

## EFFECT OF THE AQUEOUS EXTRACT OF *EUPHORBIA GUYONIANA* (EUPHORBIACEAE) ON PATHOGENIC BACTERIA FROM LAND-BASED SOURCES

BOUMAZA, S.\* – BOUCHENAK, O. – YAHIAOUI, K. – TOUBAL, S. – EL HADDAD, D. – ARAB, K.

Laboratory of Valorization and Conservation of Biological Resources (VALCOR), Department of Biology, Faculty of Sciences, University of M'hamed Bougara, 3500 Boumerdes, Algeria  
(phone: +213-24-912-951; fax: ++213-24-912-951)

\*Corresponding author

e-mail: sarah.boumaza1@hotmail.fr; phone: +213-66-531-4176; fax: +213-24-951-738

(Received 27<sup>th</sup> Mar 2018; accepted 12<sup>th</sup> Jun 2018)

**Abstract.** *Euphorbia guyoniana* is a medicinal plant endemic to Algeria. It is used by local populations for its medicinal properties. This study was to determine the antibacterial effect of the aqueous extract from *Euphorbia guyoniana* on pathogenic bacteria of telluric origin. The aqueous extract was obtained by confrontation with organic solvents method with a yield of 1.7%. The total content of the flavonoid extract was evaluated by the method of Aluminum Trichloride and was found to be 0.31 mg EQ/gE. The extract obtained was characterized by Infrared Spectroscopy and revealed a richness of phenol, aldehyde and ester then analyzed by High-Performance Liquid Chromatography, which allowed us to identify 27 flavonoid compounds. The extract was tested by the diffusion method on agar, on 12 bacterial strains isolated from a henhouse and identified by VITEK. These strains seem to be sensitive to the flavonoids of *Euphorbia guyoniana* with MICs varying from 1.47 to 61.78 mg/ml. The order of sensitivity of the bacterial strains to the extract is represented in the following order: *Staphylococcus aureus* > *Streptococcus faecalis* > *Escherichia coli*. Thus, the flavonoids of *Euphorbia guyoniana* may be an alternative to chemical control of certain pathogenic microorganisms.

**Keywords:** *Euphorbia guyoniana*, flavonoids, antibacterial activity, HPLC, VITEK

### Introduction

Within microbial communities in the soil, there may be some microorganisms that are pathogens for plants, animals and humans (Raaijmakers, 2009). Soil is a natural habitat that can contain some primary and opportunistic pathogenic bacteria. The rhizosphere may contain certain opportunistic bacteria such as *Burkholderia spp.*, *Ochrobactrum spp.* and *Stenotrophomonas spp.* (Berg et al., 2005). *Pseudomonas aeruginosa* is a highly encountered bacterium in the soil (Colinon et al., 2013). Some primary pathogenic bacteria are natural soil inhabitants, such as *Bacillus cereus* and *Bacillus anthracis*, which can cause serious diseases in humans (food poisoning and pneumonia) (Ticknor et al., 2001; Reis et al., 2014). Other bacteria are highly pathogenic to humans such as *Clostridium botulinum* and *Clostridium tetani* (Smith, 1978, 1979). There are certain categories of bacteria with a saprophyte life in soil such as *Listeria monocytogenes* (Freitag et al., 2009). The importance of these pathogens from land-based sources is growing with the increasing practice of monoculture, mainly in the Mediterranean regions (Tramier, 1986).

Control of pathogens from land-based sources has always been difficult, however the use of biocidal soil disinfection products such as chloropicrin and methyl bromide has been found to be very dangerous for humans and useful organisms' cultures. Fortunately, these products have been definitely banned, which does not solve the problem of the control of diseases of land-based origin. Vector control during epidemics

is achieved through chemical insecticides, but their use continues to give rise to a high level of contamination and ecological imbalance due to the appearance of resistance. This is why the World Health Organization (WHO) insists on the search for new methods of control that are basically biological.

*Euphorbiaceae* contains several families of chemical compounds such as alkaloids (De Nazare et al., 2005), flavonoids, Cyanogenetic compounds (Hunsa et al., 1995), ellagic acid (Mavar et al., 2004) Saponins (Tripathi and Tiwari, 1980) and terpenes (Mazoir et al., 2008). Among the species endemic to Algeria, *Euphorbia guyoniana* had a particular importance in the pharmacopoeia. According to Bellakhdar (1997), it is used by many Saharan populations against poisonous bites and stings and various infections. The latex of the plant is used to attack warts and to extirpate thorns.

This study attempts to establish for the first time the effect of the aqueous extract of a medicinal plant (*Euphorbia guyoniana*) on the human bacterial pathogen in the telluric environment for the purpose of biological control.

## Materials and methods

### *Plant material*

Experiments were carried out on the aerial and underground parts of *Euphorbia guyoniana*, collected from the Ghardaïa region (South Algeria) in February 2016. The botanical identification of the species was carried out at the botanical laboratory of the Higher National School of Agronomy (ENSA) in El-Harrach (Algeria). The whole plant (stems, flowers, leaves and roots) was used for the preparation of the extract. The plant material was ground after drying at ambient temperature in dark place in order to preserve the integrity of the molecules. The obtained ground product was stored in a hermetically sealed flask (*Photo 1*).



*Photo 1. Aerial part of Euphorbia guyoniana*

### *Extraction procedure*

The extraction of flavonoids was carried out according to the Bruneton protocol (1999). The principle of this technique is based on the treatment of the plant material

with various solvents. It is based on the degree of solubility of flavonoids in organic solvents. The recovered aqueous extract was stored in the dark in hermetically sealed vials and subjected to chemical and biological analysis.

