Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(12):14-18
(http://derpharmachemica.com/archive.html)

Chemical Compositions and Antibacterial Activity of Different Extracts of *Tribulus terrestris* growing in Morocco and Yemen

A. Al Maofari[1,2], Z. Mennane[4], A. Hakiki[4], M. Mosaddak[3] and S. EL Hajjaji[1]

[1]*Laboratory of Spectroscopy, Molecular Modeling, Materials and Environment, Faculty of Sciences, University Mohamed V, Av Ibn Battouta, BP1014, Rabat 10000, Morocco*
[2]*Laboratory of Physical-chemistry Faculty of Education, Art & Sciences, Amran University, Yemen*
[3]*Laboratory of Naturally Substances, FSR, Rabat, Morocco*
[4]*Laboratory of Medical Bacteriology, INH, Rabat, Morocco*

ABSTRACT

The antibacterial properties of plant extracts have been investigated in order to suggest them as potential tools to overcome the microbial drug resistance and the increasing incidence of foodborne diseases problems. The aim of this research is to study antibacterial activity of ethanol and hexane extracts from Tribulus Terrestris growing in Rabat of Morocco and Sa'adh of Yemen against ten pathogens bacteria. Soxhlet extraction technique was used to extract the organic extracts, using ethanol and hexane as solvent extract. Obtained extracts were analyzed by Gas chromatography (GC) and Gas chromatography-mass spectroscopy (GC-MS). GC and GC/MS results showed that oleic acid and stearic acid are major compounds of Tribulus Terrestris.

Keywords: *aromatic plant, natural extract, antibacterial, food-borne diseases, Tribulus Terrestris*

INTRODUCTION

The development of drug resistance as well as the appearance of side effects of certain antibiotics has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages[1,2]. Thus, the food industry at present uses chemical preservatives to prevent the growth of food borne and spoiling microbes and to extend the life of foods. Mainly due to undesirable effects such as toxicity and carcinogenicity of synthetic additives, interest has considerably increased for finding naturally occurring antimicrobial compounds suitable for use in food[3-9]. With antimicrobial studies, the chemical composition should ideally be used to correlate any structure activity relationships[10].

Tribulus Terrestris is a natural herb used for treating many diseases like hypertension[11]. It is a member of the Zygophyllaceae family, and an annual herb found in many tropical and moderate areas of the world, including the U.S. and Mexico, the Mediterranean region, and throughout Asia[12]. *Tribulus Terrestris*, is also known as Puncture Vine, It contains steroidal saponins, and act as a natural testosterone enhancer. *Tribulus Terrestris* increases testosterone through increasing lutenizing hormone (LH). There is good confidence that *Tribulus Terrestris*is useful as a sexual enhancement herb[13]. In middle east *Tribulus Terrestris*is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, lithon-triptic and urinary antiinfectives[14-16].

In this paper we report the chemical compositions and the antibacterial activity of ethanol, hexane and water extracts of Yemen and Morocco herb *Tribulus Terrestris.*

MATERIALS AND METHODS

2.1. Plant material

Tribulus Terrestris was collected from sa'adh, in the North of Yemen, and Rabat, capital city of Morocco, in December 2010. *Tribulus Terrestris* samples were air-dried at room temperature for 15 days and then used for soxhlet extraction

2.2. Preparation of the *Tribulus Terrestris* extracts

2.3.1. Water extract

Firstly, the powdered Tribulus Terrestris (50g) was extracted using boiling water (250 mL) for 30 min, then the decocted was filtered, finally, freeze-dried[17].

2.3.2. Organic extracts

Both hexane and ethanol extracts were obtained by classical Soxhlet extraction of 100 g of aerial parts during8 h. About 700 mL of each solvent was used. These two organic extracts, corresponding to two different polarity components, were concentrated to dryness and finally the extracts were kept at 4° C [17, 18].

2.4. Gas Chromatography–Mass Spectrometry (GC–MS).

About 10 µL of sonicated anise extract in mixture with methanol, chloroform and n-hexane was analyzed by GC–MS using Ultra Trace GC (Themo-Fisher Scientific) equipped with i) a polaris Q Themo-Fisher Scientific as mass spectra detector ii) a VP-5 capillary fused silica column (30 m, 250 µm, 25 µm film thickness). The oven temperature was held at 60°C for 2 min and then programmed with the rate of 16°C/min to reach 280°C in 20 min. Additional operating conditions are the following: He (99.99 %) as carrier gas, 76 kP as inlet pressure, linear velocity:20cm/s; injector temperature: 220°C; detector temperature: 300 °C and 1:25 as split ratio.

