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Research Article

POLYPHENOLIC CONTENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF AQUEOUS EXTRACTS OF *Eucalyptus globulus* L. and *Trigonella foenum-greacum* L.

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ABSTRACT. In Algeria, several medicinal plants from different family have been used as traditional medicines, where they are prepared as decoctions, and some are eaten as salads. Two medicinal plants were screened namely: *Eucalyptus globulus* L. and *Trigonella foenum-greacum* L. belongs to the family *Myrtaceae* and *Fabaceae* respectively; for evaluation of free radical scavenging and antibacterial activities of the aqueous extract (EGAE, TFAE) of the leaves and seeds of sample plants. Antioxidant activity was assessed using the DPPH test. Moreover, the antibacterial effect of extracts was evaluated according to the agar disk diffusion method against three bacterial strains. Quantitative estimation of total polyphenols, flavonoid showed that the extracts were rich in these compounds (280.63 µg EAG/mg of extract; 37.28 µg EQ/mg of the extract); (272.64±0.09 µg EAG/mg of the extract; 95.85±0.007 µg EQ/mg of the extract) respectively, with a yield of 8.63% and 55.24%, respectively. Furthermore, the extracts showed a very strong anti-radical activity against the radical DPPH even greater than that of BHT (IC_{50}=18.9±1.5 µg/ml; 3.4±0.3 µg/ml, respectively against BHT: IC_{50}=5.7±1.2 µg/ml). The results revealed that extracts showed antibacterial activity against Gram-positive bacteria of varying degrees, whereas there is no obvious effect on Gram-negative bacteria. In conclusion, the aqueous extract of these plants has excellent antioxidant activity and a strong antibacterial effect; these plants may also be subjected to the isolation of therapeutic antimicrobials and further pharmacological assessment.

Keywords: *Antibacterial effect, Antioxidant activity, Eucalyptus globulus* L., *Polyphenols, Trigonella foenum-greacum* L.

INTRODUCTION

Oxidative stress is characterized as a profound imbalance between the oxidative systems and the antioxidant capacities of the organism. This imbalance is the result of either an exaggerated development of oxidizing agents or alteration in the defense system [1, 2]. Free radicals such as superoxide anion (O_2), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2) are recognized at moderate concentrations for their various physiological functions, ranging from transduction of signal cell to pathogen defense. However, they can be reacted with several molecules, such as protein and lipids at important quantities,

leading to the appearance of multiple chronic diseases such as cancer, inflammation, atherosclerosis, and diabetes [3, 4].

Epidemiological studies indicate that the intake of fruit and vegetables could reduce the conditions associated with chronic diseases [3]. Besides, several screening studies have been invested in the search for new antioxidant molecules from plant extracts to enhance the antioxidant defense system [5]. For example, various studies focus on the extraction of natural antioxidants from medicinal plants that can replace synthetic additives that could be carcinogenic and even toxic to the consumer [6].

Another emergence is resistance to antibiotics, as a result of the massive and sometimes abusive use of antibiotics. This led to strong consumer demand for new antibiotics against pathogenic germs and encouraged scientists to use phytotherapy, intending to have molecules with antioxidant properties and antimicrobials [7, 8].

Eucalyptus globules L (family of *Myrtaceae)* is one of such plant which is aromatic and medicinal plants. It is a tree of 30 to 35 m, up to 100 m [9]. Its trunk consists of bark with a dark base, rough, high, smooth, grayish, and has erect branches [10]. Eucalyptus contains several biologically active compounds such as essential oils (Terpene oxides: 1,8-cineole; monoterpenes: alpha-pinene, limonene, gamma-terpinene, paracymene; sesquiterpenes: aromadendrene; sesquiterpenols: globulol), flavonoids, phenol acids, and tannins. Eucalyptol or 1,8 cineole is the majority compound with a concentration of 70 to 85% [11, 12].

Trigonella foenum-graecum L (Fenugreek) is an annual plant of the *Fabaceae* family, can reach 50 cm high, has an erect stem, branching, leaves petiolate, alternate, three-compound oval toothed leaflets [13]. The flowers are axillary, solitary or in pairs, of type papilionaceous, pale yellow to light purple triangular in shape (hence the name Trigonella). Fenugreek seed is rich in protein (20 to 30%), amino acids such as 4-hydroxyisoleucine (0.1 to 0.3% by weight of the dry drug), carbohydrates (20 to 45%) mainly mucilaginous fibers, and lipids (7 to 10%). It contains sterols (cholesterol, sitosterol, etc.), sapogenins (0.1-2.2%), trigonelline (methylbetaine, 0.37%), phosphorus, calcium, iron, β-carotene and an essential oil (around 0.015%) but also with volatile constituents (sesquiterpenes, lactones, etc.) [14].

This investigation aimed to determine the content of polyphenols and flavonoids in plants' aqueous extracts and to examine the suitability of the proposed method of testing natural antioxidants and antimicrobials.

MATERIALS AND METHODS

Plant material

The leaves of the "*Eucalyptus globulus*"(Kalytous) and the seeds of "*Trigonella feonum-graecum*" (El-helba) were collected at the end of April and the beginning of May, 2014 in the region of Ras-El-Oued in Setif. The plant parts were cleaned, dried in the dark in a well-ventilated place, then crushed and stored in the dark until use.

Microorganisms

The bacteria studied were selected for their high frequency of contaminating foodstuffs and for their pathogenicity. They were provided to us by the laboratory of Microbiology, University of Msila. Algeria. They are maintained by subculturing on nutrient agar favorable to their growth for 24 h at 37 °C. Two Gram-positive bacteria: *Staphylococcus aureus* ATCC 6536, *Bacillus subtilis* ATCC 6633 and one Gram-negative bacteria: *Escherichia coli* ATCC 8739 were tested.

