Biological activities of *Juniperus phoenicea* essential oil and impact on *in vitro* ruminal fermentation in sheep

SELMI HOUCINE[1]*, DHIFALLAH AMENI1, JEDIDI SABER[1], BAHRI AMANI[2], ABBES CHAABANE[1] AND ROUISSI HAMADI[2]

[1] Laboratory of Sylvo-Pastoral Resources, University of Jendouba, Sylvo-Pastoral Institute of Tabarka, Tunisia
[2] Higher School of Agriculture of Mateur, University of Carthage, Tunisia

SUMMARY

Juniperus phoenicea L. is a medicinal plant belonging to the Cupressaceae family. The present study was conducted to evaluate the *in vitro* antioxidant and antimicrobial activities of *Juniperus phoenicea* essential oils (JPEO) and its effects on fermentation kinetics in sheeps. Our result firstly indicated that the use of GC-MS allowed to the identification of 48 compounds in JPEO and the principal compound is α-pinene. JPEO is characterized by an excellent antioxidant (IC_{50} = 93.23 µg/mL) against the DPPH radical, but still lower when compared to that of ascorbic acid (IC_{50} = 61.30 µg/mL), used as reference antioxidant molecule. Importantly, the JPEO showed a significant effect against the entire tested bacterial flora and the highest zone of inhibition was found against *Bacillus subtilis* (zone of inhibition 2.85 ± 0.02 mm). A variation in the antimicrobial properties of JPEO according to the microorganism species was observed. Indeed, the zone of inhibition showed a great effect against Gram+ strains compared to the Gram-. Interestingly, the different doses of JPEO with sheep ruminal fluid exert a significant effect, but in a way that results in inhibiting ruminal gas production (GP) after 24 h incubation, and therefore the volatile fatty acids (AGV), organic matter digestibility (OMD), and metabolizable energy (ME) significantly decreased with increasing essential oil level. We concluded that the incorporation of essential oils in the ration did not improve the digestibility parameters of the sheep.

KEY WORDS

Juniperus phoenicea, essential oils, antioxidant capacity, antibacterial activity, digestibility.

INTRODUCTION

Improving digestibility, ruminal fermentation parameters as well as the production performance levels of farm animals requires an upgrade of the quality of feed for the latter [1]. Indeed, rations traditionally composed of a single raw material do not meet the needs of the herd [2]. This mismatch between inputs and needs significantly influences production levels. On the other hand, the uses of feed additives, antibiotics and synthetic antioxidants have adverse effects on animal health and the microbial ecosystem in the rumen [3]. Moreover, their uses were prohibited in the European Union (Regulation 1831/2003 / EC). This has led to the reappearance of pathogens responsible for diseases and losses [4] or by the quantity and quality of Nitrate released into the environment (Nitrate Directive, 91/676 / EU) and gas emissions [5].

So we resort to the search for other natural antioxidants to improve ruminal conditions and decrease the production of greenhouse gases in the first place and consequently the growth of the productivity of farm animals.

Historically, humans have used essential oils extracted from medicinal and aromatic plants daily for perfume, cooking and healing. The current craze for essential oils is not just scientific. Essential oils are therefore gaining ground in many fields: cosmetics, agrifood, and well-being and of course health and animal feed.

Several studies on the use of essential oils in animal nutrition and their impacts on products of animal origin have been set up [6,7] while their effects on ruminal fermentation and greenhouse gas emissions remain negligible.

Juniperus phoenica (Cupressaceae family) is a shrub or tree that grows in the northern hemisphere and has a typical Mediterranean distribution [8]. The Phytochemical investigation of this plant revealed a richness in essential oil, carbohydrates, glycosides, sterols, terpenes, and flavonoids [9,10]. Due to its richness in phenolic compounds *J. phoenicea* is characterized by a significant diuretic action as its use in folk medicine [11], antibacterial and antidiabetic properties [9, 12].

Hence, the current investigation aimed to evaluate the antioxidant and antibacterial capacities of *J. phoenicea* essential oils as well as their effects on *in vitro* ruminal fermentation of oat hay and gas production in sheep.

MATERIAL AND METHODS

Plant collection

The aerial part of *Juniperus phoenicea* was sampled in February 2020 from the region of Tabarka (North West Tunisia) characterized by a Pluvio thermal Quotient of Emberger (Q2 = 2000P / $M^2 - m^2$) of the order of 158.8 with an altitude 108

Corresponding Author:
Houcine Selmi (houcine_selmi@live.fr).

m, a longitude w = 36 * 55'48 .4 and a latitude E = 008 * 48 '04.5.