### ***Colorimetric determination of the flavonoic extract***

The content of *Euphorbia guyoniana* in flavonoids was determined by the method of Aluminum Trichloride (AlCl<sub>3</sub>) cited by Bahorun et al. (1996); Djeridane et al. (2006) and Ayoola et al. (2008). This method is based on the formation of an aluminum flavonoid-ion complex having a maximum absorbance at 430 nm. The concentration of flavonoids was calculated from the calibration curve established with Quercetin and expressed in equivalent milligrams of Quercetin per gram of extract weight (mg EQ/gE).

### ***Infrared spectroscopy analysis of the extract***

The infrared spectrum of the aqueous extract, for a frequency range between 400 and 4000 cm<sup>-1</sup>, was obtained by a NICOLET 560 type spectrometer.

### ***High performance liquid chromatography (HPLC)***

Qualitative analysis of the aqueous extract was realized using HPLC. The apparatus consisted of a Young Line YL9100 liquid phase chromatograph, equipped with a YL 9101 quaternary pump with integrated degasifier YL 9101, a UV/Visible detector YL 9120 and a YL 9131 oven. The column used was Agilent eclips XDB C 18 (5 µm) with a length of 25 cm and an internal diameter of 4.6 mm. The mobile phase was a mixture of ultrapure water / acetonitrile / acetic acid (50:47:2.5) in an isocratic system with a flow rate of 1 ml/min. The volume of extract and standards injected was 20 µl. The detection of the compounds was done with a UV detector at a wavelength of 280-320 nm.

### ***Isolation and identification of pathogenic bacteria***

The bacteria were isolated from the soil of a henhouse in the Bouira region (Algeria). Soil sampling was carried out by the suspension-dilution method described by Vidhyasekaran et al. (1997). The identification of bacteria was made by fresh macroscopic observation based on morphological criteria of the colony, microscopic observation including methylene blue staining and Gram staining, biochemical galleries and confirmed by VITEK.

Several bacterial strains have been identified in this soil, such as *E. coli* which is a commensal bacterium of the human digestive tract as well as of many animals. At a rate of 10<sup>7</sup> to 10<sup>9</sup> Colony Forming Unit (CFU) per gram of faeces, it accounts for 80-90% of the most dominant species of the aerobic bacterial flora of the human intestine (Tenailon et al., 2010). These strains are both responsible for intestinal and extra-intestinal infections (urinary infections, bacteremias, meningitis ...) (Locatelli., 2013). It is mainly via the natural excretion of fecal matter by animals that these pathogenic bacteria have been introduced into aquifers, rivers and soil (Solo-Gabriele et al., 2000; An et al., 2002; Byappanahalli et al., 2006).

*Enterococcus faecalis* is also a commensal bacterium of the intestines of humans and warm-blooded animals. It is a species found in human excreta at concentrations ranging

from  $10^5$  to  $10^7$  per gram of faeces (Noble, 1978; Leclerc et al., 1996). It is also found in faeces of animals such as cattle, poultry, pigs and sheep, but to a lesser extent (Leclerc et al., 1996; Franz et al., 1999; Wheeler et al., 2001). This species is an opportunistic pathogen, affecting only individuals with weakened immune systems, particularly in hospitals (Morrison et al., 1997). It may cause endocarditis, bacteremia, meningitis, urinary tract infections, intraabdominal infections and surgical wound infections (Chenoweth and Schaberg., 1990; Jett et al., 1994). Because of its natural presence in the intestines and fecal matter of humans and animals, this bacterium is frequently found in soil and on plants (Mundt., 1961; Fujioka et al., 1998; Byappanahalli et al., 2012; Valenzuela et al., 2012; Ran et al., 2013).

*Staphylococcus aureus* is a ubiquitous bacterium that is found specifically on mucous membranes, the nasopharyngeal sphere and skin of warm-blooded animals and humans (Ostyn et al., 2012). Staphylococci producing coagulases are essentially represented by the species *Staphylococcus aureus*. In addition to food poisoning and nosocomial infections in humans, this species may cause clinical and subclinical mastitis in ruminants, particularly cows, which is a common reason for milk contamination (Ostyn et al., 2012).

### ***Evaluation of the resistance of isolated bacteria to antibiotics***

The resistance of the isolated bacteria was tested by synthetic antibiotics, using the Muller Hinton agar diffusion method. The antibiotics used are Erythromycin (E<sub>15</sub>) (15 mcg) Ciproflaxacin (Cip<sub>5</sub>) (5 mcg) Clindamycin (Cl<sub>25</sub>) (25 mcg) Nalidixic Acid (Na<sub>30</sub>) (30 mcg) and Carbenicillin (Cb<sub>100</sub>) (100 mcg). Areas of inhibition were determined according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2006) and bacteria were classified as resistant or susceptible to antibiotics.

### ***Evaluation of the antibacterial activity***

The evaluation of the antibacterial activity of the aqueous extract of *Euphorbia guyoniana* was carried out by the diffusion method in agar medium recommended by several authors (Belaiche, 1979; Garbonnelle et al., 1987; Joffin and Leyral, 2014; Koba et al., 2004). Petri dishes containing the Muller Hinton agar were inoculated with a quantity of bacterial suspension (0.5 McFarland), according to the recommendations of the NCCLS. Sterilized paper discs of 6 mm of diameter, impregnated with 10 µL of extract were placed on the surface of agar. The plates were kept for 2 h at 4 °C and then incubated overnight at 37 °C. The sensitivity of the strain to the extract is manifested by the size of the diameter of the bacterial-free zone surrounding the disc. The antimicrobial activity was determined by measuring the Minimum Inhibitory Concentrations (MICs). Three replicates are performed for each bacterium.

### ***Statistical analysis***

The results were expressed as mean ± Standard Error of Mean (M ± ESM). The statistical analysis was performed using the Statistica software<sup>®</sup> (version 6, Genstat Conseils Inc., Montreal). After the analysis of the variance, the comparison of the averages is performed by the student's test for matched samples. The test is considered statistically significant when the value of p is ≤ 0.05, for a confidence interval of 95%.

