2	3 583	Medium, narrow	O−H	Stretch (free)	Alcohol
3	3 510	Medium	O−H	Stretch (free)	Alcohol
4	3 444	Medium	O−H	Stretch (bonded)	Polymeric
5	3 379	Weak	O−H	Stretch	Carboxylic acid
6	3 344	Medium	O−H	Stretch	Carboxylic acid
7	3 286	Weak	O−H	Stretch	Carboxylic acid
8	3 224	Weak	O−H	Stretch	Carboxylic acid
9	3 170	Weak	O−H	Stretch	Carboxylic acid
10	3 136	Weak	O−H	Stretch	Carboxylic acid
11	3 105	Weak	C−H	Stretch	Alkene
12	3 020	Weak	C−H	Stretch	Alkene (medial)
13	2 978	Weak	C−H	Stretch	Alkane
14	2 870	Weak	C−H	Stretch	Methyl ($-CH_3$)
15	2 823	Weak	C−H	Stretch	Alkane
16	2 673	Weak	O−H	Stretch	Carboxylic acid
17	2 596	Weak	O−H	Stretch	Carboxylic acid
18	2 542	Weak	O−H	Stretch	Carboxylic acid
19	1 755	Weak	C=O	Stretch	Ester
20	1 616	Weak	C=C	Stretch	Alkene
21	1 207	Weak	C−O	Stretch	Ester
22	1 165	Weak	Si−O−C	Asymmetric Stretch	Organic siloxane
23	960	Weak	=C−H	Bend (out of plane)	Alkene (*Trans*)
24	899	Weak	=C−H	Bend	Alkene
25	795	Weak	=C−H	Bend	Alkene
26	756	Weak	=C−H	Bend	Alkene
27	714	Weak	=C−H	Bend	Alkene
28	667	Medium	O−H	Bend (out of plane)	Alcohol
29	621	Medium	O−H	Bend (out of plane)	Alcohol

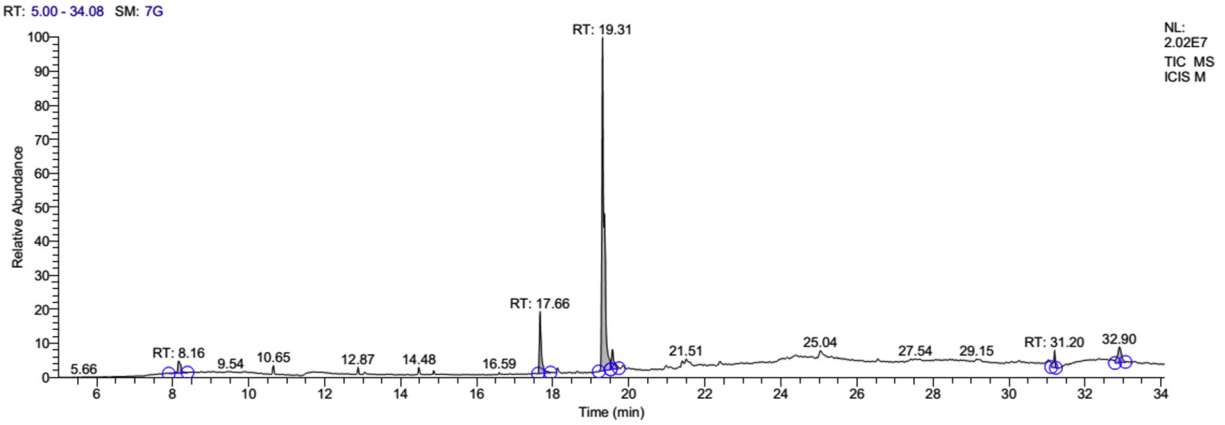

Figure 1. GC–Mass Spectrum of Sc–CO_2 extract of *T. foenum graecum* L.