Essential oil extraction

The extraction of essential oil is carried out according to the method of distillation with the training in water vapor for 3 hours at a temperature of 90 °C. The plant material was immersed in water and heated to a boil, according to the method recommended in the European Pharmacopoeia [13]. After which the essential oils were evaporated with water vapor and finally collected in a condenser and stored in a dark glass bottle (sealed brown vials) at 4 °C until chemical analysis [14].

Gas chromatography-mass spectrometry (GC - MS)

Juniperus phoenicea essential oils (JPEO) were subjected to GC-MS analysis using Trace GC ULTRA / Polaris Q (GC - MS, Thermo Electron). The column was a VB-5 (5% phenyl / 95% dimethylpolysiloxane) with film thickness of 0.25 m, a length of 30 m and an internal diameter of 0.25 m was used with helium as carrier gas. The GC oven temperature was kept at 50 C for 5 min and programmed to 250 C for 3 min at rate of 4 ° C / min and programmed to 300 C at rate of 25 C / min. The injector temperature was set at 250 C. Split flow was adjusted at 50 mL / min. MS were taken at 70 eV. Mass range was from m 20-350. A library search was carried out using the "Wiley GC / MS Library", Nist and Pmw. The sample was dissolved in Hexane.

Identification of volatile compounds in JPEO

The volatile compounds of essential oils have been identified by calculating their retention index (IR) from a range of linear alkanes (C8- C25) injected into the same analytical conditions [15]. The calculation of the indices of retention of volatile compounds is given by the following formula:

$$\mathbf{IR = [n + (TRi- TRn) / (TR (n + 1) - TRn)] * 100}$$

IR: unidentified peak retention index
n: number of carbon atoms of the aliphatic hydrocarbon eluted just before the peak at identify
TRi: retention time of unidentified peak.
TRn: retention time of the eluted aliphatic hydrocarbon just before the peak to be identified
TRn + 1: retention time of the eluted aliphatic hydrocarbon just after the peak to be identified.

DPPH radical-scavenging

The antioxidant capacity of JPEO was performed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Briefly, various concentrations of JPEO (10, 20, 50, 75, 150, 200 250 and 300 µg/mL) were added to 1 mL of methanol solution of DPPH (0.1 mM) and incubated at 27 °C for 30 min [16]. The absorbance of the sample was measured at 517 nm. DPPH radical-scavenging activity (RSA), expressed as percentage was calculated using the following formula:

$$RSA\ (\%) = A_{DPPH} - (A_{sample} - A_{control}) / A_{DPPH} \times 100.$$

Antibacterial activity

Bacterial strains and growing conditions

Antibacterial activity of JPEO was tested according to [17] against certain bacterial strains provided by the microbiology laboratory of the Sylvo-pastoral institute of Tabarka. These strains are composed of two Gram-positive bacteria *Listeria monocytogenes* (foodstuff 2132) and *Bacillus subtilis* ATCC 6633 and two Gram-negative bacteria *Salmonella enterica* (foodstuff) and *Escherichia coli* ATCC 25922.
The pre-cultures of the strains are composed of 20 mL of liquid NB (Nutrim Broth) and 100 L of the bacterial strain, the mixture is incubated at 37 °C. overnight in a water bath with shaking.

Preparation of culture medium

The culture medium used is NB (Nutrient Broth). To prepare the solid medium, 25 g of powder NB and 15 g of agar were dissolved in 1 L of distilled water. Once the medium is well stirred, it is autoclaved at 121 °C. for 1 h. Finally, 20 mL of the mixture was put in each Petri dish (diameter = 90 cm) [17].

Well method preparation

This method has been described by Guven [17]. It is based on the solid medium diffusion technique, which consists in inoculating the bacteria at a rate of 100 L/dish from a pre-culture prepared. Let stand for 2 hours at room temperature. Then, 6 mm diameter wells are dug using a sterile Pasteur pipette and are filled with 60 L of the corresponding essential oil and two control wells, one positive (the antibiotic: Gentamicin) and the other negative (ethanol). The petri dishes thus prepared are incubated at 4 ° C for 3 to 4 hours to allow the diffusion of the essential oils present in the wells. Lastly, the dishes will be incubated in an oven at 37 ° C for 48 hours. The Antibacterial activity of the essential oil is measured in terms of the diameter of the zone of inhibition that surrounds the wells using a caliper.