## Results

### *Extraction yield on flavonoids*

Aqueous extract containing flavonoids was obtained with a yield of flavonoids of 1.7%. The reason of using the aqueous extract for the study of the antibacterial activity in spite of its low rate is its richness in very polar flavonoids.

### *Colorimetric determination of flavonoids*

The content in flavonoids is reported in equivalent mg of quercetin/g of the plant. The concentration of the flavonoid in the aqueous extract is 0.31 mg EQ/gE.

### *Infrared spectroscopy analysis of flavonoid extracts*

The results of the infrared characterization of the aqueous extract are shown in the Table 1.

**Table 1.** Infrared analysis of the flavonoid extract

Wave length (cm <sup>-1</sup> )	Bonds	Nature of the bond	Function
3406.58	-OH free	Broad band	Phenol
1609.47	C=O	Mean band	Aldehyde
1079.29	C-O	Weak band	Ester

In the aqueous extract of *Euphorbia guyoniana*, and referring to the work of Mabry et al. (1970), the broad band around 3406.58 cm<sup>-1</sup> is associated with the elongation vibration of the OH bond (phenol function). The mean band at 1609.47 cm<sup>-1</sup> corresponds to the elongation vibration of the C=O bond (aldehyde function). Finally, a weak band of 1079.29 cm<sup>-1</sup> is associated with the elongation vibration of the C-O bond (ester function).

### *HPLC*

The HPLC analysis revealed the presence of 41 compounds, of which 27 could be identified in the flavonoid extract of *Euphorbia guyoniana* (Fig. 1, Table 2).

**Table 2.** HPLC of the flavonoid extract of *Euphorbia guyoniana*

Number	R <sub>T</sub> (Min)	%	Compound name
01	34.203	0.4	P-coumaric acid
02	46.703	1.0	Rosmarinic acid
03	62.753	2.0	Quercetin
04	17.237	9.5	Gallic acid
05	27.320	0.4	caffeic acid
06	41.137	0.3	Rutin
07	50.403	19.1	Ellagic acid
08	56.103	6.6	Myricetin
09	27.320	0.4	Syringic acid
10	37.903	0.2	Ferulic acid

Number	R <sub>T</sub> (Min)	%	Compound name
11	64.670	4.0	Kaempferol
12	23.520	0.3	Proanthocyanidin dimer
13	41.137	0.3	Myricetin 3-O-glucoside
14	61.787	1.5	Amentoflavone
15	45.320	1.6	Isoferulic acid
16	47.953	2.5	Quercetin-3-β-Ogalactoside
17	49.470	1.5	Luteolin-7-β-Oglucoside
18	56.103	6.6	Quercetin-3-O-α-rhamnoside
19	73.337	1.5	Luteolin
20	10.187	4.4	Hydroxytyrosol
21	58.370	3.0	Apigenin-7-Oglucoside
22	54.737	4.7	Apigenin-7-Orutinoside
23	49.470	1.5	Luteolin-7-Oglucoside
24	23.520	0.3	Salicylic acid
25	43.053	0.3	Benzoic Acid
26	47.953	2.5	M-coumaric acid
27	58.370	3.0	O-coumaric acid

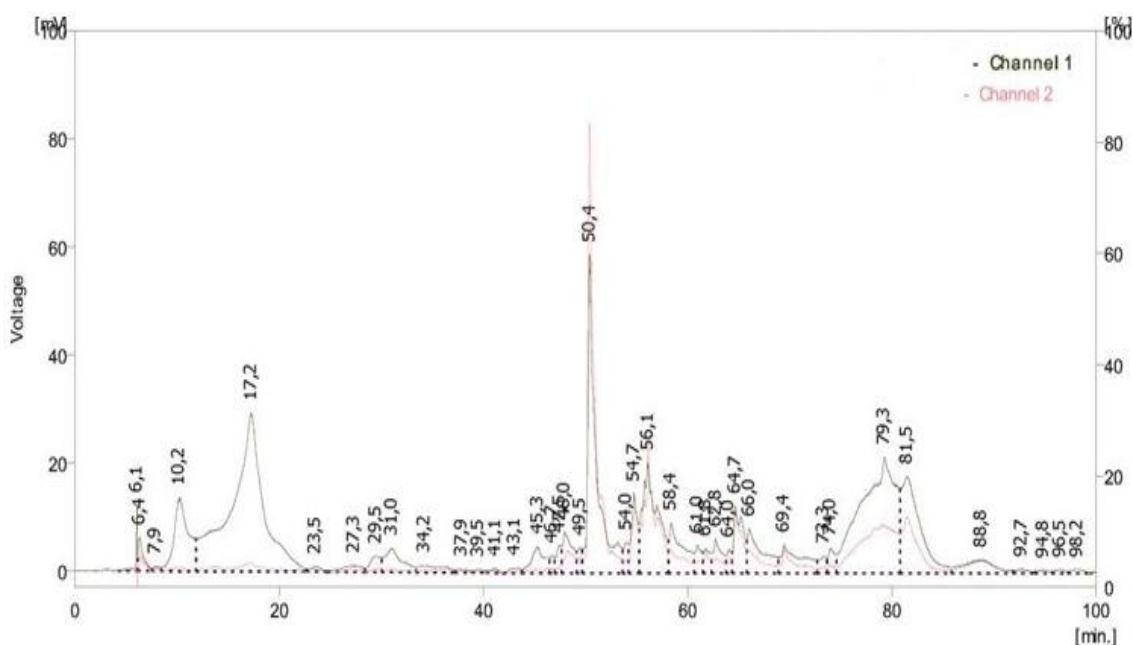


Figure 1. Chromatogram of HPLC applied to flavonoids

The analysis showed that the aqueous extract of *Euphorbia guyoniana* is rich in molecules with antibacterial activity. The major compounds are ellagic acid (19.1%), gallic acid (9.5%), Myricetine and Quercetin-3-O-α-rhamnoside (6.6%), Apigenin-7-Orutinoside (4.7%), Hydroxytyrosol (4.4%) and Kaempferol (4.0%), The other identified molecules are present at rates of less than 3%.