3.5. Pharmacological analysis

3.5.1. Anti-bacterial activity analysis

The study of anti-bacterial activity of Sc–CO_2 extract was conducted by using two bacteria belong to gram negative bacteria namely *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) and two bacteria belong to gram positive bacteria namely *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes*. Four standard drugs namely gentamycin, chloramphenicol, ciprofloxacin and norfloxacin were also studied for comparison. The extract showed moderate inhibition towards *E. coli* and *P. aeruginosa* compared with the standard drug chloramphenicol and less inhibition towards them compared with the remaining standard drugs. Sc–CO_2 extract exhibited moderate inhibition in case of *S. pyogenes* compared with the standard drugs chloramphenicol and ciprofloxacin and less inhibition compared with gentamycin and norfloxacin. In case of *S. aureus*, the extract exhibited less inhibition compared with all standard drugs. MIC values of the extracts and the MBC values of the standard drugs for every type of bacteria used were showed in Table 4.

3.5.2. Anti-fungal activity analysis

The anti-fungal activity of Sc–CO_2 extract was studied against three fungus called *Candida albicans* (*C. albican*), *Aspergillus niger* and *Aspergillus clavatus*. Two standard drugs namely nystatin and greseofulvin were used for comparison. Sc–CO_2 extract showed great and remarkable inhibition for *C. albicans* with minimum fungicidal concentration (MFC) of 250 μg/mL compared with the standard drug gresofulvin with MFC of 500 μg/mL. The result indicated that the extract worked better than the standard drug against the growth of *C. albicans* since it required half MFC value of the standard drug. MFC of the extract showed less inhibition against the growth of the other fungus compared with the standard drugs. The MFC values of the extract and MFC values of the standard drugs for every fungi used were showed in Table 5.

Table 4
Anti-bacterial activity of Sc–CO_2 extract (μg/mL).

S. No	Type of bacteria	MIC of extract	MBC of standard drugs			
			A	B	C	D
1	*E. coli* (MTCC 443)	100	0.05	50	25	10
2	*P. aeruginosa* (MTCC 1688)	125	1.00	50	25	10
3	*S. aureus* (MTCC 96)	250	0.25	50	50	10
4	*Streptococcus pyogenes* (MTCC 442)	125	0.50	50	50	10

A = gentamycin, B = chloramphenicol, C = ciprofloxacin, D = norfloxacin.

Table 5
Anti-fungal activity of Sc–CO_2 extract (μg/mL).

S. No	Type of fungi	MFC of extract	MBC of standard drugs	
			Nystatin	Greseofulvin
1	*C. albican* (MTCC 227)	250	100	500
2	*Aspergillus niger* (MTCC 282)	1 000	100	100
3	*Aspergillus clavatus* (MTCC 1323)	1 000	100	100

Table 3
GC–MS analysis of Sc–CO_2 extract.

S. No	Retention time	Compound name	Molecular formula	Area (%)
1	19.31	Methyl 9-*cis*,11-*trans*-octadecadienoate	$C_{19}H_{34}O_2$	72.28
		Methyl 10-*trans*,12-*cis*-octadecadienoate	$C_{19}H_{34}O_2$	
2	17.66	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	11.26
		Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	
		Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	
3	32.90	Campesterol	$C_{28}H_{48}O$	5.57
4	19.56	Methyl stearate	$C_{19}H_{38}O_2$	4.77
		Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	
		Heptadecanoic acid, 9-methyl-, methyl ester	$C_{19}H_{38}O_2$	
5	8.16	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	3.61
6	31.20	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	2.52

3.5.3. Anti-tuberculosis and anti-malarial activities

Sc–CO_2 extract was examined against *Mycobacterium tuberculosis* to evaluate the anti-tuberculosis activity. Two standard drugs called isoniazid and rifampicin were used for comparison. Their susceptibility was found to be 0.20 and 0.25 μg/mL. The extract showed no considerable inhibition compared with these two standard drugs since its MIC value were found to be 100 μg/mL. Additionally, Sc–CO_2 extract was tested for anti-malarial activity against *Plasmodium falciparum* strain. Chloroquine and quinine as standard drugs were used for comparison. Their susceptibility was found to be 0.020 and 0.268 μg/mL. The extract showed MIC of 0.29 μg/mL so it showed less inhibition compared with chloroquine but on the other hand MIC of the extract was near to the MIC of quinine.