Ruminal fermentation and kinetics of gas production

The rumen content is then homogenized and filtered to remove the solid phase. The contents of the flask were emptied into an industrial mixer and purged simultaneously with CO_2 to maintain anaerobic conditions [18]. After mixing, the rumen fluid was transferred to a 100 ml glass syringe. In each syringe we mixed: 10 mL of filtered rumen juice, 20 mL of artificial saliva and 300 mg of crushed substrate (3 replicates per sample). Then the syringe were stored in a water bath at 39 °C, purged with CO_2 and continued as recommended by Goering and Van Soest [19]. The digestibility of organic matter (DOM) is calculated using the formula proposed by Menke and Steingass [20]. The metabolizable energy (ME) content as well as the volatile fatty acids produced, was calculated according to the method of Makkar [21].

$$DOM = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651\ MM$$
$$ME\ (MJ / kg\ DM) = 2.20 + 0.136GP + 0.057CP$$
$$VFA\ (mmol / syringe) = 0.0239GP - 0.0601$$

Statistical analysis

The results of the effects of doses of *Juniperus phoenicea* EO on the measured parameters (antiradical activity, anti microbial and ruminal fermentation parameters) were subjected to analysis of variance according to the procedure GLM of SAS [22] and compared by the multiple range test of Duncan [23]. The characteristic parameters of the gas production kinetics were predicted according to the non-linear regression model of Orskov and McDonald [24]: $Y = a + b * (1-e^{-ct})$.

Table 1 - Phytochemical composition of *Juniperus phoenicea* essential oils (JPEO): 48 compounds were identified in the essential oils as a result of Gas Chromatography-Mass Spectrometry Analysis. RT: Retention time.

Pic	Components	Laughed	Compositions No. (%)
1	Androst-4-en-3-one	708	0.215
2	Hexadecanoic acid	717	0.587
3	δ-Cadinene	727	2.944
4	Podocarp-7-en-3-one	730	0.289
5	Caryophyllene oxide	732	0.106
6	β-Elemene	733	2,152
7	1,5,5-Trimethyl-6-methylene	745	4,702
8	Androstan-3-ol	750	0.016
9	Retinol	753	0.370
10	Octadecanoic acid	757	0.248
11	Ledene alcohol	758	0.237
12	Bornyl chloride	759	0.123
13	Aristolen epoxide	768	0.245
14	1-Naphthalenol	771	0.885
15	Terpinolene	773	4.227
16	Tricyclene	776	0.357
17	Longifolene- (V4)	783	2,880
18	Eucayptol	787	0.403
19	τ-Muurolol	791	0.805
20	Murolan	792	0.119
21	Himachala-2,4-diene	796	0.141
22	Cyclohexane	802	3.332
23	Humulan-1,6-dien-3-ol	803	0.373
24	δ- Silienne	805	0.010
25	2,3-Dihydroxydroxypropyl elaidate	808	0.411
27	p-Cymene	814	2.001
26	Cubenol	816	0.486
28	2-Cyclohexen-1-ol	825	0.151
29	Bornyl acetate	827	0.131
30	D-Limonene	828	0.440
31	1R, 4S, 7S, 11R-2,2,4,8-Tetrame	831	3,791
32	Santolinatriene	836	0.265
33	Isopulegol acetate	842	0.337
34	Bicyclosesquiphellandrene	845	1,770
35	Cyclohexene	846	1,024
36	1,3,8-p-Menthatriene	847	0.039
37	Azulene	849	3.366
38	α-Pinene	851	20,245
39	Camphene	852	0.256
40	Ylangene	860	0.021
41	Myrcene	861	3,253
42	α-Cubebene	862	0.253
43	trans-linalool oxide	865	3,727
44	Copaene	870	0.973
45	β-Pinene	880	5.683
46	Naphthalene	885	6,659
47	3-Carene	887	0.091
48	Thujopsene	896	0.330
	Total identified		**99.86**

RESULT AND DISCUSSION

Chemical composition of Juniperus phoenicea essential oils

The chemical composition of *Juniperus phoenicea* essential oils (JPEO), reported in the Table 1, allowed to the identification of 48 components and the α-pinene is the major compound. Indeed, this oil is characterized by a high level of a hydrocarbon monoterpene (α-pinene). The study of the chemical composition of the oil from this same plant, but from other origins, has been the subject of several researches works [25]. The results obtained for the various works show that this oil is formed mainly of α-pinene. However, in the hydrodistillation from dried leaves showed that the germacrene D (12.6 %), (E)-caryophyllene (7.2 %) are the major component [9]. The other compounds differ from one region to another and even from one country to another and this variation are linked to pedoclimatic, environmental and genetic parameters [26].

Antioxidant activity of Juniperus phoenicea essential oils

The antioxidant activity of JPEO was tested by the radical method using a spectrophotometer. Result obtained is presented in the form of a straight line whose equation is presented in Table 2 and Figure 1. The JPEO is endowed with an important antioxidant activity. It is almost balanced with the antioxidant activity of ascorbic acid used as a reference molecule.