### Identification of isolated bacteria

The results of the identification of isolated bacteria are shown in *Tables 3* and *4*.

In total, twelve bacterial strains are isolated and identified from a henhouse soil belonging to three species: *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive) and *Enterococcus faecalis* (Gram positive).

**Table 3.** Identification of bacterial strains

Sample	Macroscopic appearance		Microscopic appearance		Culturing		Biochemical tests	
	Form	Size	Fresh state	Gram staining/ Grouping mode	Culture media	Conditions	Oxidase	Catalase
01	Very small colony Pink	1 mm	Cocci immobile	G + Bunch of grapes	CHAPMAN	24 h at 37 °C	-	+
02	Large colony Yellowish	3 mm	Bacillus mobile	G- Isolated	HEKTOEN	24 h at 37 °C	-	+
03	Large colony Orange	4 mm	Bacillus mobile	G- Diplocoque	HEKTOEN	24 h at 37 °C	-	+
04	Small colony Transparent	1.5 mm	Cocci immobile	G+ Chain	BEA	24 h at 37 °C	-	-
05	Large colony Salmon	3 mm	Bacillus mobile	G- Diplocoque	HEKTOEN	24 h at 37 °C	-	+
06	Large colony Yellowish	4 mm	Bacillus immobile	G- Clusters	HEKTOEN	24 h at 37 °C	-	+
07	Small colony Golden	1.5 mm	Cocci immobile	G+ Bunch of grapes	CHAPMAN	24 h at 37 °C	-	+
08	Very small colony Yellowish	0.5 mm	Cocci immobile	G+ Bunch of grapes Isolated	CHAPMAN	24 h at 37 °C	-	+
09	Small colony Whitish	1.5 mm	Cocci immobile	G+ Chain Diplocoque	BEA	24 h at 37 °C	-	-
10	small colony Yellowish	1.5 mm	Cocci immobile	G+ Bunch of grapes	CHAPMAN	24 h at 37 °C	-	+
11	Small colony Transparent Surrounded by a black halo	1.5 mm	Cocci immobile	G+ Chain Isolated	BEA	24 h at 37°C	-	-
12	Small colony transparent	0.5 mm	Cocci immobile	G+ Chain	BEA	24 h at 37°C	-	-

+: presence; -: absence; G+: Gram positive; G-: Gram negative

**Table 4. Biochemical galleries**

Sample	Biochemical galleries													Name of the germ
	NR	Mob	Gaz	Glu	Suc	Lac	H2S	Urease	Indol	CIT	LDC	ODC	ADH	
01	+	-	-	+	+	+	-	+	-	+	/	/	/	<i>Staphylococcus aureus</i> (Staphylococcaceae)
02	+	+	+	+	+	+	-	-	+	-	+	+	-	<i>Escherichia coli</i> (Enterobacteriaceae)
03	+	+	+	+	+	+	-	-	+	-	+	-	-	<i>Escherichia coli</i> (Enterobacteriaceae)
04	+	-	-	+	+	+	-	-	-	-	-	+	+	<i>Enterococcus faecalis</i> (Streptococcaceae)
05	+	+	+	+	+	+	-	-	+	-	+	+	-	<i>Escherichia coli</i> (Enterobacteriaceae)
06	+	+	+	+	+	+	-	-	+	-	+	-	-	<i>Escherichia coli</i> (Enterobacteriaceae)
07	+	-	-	+	+	+	-	+	-	+	/	/	/	<i>Staphylococcus aureus</i> (Staphylococcaceae)
08	+	-	-	+	+	+	-	+	-	+	/	/	/	<i>Staphylococcus aureus</i> (Staphylococcaceae)
09	+	-	-	+	+	+	-	-	-	-	-	+	+	<i>Enterococcus faecalis</i> (Streptococcaceae)
10	+	-	-	+	+	+	-	+	-	+	/	/	/	<i>Staphylococcus aureus</i> (Staphylococcaceae)
11	+	-	-	+	+	+	-	-	-	-	+	+	+	<i>Enterococcus faecalis</i> (Streptococcaceae)
12	+	-	-	+	+	+	-	-	-	-	+	+	+	<i>Enterococcus faecalis</i> (Streptococcaceae)

+: presence; -: absence; /: unaccomplished

### Antibacterial activity

Isolated strains used to evaluate antimicrobial activity showed an important resistance to the extract, *Tables 5 and 6* show the results.

The results showed that the antibacterial activity of the aqueous extract of the plant differs from one strain to another. An important antibacterial activity of the extract is observed for *Staphylococcus aureus* with zones of inhibition ranging from 21 ( $\pm$  1) to 31.33 ( $\pm$  1.5) mm, and positive controls for Erythromycin, Ciproflaxacin And clindamycin which showed respectively clear inhibition zones of 6 ( $\pm$  0) to 34 ( $\pm$  1) mm, 6 ( $\pm$  0.5) to 36 ( $\pm$  0.5) mm and 6 ( $\pm$  0) to 20 ( $\pm$  0.5) mm according to the strain (*Fig. 2*). A high sensitivity of *enterococcus faecalis* to the extract was noted with zones of inhibition ranging from 22 ( $\pm$  2) to 28.33 ( $\pm$  1.2) mm, and also positive controls for Erythromycin, Ciproflaxacin, clindamycin, And nalidixic acid with inhibition zones of 6 ( $\pm$  0.5) to 20 ( $\pm$  2.6) mm, 6 ( $\pm$  0) to 34 ( $\pm$  0) mm, 6 18 ( $\pm$  0) mm and 6 ( $\pm$  0.5) to 15 ( $\pm$  0) mm respectively (*Fig. 3*). Finally, a lower sensitivity was observed in *Escherichia coli* for the extract with an inhibition zone between 18.33 ( $\pm$  1.5) and 28.66 ( $\pm$  1.5) mm,



and positive controls for Ciproflaxacin, Clindamycin and carbenicillin with inhibition zones of 40 ( $\pm$  0) to 42 ( $\pm$  1) mm, 12 ( $\pm$  1) to 25 ( $\pm$  0) mm, and 8 ( $\pm$  0) to 32 ( $\pm$  1) mm respectively (Fig. 4).