4. Discussion

Many studies aim to extract the seed of *T. foenum graecum* L. for its medicinal significant in the treatment of various diseases. However, majority of these studies use the traditional methods for achieving the extraction. Up to now, few studies have turned towards the extraction of the seeds of *T. foenum graecum* L. by using a modern method such as supercritical fluid extraction, which give this study a considerable importance in the research field regarding the extraction of herbal drugs to get the maximum benefits of the medicinal plant and to solve the human health problems away from the side effects caused by other synthetic medicine. One of these studies conducted by Boganovic *et al.* [18] indicated that the best extraction yield was of percentage 2.57% and was obtained at 42.6 °C and 27.0 MPa. Another study performed by Das and Panda [19] showed that three isothermal (40 °C, 50 °C and 60 °C) and three isobaric (10, 20 and 30 bars) were applied. The percentage of the extraction yield was 3.65% which achieved at optimal condition 40 °C, 30 bars for 85 min. Both studies conflict with the result obtained in the present context in the optimal condition although the percentage of the extraction yield is approximate. The result in the current study was near to the study conducted by Tang *et al.* [20] which achieved the optimal condition at 55 °C and 25 MPa. The outcome of this study showed that the highest percentage of the accumulative yield was 3.111%, which was achieved at optimal condition 60 °C and 10 MPa. The pressure had a negative effect on the extraction process whereas the temperature played a significant role in increasing the extraction efficiency. FTIR analysis was successfully applied to detect the functional groups and accordingly the phytochemical compounds which may present in the extract that achieved the highest percentage yield and GC–MS analysis revealed the chemical composition of this extract which included methyl ester of conjugated linoleic acid as the major bioactive principle with concentration of (72.28%), followed by saturated FAMEs (16.03%), steroids (8.09%) and organic siloxane compound (3.61%). GC–MS analysis is the first step towards understanding the nature of active components in the medicinal plant [21] and spectrophotometric technique is rapid, easy to use, accurate, and not costly as compared with other techniques for the identification of drugs in different samples [22,23]. In terms of percentage amount, the highest concentration was detected with 72.28% at retention time 19.31 min. Two isomers compounds belonging to methyl esters of conjugated linoleic acid were expected to be either methyl 9-*cis*,11-*trans* octadecadienoate which are known with anti-cancer activity [24] or methyl 10 *trans*,12-*cis*-octadecadienoate whose biological activities have not been reported yet. The second bioactive constituent appeared at retention time 17.66 min with area percentage of 11.26%. Three components belong to saturated FAMEs were given according to the library search and one of them may present. The first expected compound is hexadecanoic acid methyl ester, which is also called as methyl palmitate or palmitic acid methyl ester. This compound is familiar with numerous of biological activities like anti-bacterial, anti-fungal [25] anti-oxidant, anti-inflammatory and decrease of blood cholesterol [26]. In addition, it is considered as hypocholesterolemic nematicide, pesticide, 5-alpha reductase inhibitor, hemolytic and anti-androgenic flavor. It is further reported as anti-histaminic, anti-eczemic anti-arthritic, anti-coronary, hepatoprotective, insectifuge and cancer preventive [26,27]. The second expected compound is pentadecanoic acid, 14 methyl, methyl ester, which is also called as methyl, 14 methyl pentadecanoate or methyl isohexadecanoate. It is one type of palmitic acid methyl ester and is known with anti-oxidant [21], anti-fungal and anti-microbial activities [28]. The third expected compound is pentadecanoic acid, methyl ester, a type of fatty acid methyl ester and is reported for its anti-microbial and anti-fungal activities [25]. The third bioactive compound is campestrol which appears at retention time 32.90 min with area percentage of 5.57%. It is a steroid and it plays an important role as hypocholesterolemic, also it has anti-oxidant activity [29]. The fourth highest concentration appears at retention time 19.56 min with area percentage of 4.77%. Three compounds belong to FAMEs are reported, methyl stearate; heptadecanoic acid, 16 methyl–methyl ester or heptadecanoic acid, 9 methyl, methyl ester and one of them may present. It is found in the literature that methyl stearate has anti-diarrheal cytotoxic and anti-proliferative activity [30] but the biological activities of the other two components are not reported. The fifth active principle is cyclopentasiloxane, decamethyl which is detected at retention time 8.16 min with percentage area of 3.61%. It is a siloxane compound and it is used for skin care, cosmetics and induction of hepatic xenobiotic metabolizing enzyme [31]. The last expected bioactive compound is 1-monolinoleoylglycerol trimethylsilyl ether which appears at retention time 31.20 min with percentage area of 2.52%. It is known with majority of biological activities like anti-microbial, anti-oxidant, anti-arthritic, anti-asthma, anti-inflammatory and anti-diabetic. It can also be considered as a diuretic [32]. A modern study done by Senthil *et al.* [33] declares that it is a bioactive compound against type 2 diabetes.