So the JPEO can be used as a natural antioxidant and can even replace other synthetic antioxidants that have adverse effects. The JPEO has antioxidant activity which may be related to its chemical composition. It is difficult to attribute this activity to a single compound since a synergistic effect between the different compounds can take place. Our results are in line with those of Meddini et al [27]. In addition, Mansouri et al [28] have shown that the leaves methanolic extract of this plant is endowed with a low antioxidant activity (IC_{50} = 12.57 µg/ mL) compared to essential oils.

Antibacterial activity of Juniperus phoenicea essential oils

The results recorded in Table 3 show that JPEO exhibits significant antimicrobial activity against the four strains tested despite their morphology and their Gram. This activity depends on the essential oil (p<0.0001) and the strain (p <0.0001). The results show that the Gram negative bacteria are more resistant than the Gram positive bacteria. Indeed, *Escherichia coli* were inhibited from the 30µl concentration, *Bacillus*is inhibited from 10 µl. Gram positive bacteria have been shown to be more sensitive than Gram negative. This result corroborates with that found by Bouzouita et al [25]. This sensitivity of Gram positive bacteria to essential oils is linked in particular to the nature of the membrane of the bacterium which is hydrophobic lipopolysaccharide, which causes a destabilization of the

Table 2 - IC_{50} values of the DPPH radical inhibition of *Juniperus phoenicea* essential oils (JPEO) and ascorbic acid.

	IC_{50} (µg/ml)
Juniperus phoenicea essential oils	93.23^{a} ± 2.41
Ascorbic acid	61.30^{b} ± 0
P> F	0.0022

Table 3 - Inhibition zone diameter (IZD) of *Juniperus phoenicea* essential oils (JPEO) against four reference strains.

Doses(µl)	*Escherichia coli*	*Salmonella enterica*	*Listeria monocytogenes*	*Bacillus subtilis*
100	$1.7^{d} \pm 0.12$	$2.17^{c} \pm 0.03$	$2.32^{b} \pm 0.45$	$2.85^{a} \pm 0.02$
80	$1.3^{c} \pm 0.14$	$2.05^{b} \pm 0.21$	$2.07^{b} \pm 0.67$	$2.2^{a} \pm 0.01$
60	$1.07^{d} \pm 0.07$	$1.95^{b} \pm 0.77$	$1.77^{c} \pm 0.17$	$2.15^{a} \pm 0.11$
30	$0.95^{d} \pm 0.17$	$1.77^{b} \pm 0.035$	$1.6^{c} \pm 0.24$	$2.1^{a} \pm 0.07$
10	$0.75^{d} \pm 0.08$	$1.6^{b} \pm 0.17$	$1.32^{c} \pm 0.88$	$1.75^{a} \pm 0.11$
Gentamicin	2.64 ± 0.007	3.64 ± 0.098	3.67 ± 0.15	4.08 ± 0.25
Bacteria effect			<0.0001	
Dose effect			<0.0001	

a, b,c and d: The means in the same column, for the same test and bearing different letters are significantly different (α=0.01)

structure and an increase in membrane permeability [29]. These changes lead to the leakage of ions and intracellular compounds [30,31].

This antimicrobial activity observed for essential oils *Juniperus phoenicea* can be attributed to their predominant compounds. It has been reported that α-pinene, which is the major compound of *Juniperus oxycedrus*, exhibits several biological activities, it is antibacterial, anti-inflammatory, antiviral, expectorant, sedative, herbicide and insect repellent [32,28]. This suggests that it seems to be the determining element of the activity observed against the microorganisms tested in this study.

Ruminal fermentation and kinetics of gas production

Kinetics of gas production

The effect of *Juniperus phoenicea* essential oil on the kinetics of gas production was totally in contradiction with what is mentioned in the bibliography. Indeed, the doses of the latter exert a significant effect, but in a way, which leads to inhibit the production of ruminal, gas, by the fact that the values of control (C0) are the largest compared to the others in the three tests (Table 4). It can be seen from the figure that gas production is rapid, increasing but from 24 hours the fermentation rate becomes more stable, hence obtaining a plateau.

The curve of C0 is the largest in value and the fastest to reach its stability, followed by the dose of essential oil at the same rate but in value lower than that of C0.

Gas production after 24 hours of incubation was significantly reduced. These results are in agreement with those obtained by Arhab et al [33] with the essential oil of *Juniperus phoenicea*. The low gas production of the ration incubated with different levels of EO is thought to be due to the antimicrobial activity of the EO compounds, according to Derwich et al [34], the components can act individually or synergistically to selectively inhibit on the activity of microorganisms and limit fermentation.