2.5. Antibacterial activity

2.5.1. Bacterial strains

Evaluation of antibacterial activity was carried out using isolated standard Gram- positive and Gram-negative strains as indicated in Table1. The microorganisms were storied on Muellere Hinton Agar (Bio-Rad) at 4 °C and were obtained from the culture collection of the Laboratory of Medical Bacteriology, INH, Rabat, Morocco. The nutrient broth (Bio-Rad) and the Muellere Hinton agar were used, respectively, for growing and diluting the microorganism suspensions for the antimicrobial assays.

Table 1: Bacterial strains used

Bacterial groups	Bacterial strains tested	Origin
Cocci Gram-positive	*Streptococcus pyogenes*	urinary infection
	Streptococcus sanguinis	skin infection
	Staphylococcus epidermidis	urinary infection
	Methicillin-resistant Staphylococcus aureus (MRSA)	Nosocomial infection
Bacill Gram-negative	*Pseudomonas aeruginosa*	urinary infection
	Acinetobacter baumannii	Nosocomial infection
	Salmonella enteritidis	foodborne illess
	Hafnia alvei	urinary infection
	Yersinia enterocolitica	foodborne illess
	Escherichia coli	infection vogiales

2.5.2. Extract dissolution

The water and organic extracts were dissolved in water, DMSO (30%) for 0.1g/mL of initial concentration was obtained.

2.5.3. Antibacterial assay

Antibacterial activities of different extracts obtained from plant were assessed both using the agar well diffusion method,[19]and were prepared in the plates with the help of a cork-borer (0.6 cm). Finally, 50 µL of the tested compound were introduced into the well, and then the plates were incubated for 24h at 37 °C. The diameter of the visible zone showed the absence of growth. For each bacterial strain studied, controls were maintained. The result was obtained by measuring the zone diameter and experiments were done three times. The mean values are presented.

2.5.4. Determination of minimum inhibitory concentration (MIC)

The determination of MIC of the CFS against microbial strains pathogen is performed according to the technique in microtiter plates, showed in Table 4[20].

RESULTS AND DISCUSSION

3.1. Extraction yield

The Tribulus Terrestris extracts with different solvents showed the highest extraction efficiency with water, and then with ethanol. As described in Table 2, the hexane extract was present at low concentration. The highest extraction efficiency was obtained in polar solvents and consequently possessed polar constituents of the Tribulus Terrestris.

Table 2: Yield of extracts from *Tribulus Terrestris* collected from sa'adh and Rabat in Yemen and Morocco, respectively.-

Extracted fractions	Yield (%)
Yemen water extract	10,66
Moroccan water extract	9,66
Yemen ethanol extract	7,59
Morocco ethanolextract	7,15
Yemen hexaneextract	3,27
Moroccan hexaneextract	3,05

3.1 Extract analysis

For the investigation of the extracts of *Tribulus Terrestris*, the extracts were obtained by soxlhet technique. It had a green color, odor at room temperature and its output to the dry plant material was found to be 3,27%,7,59% and 10,66% for hexane , ethanol and water extracts of *Tribulus Terrestris* respectively. The composition of the ethanol and hexane were determined by gas chromatography-mass spectrometry on the basis of the GC retention times as summarized in Tables 3 and 4. The structures of the major compounds are presented in Figure 1.

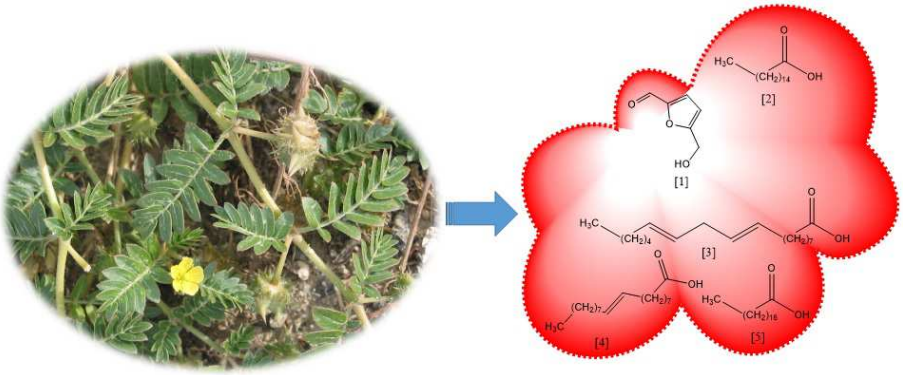

Figure 1: Chemical structures of Hydroxy methyl furfural(1), palmitic acid(2), linoleic Acid(3), oleic acid(4)stearic acid(5)