Sc–CO_2 extract of *T. foenum graecum* L. is screened for the first time to discover its pharmaceutical characteristics according to the best of our knowledge. Antimicrobial activities are prevalent and significant methods in diagnosing and dominant the mischievous microorganisms during the therapy of infectious sicknesses and in food deterioration. The active constituents of natural plants extracts with anti-microbial activities usually intervene with growth and metabolism of microorganisms and prohibit them from contamination [34]. Gram negative organisms are found to be resistant to majority of the medicines and natural agents. The reason behind that refers to their outer membranes which act as selective barriers for the crossing of molecules in and out of the cell [35]. This is an effective permeability barrier intercept the penetration of amphipathic compounds and multidrug resistance pumps which rejects throw toxins

across this barrier [36]. However, in the current study the extract inhibits the growth of *E. coli* and *P. aeruginosa* from gram negative bacteria. In addition, the extract exhibits a noticeable anti-fungal activity since it acts more superior than the synthetic drug in the inhibition of the growth of *C. albican*. The extract has low inhibitory effect towards *M. tuberculosis* but a better inhibitory action towards *P. falciparum* strain since the MIC value indicates the ability of Sc–CO_2 extract to act as anti-malarial agent. There are some studies for the anti-biological activities of *T. foenum graecum* L. seeds but majority of them are conducted for extracts obtained by traditional methods. Nandagopal *et al.* [37] extracted the seeds applying different solvents and it was reported that ethanolic extract exhibited more considerable inhibition than chloroform, water, benzene and acetone extracts. The seeds extracts have more inhibitory action against *Klebsiella pneumonia* and *P. aeruginosa*, *E. coli*, *S. aureus* and *Salmonella typhi*. Chalghoumi *et al.* [38] explained that aqueous extract of *T. foenum graecum* L. has not showed any effect towards the growth inhibitory of the pathogenic bacteria. However, the extracts obtained by organic solvents have exhibited low to moderate high effect towards the growth inhibitory. It is reported that *T. foenum graecum* L. seeds crude extracts may possess anti-bacterial activity against *E. coli* depending on the extraction solvent. It is in agree with the result obtained in the current scenario, which confirms the availability of different bioactive compounds in high concentration in Sc–CO_2 extract of *T. foenum graecum* L. seeds. These compounds may be responsible of the pharmacological effects of the extract and enable it to play a major role as biotherapeutic agent and alternative natural drug to treat the health sicknesses caused by *C. albican*.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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