Prediction of the digestibility of Organic Matter (d MO), the production of Volatile Fatty Acids (VFA), and Metabolizable Energy (ME)

An effect is observed at the levels of the decrease in VFAs, MO

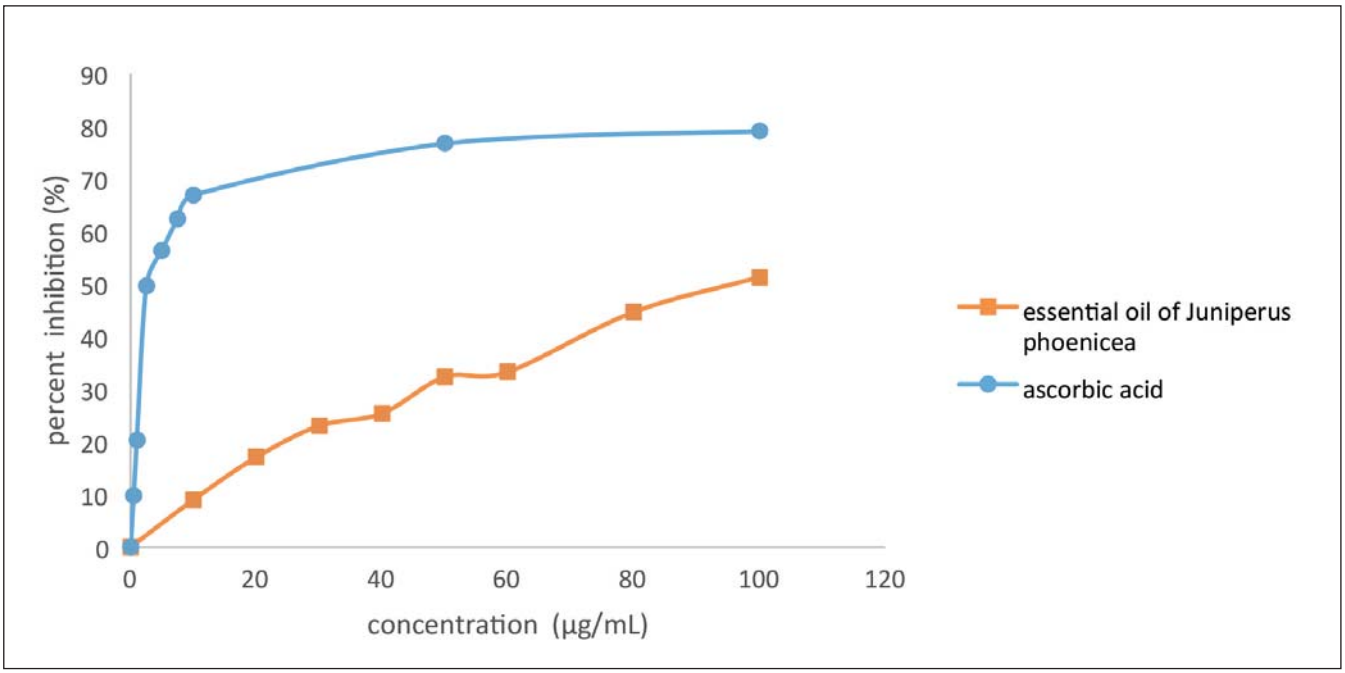

Figure 1 - A Free radical-scavenging activity of *Juniperus phoenicea* essential oils (JPEO) and ascorbic acid on 2,2-diphenyl-1- picrylhydrazyl (DPPH).

Table 4 - Gas production kinetics according to different doses.

Doses(µl)	a	b	c
C_0	0.3597 ± 0.001	35.466[a] ± 0.256	0.0492 ± 0.002
10	-0.092 ± 0.392	20.840 ± 0.248	0.0968 ± 0.002
20	0.7324 ± 0.001	12.920 ± 0.002	0.0977 ± 0
30	2.4911[a] ± 0.001	11.134 ± 0.003	0.0388 ± 0
40	-0.3880 ± 0.001	8.513 ± 0.005	0.3011[a] ± 0
50	-0.0991 ± 0.345	8.594 ± 0.002	0.2504 ± 0.005
P> F	<0.0001	<0.0001	<0.0001

a, b and c: The means in the same column, for the same test and bearing different letters are significantly different.(α=0.01)

Table 5 - Prediction of the digestibility of Organic Matter (d MO), the production of Volatile Fatty Acids (VFA), and Metabolizable Energy (ME) according to different doses.