**Table 5.** Antibiotic and extract susceptibility tests

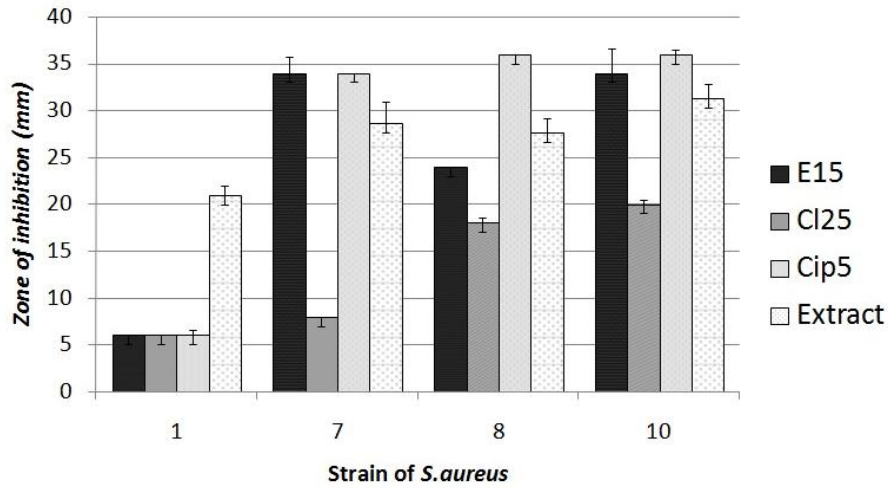
Strain		Areas of Antibiotic Inhibition					Areas of extract inhibition
		E <sub>15</sub>	Cl <sub>25</sub>	Cip <sub>5</sub>	Cb <sub>100</sub>	NA <sub>30</sub>	
<i>Escherichia coli</i>	01	/	16 (S)	41 (S)	13 (S)	/	28 (S)
	02	/	12 (S)	42 (S)	32 (S)	/	19 (S)
	03	/	16 (S)	42 (S)	24 (S)	/	20 (S)
	04	/	25 (S)	40 (S)	8 (S)	/	18 (S)
<i>Staphylococcus aureus</i>	01	6 (R)	6 (R)	6 (R)	/	/	21 (S)
	02	34 (S)	8 (S)	34 (S)	/	/	28 (S)
	03	24 (S)	18 (S)	36 (S)	/	/	27 (S)
	04	34 (S)	20 (S)	36 (S)	/	/	32 (S)
<i>Enterococcus faecalis</i>	01	6 (R)	6 (R)	6 (R)	/	6 (R)	28 (S)
	02	18 (S)	12 (S)	16 (S)	/	15 (S)	22 (S)
	03	20 (S)	18 (S)	34 (S)	/	6 (R)	28 (S)
	04	6 (R)	6 (R)	6 (R)	/	6 (R)	26 (S)

S: sensitive; R: resistant; /: unaccomplished

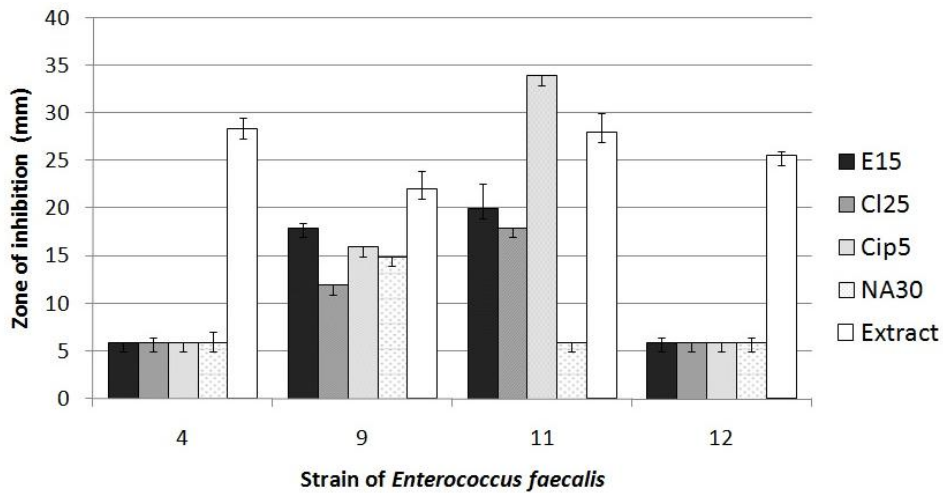
**Table 6.** Values of minimum inhibitory concentrations

Strain	MIC <sub>s</sub> (mg/ml)	
<i>Escherichia coli</i>	01	61.78
	02	55.12
	03	55.12
	04	50.23
<i>Staphylococcus aureus</i>	01	1.47
	02	8.88
	03	3.25
	04	4.75
<i>Enterococcus faecalis</i>	01	23.23
	02	25.87
	03	19.87
	04	17.05

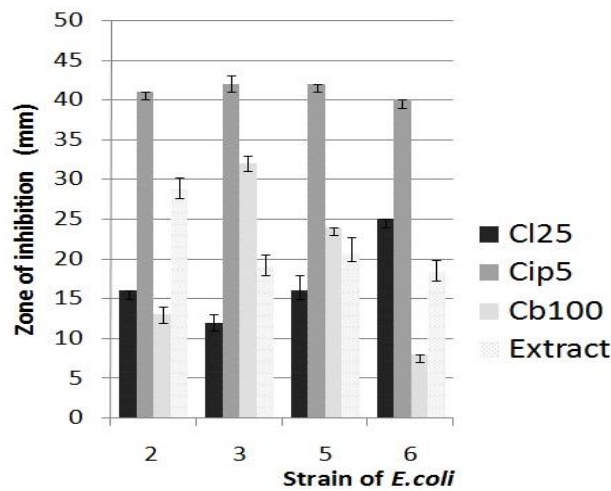
From the results obtained above, it is clear that the aqueous extract of *Euphorbia guyoniana* is much more active against the different microbial strains than the synthetic antibiotics by exhibiting larger zones of inhibition. These results are confirmed by significant tests with  $p < 0.05$ . Except for Ciproflaxacin, which exhibits slightly larger inhibition zones on the *Escherichia coli* strains compared to the extract. This important antibacterial activity is due to the richness of the extract in flavonoids, known to be effective antibacterial substances.