Table 3. Chemical composition of Tribulus Terrestris. Ethanolic extract

Compound	**Amount**% M	RT(min)
Hydroxy methyl furfural	trace	8.81
palmitic acid	13.43	12.23
linoleic Acid	46.27	14.33
oleic acid	31.34	18.53
stearic acid	5.97	21.34

Table 4. Chemical composition of Tribulus Terrestris. hexane extract

Compound	**Amount**% M	RT(min)
Thujone	trace	8.96
Hydroxy methyl furfural	58.69	13,78
palmitic acid	4.85	29,21
linoleic Acid	3.56	32,41
oleic acid	29.20	32,62
stearic acid	1.07	33,55

3.3. The Antibacterial activity of *T. Terrestris* extracts

The antibacterial activities of the plant extracts were evaluated by measuring the inhibition zone observed around the tested materials. The inhibition zone, measured in millimeters, including the diameter of the well, was used as the criteria of the antibacterial potency. The most active extracts included the ethanol plants extract. The second most active extract against the same bacteria is the hexane extract, therefore, the water extract showed no antibacterial activity against the tested bacterial strains(Table 5).

Table 5: Antibacterial activity and minimum inhibitory concentrations (MIC) of essential oil and organic extracts derived from *anise* seeds against ten pathogens bacteria.

Microorganism	*The organic extracts*									*Antibiotic (Reference)*		
	Ethanol			*Hexane*			*Water*					
	MIC	*ID*		*MIC*	*ID*		*MIC*	*ID*		*ID*		
		M	Y		M	Y		M	Y			
Streptococcus pyogenes	100	10	10	100	9	8	-	-	0	24	S	*Oxacilline 1µg*
Streptococcus sanguinis	100	12	10	-	0	0	-	-	0	23	S	
Staphylococcus epidermidis	100	11	10	100	10	9	-	-	0	30	S	
Methicillin-resistant Staphylococcus aureus (MRSA)	100	12	11	100	10	9	-	-	0	17	R	
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	0	0	R	
Acinetobacter baumannii	100	11	11	100	9	9	-	-	0	29	S	*Ampicilline 25µg*
Salmonella enteritidis	-	-	-	-	-	-	-	-	0	18	I	
Hafnia alvei	-	-	-	-	-	-	-	-	0	18	I	
Yersinia enterocolitica	100	9	8	-	-	-	-	-	0	15	R	
E.coli	-	-	-	-	-	-	-	-	0	14	R	

MIC = Minimum inhibitory concentrations (mg/mL) ; ID =Inhibitory Diameter (mm) ; M,Y = Morocco , Yemen respectively ; R= resistance; S = sensible ; I = intermediary

Under the same experimental conditions, 50µL of all extracts (initially at 0.1 g/mL), *Salmonella enteritidis, Hafnia alveie, Pseudomonas aeruginosa, E.coli* displayed strong resistance against all the tested extracts, while *Staphylococcus aureus* was the most sensitive bacterium.

These data indicate that Gram-positive bacteria are the most sensitive strains for the different extracts**,** which exhibited their main antibacterial activity on Gram-negative bacteria [21]. Our results are in good agreement with previous works [22-24]showing a weaker activity of essential oil of Anis and Salvia tomentosa.

3.4. Minimum inhibitory concentrations (MIC).

As shown in Table 4, the MIC values of Ethanol extracts exhibited strong antibacterial effect as compared to the hexane extract. Hexane fraction extracts exhibited mild to moderate antibacterial effects, with MIC values 100mg/mL. No antibacterial effect of the water extract was observed.

CONCLUSION

Qualitative and the quantitative analysis of different extracts showed the presence of five compounds inthe whole *Tribulus Terrestris* extracts. The major components in the extracts of both *Tribulus Terrestris* of Yemen and Morocco areacid oleic and acid stearic. The bacterial activity of different extracts against ten strains of bacteria were performed using the agar well diffusion technique and showed that the *Tribulus Terrestris* extracts displayed a good antibacterial activities. These biological activities differaccording to the extract types and the tested bacteria strains. The first interesting results reported in this paper show the antibacterial activities against Gram-positive and Gram-negative strains of different fractions extracts of *Tribulus Terrestris*, and open a new interesting approach to develop plants as natural condiment and preservative for the food industry.