Doses	d MO (%)	ME (MJ / kg DM)	AGV (mmol / syringe)
C0	38.272[a] ± 8.0379	5.676[a] ± 1.229	0.525[a] ± 0.216
10	31.456[ab] ± 9.863	4.634[ab] ± 1.508	0.342[ab] ± 0.265
20	26.715 ± 7.1535	3.908 ± 1.094	0.215 ± 0.192
30	23.306 ± 3.395	3.386 ± 0.519	0.123 ± 0.091
40	23.158 ± 1.334	3.364 ± 0.204	0.119 ± 0.036
50	23.455 ± 1.796	3.409 ± 0.275	0.127 ± 0.048
P> F	0.0608	0.0608	0.0608

a, b and c: The values assigned the same letter on the same column do not differ significantly; d OM: digestibility of Organic Matter; EM: Metabolisable Energy; AGV: Volatile Fatty Acids

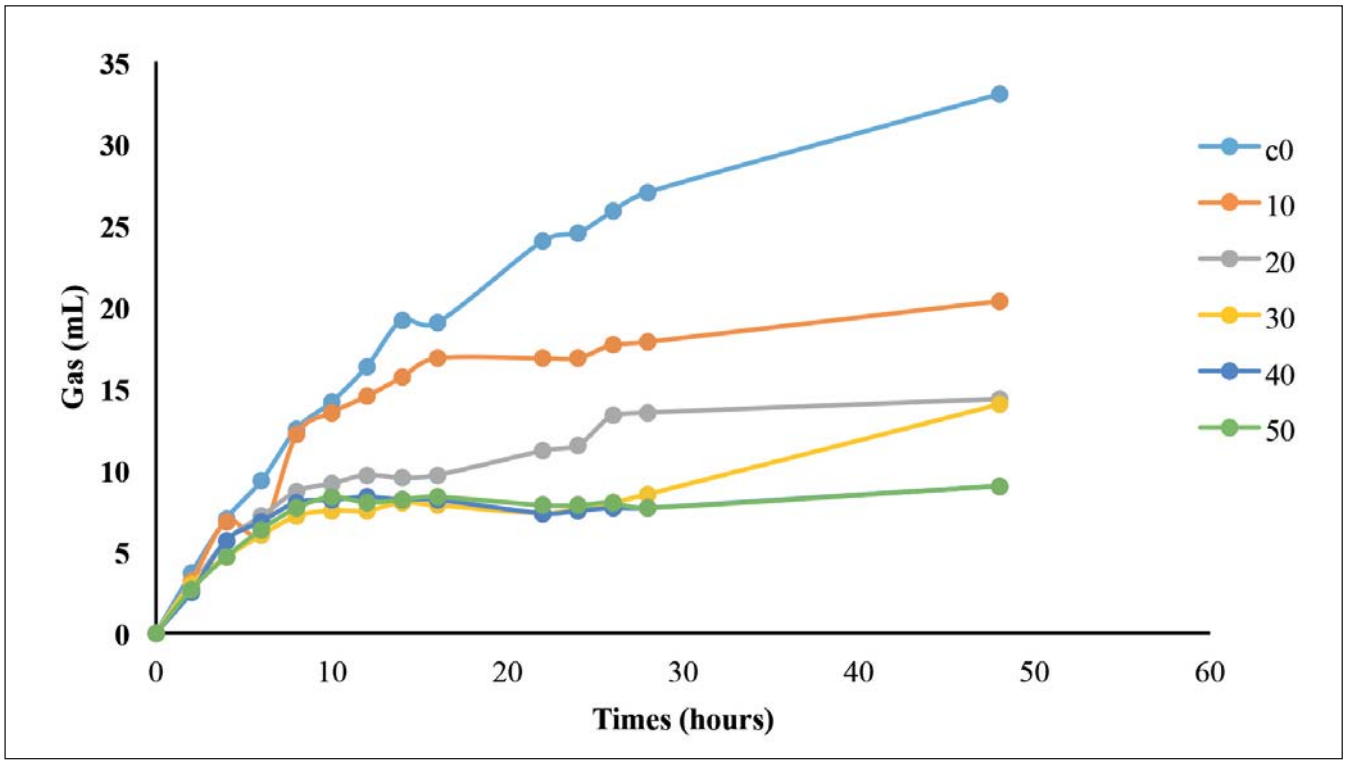

Figure 2 - Kinetics of gas production in sheep according to different doses of *Juniperus phoenicea* essential oils (JPEO).

and EM of control (C0) compared to the different doses of JPEO (Table 5).