**Figure 2.** Sensitivity of *S. aureus* strains of the extract and antibiotics



**Figure 3.** Sensitivity of the strains *Enterococcus faecalis* to the extract and antibiotics



**Figure 4.** Sensitivity of *E. coli* to the extract and antibiotics

## Discussion

The value of the flavonoid yield of the *Euphorbia guyoniana* plant found in our study (1.7%) was higher than that obtained by Kemassi (2014) for the aqueous extract of the same plant harvested from the Ghardaïa region which was obtained by Maceration with acetone, ie 0.082%. In addition, higher yields were noted by Herouini et al. (2015) for the flavonoid extract of the roots (6.3%) and the aerial part (4.3%) of *Euphorbia guyoniana* harvested in Oued sebseb (Algerian Sahara) obtained by reflux. According to Haba et al. (2008), *Euphorbia guyoniana* is a plant rich in secondary metabolites including diterpenes, triterpenes, steroids and aromatic compounds. The yield of flavonoids appears to depend on the nature of the biotope and the extraction method, knowing that the number of washes carried out in the extraction protocol could lead to substantial losses of the aglycones, hence the disadvantages of the method of extraction by solvent confrontation. Similar works have reported the existing of variability in the yield values extraction of secondary metabolite depending on the procedure of extraction (Moreira et al., 2005; Sagdic and Ozcan, 2003; Celiktaş et al., 2007; Turkmen et al., 2007).

Concerning the concentration of aqueous extract in flavonoids; Andrianarisoa and Tsirinirindravo (2009) found a concentration of approximately 63.39 µg/µl for the aqueous extract of the leaves of *Dalechampia clematidifolia* (Euphorbiaceae) harvested in Madagascar, which represents a significantly lower value to that found for the species *Euphorbia guyoniana*.

HPLC analysis by Smara et al. (2014) on the aerial parts of the same plant (*Euphorbia guyoniana*) harvested from the Oued Souf region (Algerian Sahara) revealed the presence of a hydrolyzable tannin, a single coumarin and two flavonoids (flavonol) namely Quercetin-3O-β-D-glucuronide and kaempferol-3O-β-D-glucuronide. In our study, Quercetin is detected as a molecule, and associated with galactoside, glucoside or rhamnoside. As for kaempferol, this compound is also identified in the free form.

Very little study concerning the antibacterial activity of the aqueous extract of *Euphorbia guyoniana* was carried out. Herouini et al. (2015) used separately the flavonoid aqueous extracts of the aerial and subterranean parts of *Euphorbia guyoniana* on *Staphylococcus aureus* and *Escherichia coli* isolated from several infections, and noted a less marked activity than that observed in our study. These authors obtained zones of inhibition comprised between 7 and 8 mm.

This difference in the inhibitory effect of the bacterial growth observed may be related to the richness of the extract tested in flavonoid compounds known for their antibacterial activity. In the case of this study, the important antibacterial effect observed can be attributed either to the richness of the extract of flavonoid compounds (ellagic acid, gallic acid, Myricetine, Quercetin-3-O-α-rhamnoside, Apigenin-Orutinoside, Hydroxytyrosol and Kaempferol) or to the virulence of the bacterial strain.

Moreover, the efficiency of an extract of a plant also depends on the extraction method (Moreira et al., 2005; Sagdic and Ozcan, 2003; Celiktaş et al., 2007; Turkmen et al., 2007), the part of the plant used (Yeo Sounta, 2014; Natarajan et al., 2005) and the harvest season.

## Conclusion

The evaluation of the antibacterial activity of the aqueous flavonoid extract of the medicinal plant *Euphorbia guyoniana* showed a remarkable inhibitory effect on pathogenic bacteria of telluric origin. In perspective other studies are necessary for the development of formulations for pharmaceutical use based on this extract in order to fight against these pathogens.

**Acknowledgements.** The authors gratefully acknowledge the precious help of the Center for Scientific and Technical Research in Physico-Chemical Analysis (CRAPC) of Algeria and the Department of Biology of the University of Boumerdes of Algeria for valuable technical assistance.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## REFERENCES