REFERENCES

[1] Lewis, K., & Ausubel, F. M. (**2006**). *Nature Biotechnology*, 24, 1504–1507.
[2] G. Milhau, A. Valentin, F. Benoit, M. Mallie, J. Bastide, Y. Pelissier, J. Bessiere. *Journal of Essential Oil Research* **1997**, 9, 329.
[3] Feng, W., & Zheng, X. (**2007**). *Food Control*, 18(9), 1126–1130.
[4] A. Shokri, T. Hatami, M. Khamforoushb. *J. of Supercritical Fluids*. **2011**, 58 (issue 1), 49.
[5] S. Burt. *International Journal of Food Microbiology*, **2004**, 94, 223.
[6] W. Peschel, F. Sanchez-Rabaneda, W. Dieckmann, A. Plescher, I. Gartzia, D. Jimenezet R. Lamuela-Ravento, S. Buxaderas, C. Codina.*Food Chemistry*. **2006**, 97, 137.
[7] A. Smith-Palmer, J. Stewart, & L. Fyfe. *Food Microbiology*, **2001**, 18, 463.
[8] M. Bendahou, A. Muselli, M. Grignon-Dubois, M. Benyoucef, Jean-Marie Desjobert, Antoine-François Bernardini, Jean Costa. *Food Chemistry*. **2008,** 106, 132.
[9] I.M. Bakri, C.W.I. Douglas. *Arch. Oral Biol*. **2005**, 50, 645.
[10] Vivek K. Bajpai, Sharif M. Al-Reza, Ung Kyu Choi, Jong Hwi Lee, Sun Chul Kang. *Food and Chemical Toxicology*. **2009**, 47, 1876.
[11] R. A. Mothana, U. Lindequist.*J. Ethnopharmacol*., **2005**, 96(1- 2):177–181.
[12] K, Abeywickrama, G. A. Bean. *Mycopa-thologia*., **1991**, 113: 187–190.

[13] K. Gauthaman, A. P. Ganesan, R. N. Prasad. *Journal of Alternative and Complementary Medicine.*, **2003**, 9 (2): 257–265.
[14] S. H. Majeed, M. J. Mahmood. 1st Ed. Baghdad: Dar Al-Thaowra for Publishing., **1988**, p. 40. (in Arabic).
[15] S. Aldein. Medicinal Herbs. 1st Ed. Baghdad: Dar Al-Shoun AlThaqafia Al-Aama for Publishing.**1986**, p. 70. (in Arabic)
[16] N.A. Awadh Ali, W.-D. Ju¨lich, C. Kusnick, U. Lindequist. *Journal of Ethnopharmacology* 74 (**2001**) 173–179
[17] N. Hayder, A. Abdelwahed, S. Kilani, R. Ben Ammar, A. Mahmoud, K. Ghedira, L.C. Ghedira, Mutat. *Res./Genet. Toxicol. Environ. Mutagen*. **2004**, 564 (1), 89.
[18] H. Najjaa, M. Neffati, S. Zouari, E. Ammar. C. R. *Chimie*, **2007,** 10, 820.
[19] C. Perez, M. Paul, P. Bazerque. *Acta. Bio. Med. Exp*. **1990,** 15, 113.
[20] J. N. Eloff. *Plant Medical*. **1998**, 64, 711.
[21] I.M. Bakri, C.W.I. Douglas. *Archives of Oral Biology*, **2005,** 50, 645.
[22] B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou.*Food Chemistry*,**2005,** 90, 333.
[23] A. Al Maofari, S. El Hajjaji, S.Zaydoun, B. Ouaki, R. Charof, Z. Mennane, A. Hakiki, M. Mosaddak. *International Journal of Engineering & Technology* IJET-IJENS Vol:1 5 No:01
[24] A. Al Maofari, S. El Hajjaji, A. Debbab, S.Zaydoun1, B. Ouaki,R. Charof, Z. Mennane, A. Hakiki, M. Mosaddak. *Scientific Study & Research*. **2013**, 14 (1), pp. 011 – 016