These results on AGV production and d MO are in agreement with those of [3] who observed that increasing the dose of essential oil decreased the production of AGV and d MO, this due to the fact that at high concentrations, the compounds of essential oils would cause defaunation, which would be at the origin of the decrease in the fermentation activity of mi-

croorganisms and, consequently, of the decrease in digestibility and the production of AGVs.

CONCLUSION

Our result of chemical composition of *Juniperus phoenicea* essential oils indicated that the α-pinene is the major compound. It has a very interesting antioxidant activity, the IC_{50} of which proves that this property is close to that of the reference molecule, ascorbic acid. Its antibacterial activity on all 4 strains corroborate with other studies which prove that gram negative bacterial strains are more resistant than gram positive ones. In addition, the effect of JPEO has exceeded all expectations that any essential oil improves ruminal fermentation, as this is not the case in this work. We also demonstrate that JPEO inhibited gas production and decreased the ruminal fermentation parameters. So it can be concluded that JPEO is not recommended for ruminants even at low doses.

Acknowledgements

The financial support of the Institution of Agricultural Research and Higher Education IRESA Tunisia is appreciatively acknowledged.

References

1. Selmi H., Hasnaoui M., Bahri A., Abbès C., Dallali D., Jedidi S., Rouissi H. (2018). Chemical properties, antioxidant activities and in vitro fermentation profiles of some shrubs of North Western Tunisia. Indian J. Anim. Research, 54(7): 851-855.
2. McIntosh F. M., Williams P., Losa R., Wallace R. J., Beeverand D. A., Newbold C.J. (2003). Effects of essential oils on ruminal micro-organisms and their protein metabolism. Appl. Environ. Microbial, 69: 5011-5014.
3. Benchaar C., Calsamiglia C., Chaves A. V., Fraser G. R., Colombatto D., Mc Allister T.A., Beauchemin K.A.(2008). A Review of Plant-Derived Essential Oils in Ruminant Nutrition and Production. Anim. Feed Sci. Technol, 145: 209-228
4. Alloui M. N. (2011). Les phytobiotiques comme alternative aux antibiotiques promoteurs de croissance dans l'aliment des Volailles. Livest. Res. Rural. Dev, 23(6): 133
5. Macheboeuf D., Papon Y., Arturo-Schaan M., Mousset J. I., Cherel R. (2006). Use of plant extracts (essential oils and polyphenols extract) to reduce the ruminal degradability of proteins - an in vitro essay. Renc. Rech. Ruminants, 13: 69-72.
6. Smeti S., Hajji H., Mekki I., Mahouachi M., Atti N. (2018). Effects of dose and administration form of rosemary essential oils on meat quality and fatty acid profile of lamb. Small Ruminant Research, 158: 62-68.
7. Selmi H., Bahri A., Ferchichi A., Rouissi H. (2020). Effect of supplementing Moringaoleifera essential oils on milk quality and fatty acid profile in dairy sheep. Indian J. Anim. Research, 54(7): 879-882.
8. Amaral Franco J. (1986). Flora Europea: Juniperus L., Ed. T. G. Tutin, Cambridge University Press, Cambridge, Vol. 1.
9. Medini H., Marzouki H., Chemli R., Marongiu B., Piras A., Porceda S., Tuveri E. (2008). Chemical characterization and evaluation of biological activity of essential oil of *Juniperus phoenicea* of Tunisia. J. E. Oil Bearing Plants, 11(3): 233-241.
10. El-Sawi S. A., Motawae H. M., Sleem M. A., El-Shabrawy A. O., Sleem A., Ismail M. A.(2014). Phytochemical screening, investigation of carbohydrate contents, and antiviral activity of *Juniperus phoenicea* L. growing in Egypt. J. Herbs Spices Med. Plants, 20: 83-91.
11. Salma E. S. A., Hemaia M. M., Mohamed S. A., Abdel-Rahman E.-S. E., Amanis, S. A., Amal, A. M., Maii, I. A. (2011). Investigation of lipoidal matter, antimicrobial and diuretic activities of leaves and fruits of *Juniperus phoenicea* L. growing in Egypt. Aust J. Med Herb. 23(4): 174-179
12. El-Sawi S. A., Motawae H. M., El-Shabrawy A.-R. O., Sleem M. A.-F., Sleem A. A., Abdel Naby, M., Maamoun, I. (2015). Antihyperglycemic effect of *Juniperus phoenicea* L. on alloxan-induced diabetic rats and diterpenoids isolated from the fruits. J. Coastal Life Med, 3(11): 906-909.