- [1] An, Y. J., Kampbell, D. H., Breidenbach, G. P. (2002): *Escherichia coli* and total coliforms in water and sediments at lake marinas. – Environ. Pollut. 120(3): 771-778.
- [2] Andrianarisoa, B., Tsirinirindravo, L. H. (2009): Antibacterial activities of leaf extract of *Dalechampia clematidifolia* (Euphorbiaceae). – Int. J. Biol. Chem. Sci. 3(5): 1198-1202.
- [3] Ayoola, G., Coker, H., Adesegun, S., Adepoju-Bello, A., Obaweya, K., Ezennia, E., Atangbayila, T. (2008): Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. – Trop. J. Pharm. Res. 7(3): 1019-1024.
- [4] Bahorun, T., Grinier, B., Trotin, F., Brunet, G., Din, T., Luncky, M., Vasseur, J., Cazin, M., Cazin, C., Pinkas, M. (1996): Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. – Arzneimittelforschung 46(11): 1086-1089.
- [5] Belaiche, P. (1979): Traité de phytothérapie et d'aromathérapie Tome 1. L'aromatogramme. – M. S. A., Paris.
- [6] Bellakhdar, J. (1997): La Pharmacopée Marocaine Traditionnelle. Médecine arabe ancienne et savoirs populaires. – Ibis Press, Paris.
- [7] Berg, G., Eberl, L., Hartmann, A. (2005): The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. – Environ. Microbiol. 7(11): 1673-1685.
- [8] Bruneton, J. (1999): Pharmacognosie, Phytochimie, Plantes médicinales. Tec & Doc. – Editions médicales internationales, Paris.
- [9] Byappanahalli, M. N., Whitman, R. L., Shively, D. A., Sadowsky, M., Ishii, S. (2006): Population structure, persistence, and seasonality of autochthonous *Escherichia coli* in temperate, coastal forest soil from a Great Lakes watershed. – Environ. Microbiol. 8(3): 504-513.
- [10] Byappanahalli, M., Roll, B., Fujioka, R. (2012): Evidence for occurrence, persistence, and growth potential of *Escherichia coli* and Enterococci in Hawaii's soil environments. – Microb. Environ. 27(2): 164-170.
- [11] Celiktas, O. Y., Hames Kocabas, E. E., Bedir, E., Vardar Sukan, F., Ozek, T., Base, K. H. C. (2007): Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. – Food Chem. 100(2): 553-559.
- [12] Chenoweth, C., Schaberg, D. (1990): The epidemiology of enterococci. – Eur. J. Clin. Microbiol. 9(2): 80-89.
- [13] Colinon, C., Deredjian, A., Hien, E., Brothier, E., Bouziri, L., Cournoyer, B., Hartman, A., Henry, S., Jolivet, C., Ranjard, L. (2013): Detection and enumeration of *Pseudomonas*

- aeruginosa* in soil and manure assessed by an ecfX qPCR assay. – J Appl Microbiol 114(6): 1734-1749.
- [14] De Nazaré, D., Sebastião, F., Palmeira, J., Conserva, L., Lyra Lemos, R. (2005): Quinoline alkaloids from *Sebastiania corniculata* (Euphorbiaceae). – Biochem. Syst. Ecol. 33(5): 555-558.
- [15] Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N. (2006): Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. – Food Chem. 97(4): 654-660.
- [16] Franz, C. M. A. P., Holzapfel, W. H., Stiles, M. E. (1990): Enterococci at the crossroads of food safety?. – Int J Food Microbiol 47(1-2): 1-24.
- [17] Freitag, N. E., Port, C. G., Miner, M. D. (2009): *Listeria monocytogenes* from saprophyte to intracellular pathogen. – Nat. Rev. Microbiol. 7(9): 623-628.
- [18] Fujioka, R., Sian-Denton, C., Borja, M., Castro, J., Morphew, K. (1998): Soil: the environmental source of *Escherichia coli* and Enterococci in Guam's streams. – J Appl Microbiol 85(1): 83S-89S.
- [19] Garbannelle, B., Dens, F., Marmonier, A., Pinon, G., Vargues, R. (1987): Bactériologie médicale Techniques usuelles. – SIMEP, Paris.
- [20] Haba, H. (2008): Etude phytochimique de deux Euphorbiaceae sahariennes: *Euphorbia guyoniana* Boiss. & Reut. et *Euphorbia retusa* Forsk. – PhD Thesis, University of Batna, Algeria.
- [21] Herouini, A., Kemassi, A., Ould El Hadj, M. D. (2015): Etude de l'activité biologique des extraits aqueux d'*Euphorbia guyoniana* (Euphorbiaceae) récoltée dans Oued Sebseb (Sahara Algérien). – el-wahat 8(2): 15-25.
- [22] Hunsä, P., Chulabhorn, M., Ruchirawat, S., Prawat, U., Tuntiwachwuttikul, P., Tooptakong, U., Taylor, W. C., Pakawatchai, C., Brian, W., Skelton, B. W., Allen, H. (1995): Cyanogenic and non-cyanogenic glycosides from *Manihot esculenta*. – Phytochemistry 40(4): 1167-1173.
- [23] Jett, B. D., Huycke, M. M., Gilmore, M. S. (1994): Virulence of enterococci. – Clin. Microbiol. Rev 7(4): 462-478.
- [24] Joffin, N., Leyral, G. (2014): Microbiologie technique. Dictionnaire des techniques. – Canopé-CRDP, Bordeaux.
- [25] Kemassi, A. (2014): Toxicité comparée des extraits d'*Euphorbia guyoniana* (Stapf.) (Euphorbiaceae), *Cleome arabica* L. (Capparidaceae) et de *Capparis spinosa* L. (Capparidaceae) récoltés de la région de Ghardaïa (Sahara septentrional) sur les larves du cinquième stade et les adultes de *Schistocerca gregaria* (Forskäl, 1775) (Orthoptera-Cyrtacanthacridinae). – PhD Thesis, Kasdi Merbah University, Ouargla, Algeria.
- [26] Koba, K., Sanda, K., Raynaud, C., Nenonene, Y. A., Millet, J., Chaumont Loziene, K., Venskutonis, P. R., Sipailien, A., Labokas, J. (2017): Radical scavenging and antibacterial properties of the extracts from different *Thymus pulegioides* L. chemotypes. – Food Chem 103(2): 546-559.
- [27] Leclerc, H., Devriese, L. A., Mossel, D. A. A. (1996): Taxonomical changes in intestinal (faecal) enterococci and streptococci: consequences on their use as indicators of faecal contamination in drinking water. – J Appl Microbiol 81(5): 459-466.
- [28] Locatelli, A. (2013): Prévalence de pathogènes humains dans les sols français, effet des facteurs pédoclimatiques, biologiques et du mode d'utilisation des sols. – PhD Thesis, Université de Bourgogne, Dijon, France.
- [29] Mabry, T. J., Markham, K. R., Thomas, M. B. (1970): The Systematic Identification of Flavonoids. – Springer, New York.
- [30] Mavar, M. H., Brick, D., Marie, D. E. P., Quetin-Leclercq, J. (2004): In vivo anti-inflammatory activity of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae). – J Ethnopharmacol 92(3): 209-214.