13. European Pharmacopoeia, 6th ed., council of Europe, Strasbourg (2008).
14. Hammoudi R., Dehak K., Hadj Mahammed M., Ouldelhadj M. D. (2015). Composition Chimique et activité antioxydante des Huiles Essentielles de *Deverra Scoparia* Coss. et Dur. (Apiaceae). Lebanese Sci. J. 16(2): 27-36.
15. Van Den Dooland H., Kratz, P. D. (1963). A Generalization of the Retention Index System Including Linear Temperature Programmed Gas-Liquid Partition Chromatography. J. Chromato A, 11: 463-471.
16. Ben Ammar R., Bhouri W., Ben Sghaier M., Boubaker J., Skandrani I., Neffati A., Bouhlel I., Kilani S., Mariotte A. M., Chekir-Ghedira L., Dijoux-Franca M. G., Ghedira K. (2009). Antioxidant and free radical-scavenging properties of three flavonoids isolated from the leaves of *Rhamnus alaternus* L. (Rhamnaceae): A structure-activity relationship study. Food Chem, 116: 258-264.
17. Guven D., Dapena A., Kartal B., Schmid M.C., Maas B., Van de Pas-Schoonen K. (2005). Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria. Appl. Environ. Microbiol, 71: 1066-1071.
18. Grant R. J. and Mertens D.R. (1992). Impact of in vitro fermentation techniques upon kinetics of fiber digestion. J. Dairy Sci, 75: 1263-1272.
19. Goering H. K., Van Soest P. J. (1970). Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Application. Agricultural Handbook No. 379, Agricultural Research Service, U.S. Department of Agriculture.
20. Menke K. H., Steingass H. (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev, 28: 9-55.
21. Makkar H. P. S. (2002). Development and Field Evaluation of animal feed supplementation packages. Proceedings of the final review meeting of an IAEA Technical Cooperation Regional AFRA Project, November 25-29, 2000, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Cairo, Egypt: 1-66.
22. SAS, Statistical Analysis Systems Institute, Inc, users guide version 9.0. (2009). Cary, NC, USA.
23. Duncan D.B. (1955). Multiple Range and Multiple F-Test Biometrics. 11: 1-5.
24. Orskov, E. R. and McDonald I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric.Sci. Cambridge. 92: 499-503.
25. Bouzouita N., Kachouri F., Ben Halima M., Chaabouni M. M. (2008). Composition chimique et activité antioxydante, antimicrobienne et insecticide de l'huile essentielle de *Juniperus phoenicea*. Société Chim. Tunisie. 10: 119-125.
26. Dob T., Dahmane D., Chaabane C. (2008). Chemical Composition of the Essential Oil of Juniperus phoenicea L. from Algeria. *J.* Essent. Oil Res, 20: 15-20.
27. Meddini H., Elaissi A., Khouja M. L., Chraief I., Farhat F., Hammami M., Chemli R., and Harzallah-skhiri F. (2010). Leaf Essential Oil of *Juniperus oxycedrus* L. (Cupressaceae) harvested in northern Tunisia: Composition and Intra-Specific Variability. Chem Biodi. **7**: 1254-1266.
28. Mansouri N., Satrani B., Ghanmi M., El ghadraoui L., Aafi A., Farah A. (2010). Valorisation des huiles essentielles de *Juniperus thurifera* et de *Juniperus oxycedrus* du Maroc. Phytothérapie. 8: 166-170.
29. Sikkema J., De Bont J. A. M., Poolman B. (1994). Interactions of cyclic hydrocarbons with biological membranes. *J Biol. Chem*. 269: 8022-8028.
30. Christine F., Carson J., Mee B., Riley T. V. (2002). Mechanism of action of *Melaleuca alternifolia* (Tea Tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother.46(6):1914-1920.
31. Ultee A. M., Bennikand M. H. J., Moezelaar R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Applied environm. Mcrob, 68(4): 1561-1568.
32. Duke, J. A. (1998). Phytochemical Database. USDA-ARS-NGRL (ed),Beltville agricultural research center, Belstville, Maryland, USA.
33. Arhab, R., Khenaka, K., Leulmi, N., Belaidi, H., Harzallah, B., Bousseboua, H. (2013). Effect of essential oils extracted from Saturejacalamintha, Menthapulegiumand Juniperus phoenicea on in vitro methanogenesis and fermentation traits of vetch-oat hay. Afr. J. Environm. Sci. Technol. **7**(4): 140-144.
34. Derwich E., Benziane Z., Boukir A. (2010). Chemical composition of leaf essential oil of Juniperus phoenicea and evaluation of its antibacterial activity. Internat. J. Agricul. Biol, 12(2): 199-204.