- [31] Mazoir, N. A., Benharref, M., Bailén, M., Reina, M., Onzálezcoloma, A. (2008): Bioactive triterpene derivatives from latex of two *Euphorbia* species. – *Phytochemistry* 69(6): 1328-1338.
- [32] Moreira, M. R., Ponce, A. G., Del Valle, C. E., Roura, S. I. (2005): Inhibitory parameters of essential oils to reduce a foodborne pathogen. – *Food Sci. Technol.* 38(5): 565-570.
- [33] Morrison, D., Woodford, N., Cookson, B. (1997): Enterococci as emerging pathogens of humans. – *J Appl Microbiol* 83(S1): 89S-99S.
- [34] Mundt, J. O. (1961): Occurrence of Enterococci: bud, blossom, and soil studies. – *Appl Microbiol* 9(6): 541-544.
- [35] Natarajan, K., Kumaresan, V., Narayanan, K. (2005): A check list of Indian agarics and boletes (1984-2002). – *Kavaka* 33: 61-128.
- [36] Noble, C. J. (1978): Carriage of group D streptococci in the human bowel. – *J Clin Pathol* 31(12): 1182-1186.
- [37] Ostyn, A., De Buyser, M. L., Guillier, F., Krys, S., Hennekinne, J. A. (2012): Benefits of the combined use of immunological- and PCR- based methods for determination of staphylococcal enterotoxin food safety criteria in cheese. – *Food Anal Methods* 2: 173-178.
- [38] Raaijmakers, J. M., Timothy, C. P., Steinberg, C., Alabouvette, C., Moëgne-Loccoz, Y. (2009): The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. – *Plant Soil* 321(1): 341-361.
- [39] Ran, Q., Badgley, B. D., Dillon, N., Dunne, G. M., Sadowsky, M. J. (2013): Occurrence, genetic diversity, and persistence of Enterococci in a lake superior watershed. – *Appl. Environ. Microbiol.* 79(9): 3067-3075.
- [40] Reis, A. L. S., Montanhini, M. T. M., Bittencourt, J. V. M., Destro, M. T., Bersot, L. S. (2014): Gene detection and toxin production evaluation of hemolysin BL of *Bacillus cereus* isolated from milk and dairy products marketed in Brazil. – *Braz J Microbiol* 44(4): 1195-1198.
- [41] Sagdic, O., Ozcan, M. (2003): Antibacterial activity of Turkish spice hydrosols. – *Food Control* 14(3): 141-143.
- [42] Smara, O., Julia, A., Moral-Salmi, C., Vigor, C., Vercauteren, J., Legseir, B. (2014): Flavonoids from *Euphorbia guyoniana* Boissier & Reuter. – *J. Life Sci* 8(6): 544-551.
- [43] Smith, L. D. (1978): The occurrence of *Clostridium botulinum* and *Clostridium tetani* in the soil of the United States. – *Health Lab Sci* 15(2): 74-80.
- [44] Smith, L. D. S. (1979): *Clostridium botulinum*: Characteristics and occurrence. – *Rev Infect Dis* 1(4): 637-641.
- [45] Solo-Gabriele, H. M., Wolfert, M. A., Desmarais, T. R., Palmer, C. J. (2000): Sources of *Escherichia coli* in a coastal subtropical environment. – *Appl Environ Microbiol* 66(1): 230-237.
- [46] Tenailon, O., Skurnik, D., Picard, B., Denamur, E. (2010): The population genetics of commensal *Escherichia coli*. – *Nature Rev. Microbiol.* 8(3): 207-217.
- [47] Ticknor, L. O., Kolsto, A. B., Hill, K. K., Keim, P., Laker, M. T., Tonks, M., Jackson, J. P. (2001): Fluorescent amplified fragment length polymorphism analysis of Norwegian *Bacillus cereus* and *Bacillus thuringiensis* soil isolates. – *Appl Environ Microbiol* 67(10): 4863-4873.
- [48] Tramier, R. (1986): Pathogens from land-based sources; Means of intervention and biocenosis. – *OEPP Bull* 16(2): 299-310.
- [49] Tripathi, R. D., Tiwari, K. P. (1980): Genuculatin, a triterpenoid saponin from *Euphorbia geniculata*. – *Phytochem* 19(10): 2163-2166.
- [50] Turkmen, N., Velioglu, Y. S., Sari, F., Polat, G. (2007): Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. – *Molecules* 12(3): 484-496.

- [51] Valenzuela, A. S., Benomar, N., Abriouel, H., Pulido, R. P., Cañamero, M. M., Gálvez, A. (2012): Characterization of *Enterococcus faecalis* and *Enterococcus faecium* from wild flowers. – *Anton. Leeuw. Int. J. G* 101(4): 701-711.
- [52] Vidhyasekaran, P., Sethuraman, K., Rajappan, K., Vasumathi, K. (1997): Powder formulations of *Pseudomonas fluorescens* to control pigeonpea wilt. – *Bio. Control* 8(3) 166-171.
- [53] Wheeler, A. L., Hartel, P. G., Godfrey, D. G., Hill, J. L., Segars, W. I. (2001): Potential of as a human fecal indicator for microbial source tracking. – *J. Environ. Qual.* 31(4): 1286-1293.
- [54] Yeo Sounta, O., Guessennd Kouadio, N., Meité, S., Ouattara, K., Bahi Gnogbo, A., N'Guessan, J. D., Coulibaly, A. (2014): In vitro antioxidant activity of extracts of the root *Cochlospermum planchonii* Hook. f. ex. Planch (Cochlospermaceae). – *J. Pharmacogn. Phytochem.* 3(4): 164-170.