Preparation of the extract

The extraction is carried out according to [15]. 50g of the powder from the leaves or seeds of the studied plants are macerated in 500 ml of distilled water. The mixture is kept under stirring and decoction for 10 min at 100 C°. The solution obtained was filtered through wattman paper, and then dried at 40 C°, to obtain two extracts (EGAE and TFAE) which will be stored until use. The yield is expressed as a percentage of the mass of the extract relative to the mass of the dry plant, using the following formula:

Yield%=M extract /M sample×100

Yield%: The extraction yield.

M extract: Mass of the resulting dry extract in grams.

M sample: Mass of plant material to be treated in grams.

Determination of total polyphenols

The method is based on the oxidation of phenolic compounds by the reagent Folin Ciocalteu, which is a mixture of complexes of phosphotungestic acid and phosphoomolybdic acid of yellow color. This oxidation leads to the formation of a new blue molybdenum-tungesten complex which absorbs at 765 nm, the intensity of which is proportional to the amount of polyphenols present [16]. Briefly, 100 µl of the extract is mixed with 500 µl of the Folin-Ciocalteu reagent (10%), after 04 min, 400 µl of sodium carbonate (7.5%) is added, the whole is incubated at laboratory temperature for 1h30 min then the absorbance was measured at 765 nm. the concentration of total polyphenols is expressed in µg of Gallic acid equivalent per milligram of extract (µg EAG/mg of extract).

Determination of total flavonoids

Flavonoids gave yellowish complexes in the presence of aluminum chloride, due to the free hydroxyl groups. Thus the yellow color obtained is proportional to the quantity of flavonoids in the extract[17]. Briefly, 1ml of aluminum trichloride (2% $AlCl_3$) is added to 1ml of the sample containing different concentrations. The mixture is left to react for 10 min at room temperature. The absorbance was measured at 430 nm. The concentration of total flavonoids is expressed in µg of Quercetin equivalent per milligram of extract µg EQ/mg of extract).

DPPH free radical scvenging test

The experimental protocol used is that of [18], with slight modifications. The DPPH solution is prepared by dissolving 4 mg of DPPH in 100 ml of methanol. The results are expressed as a percentage of inhibition (The half maximal inhibitory concentration: IC_{50}) of the DPPH radical. The Value IC_{50} is defined as being the concentration of the substrate which causes the loss of 50% of the activity of the DPPH. This percentage is calculated according to the following formula:

$IC_{50}\%=(A_0-A/A_0)\times 100$

Where: A_0: Absorbance of the DPPH• solution without the sample (negative control);

A: Absorbance of the DPPH• solution in the presence of the sample.

Antibacterial activity

In the nutrient broth, the microorganism was activated by inoculating a loopful of the bacteria strain, a few well-isolated and perfectly identical colonies are scraped using a sealed Pasteur pipette. Then, 10 ml of sterile physiological water is discharged and the bacterial suspension was homogenized; its opacity must be equivalent to 0.5 Mc Ferland which corresponds to 108 CFU/ml (Colony Forming Units), then diluted to obtain an inoculum of 106 CFU/ml [19].

The antimicrobial activity of the extracts was evaluated by the agar disk diffusion method [20]. The disks were impregnated with the extract (50, 75, and 100 mg/ml) for EGAE and (200, 600, and 900 mg/ml) for TFAE and a disk contains DMSO or Distilled water as a negative control placed in the center of each plate. The medium poured into Petri dishes are inoculated by swabbing from a bacterial suspension of 106 CFU/ml. A volume corresponding to 10 µl of the extracts was introduced onto the disks (6 mm). In parallel, controls are used to verify the growth of the different strains; this operation is repeated 3 times. The plates were incubated at 37 °C/24 h. The microbial growth is assessed by measuring the diameters of the zone of inhibition (mm) that form around the disks.

Three synthetic antibiotics namely: Gentamicin, Ampicillin, Oxacillin were used by the agar diffusion method to detect the sensitivity of the bacterial strains

RESULTS AND DISCUSSION

Extraction

The preparation of plant extracts was carried out by water as it is a polar solvent. Su *et al*. [21] reported that the yield of aqueous extractions increases with temperature. This can explain by the fact that water at high temperature disrupts of cells facilitating the penetration of the solvent and the solubilization of molecules [22]. Heat can, however, lead to the degradation of thermolabile molecules [23], which is why the decoction was carried out for a reduced time. The yield of this extraction is expressed as a percentage of the mass of extract relative to the mass of dry *Eucalyptus globulus*, it is of value (8.63%) in this study (Table 1). These results are superior to those of [24] Raho et al (2008) which were 1.2%, and from Pal Singh et al. (2012) [25] which was 1.8% for fresh leave. In a study carried out by [26], the results showed that the methanolic extract of *Trigonella foenum-greacum* L. has a low yield of 25.89% compared to our aqueous extract of 55.24%, The difference between the two extracts is due to the extraction techniques used, which are completely different and the chemical composition which differs from one extract to another. Furthermore, this difference in yield is linked to harsh climatic conditions (high temperature, solar exposure, drought, salinity) [15].

Many authors have noticed that dry plants give a better yield than fresh plants. Zrira *et al*. [27] reported that the leaves of *E. globulus* yielded better dry (4.29%) than fresh (3.91%). So there is a relationship between the yield and the freshness of the plant used for extraction

Determination of total polyphenols and total flavonoids

The choice to assay this family of bioactive chemicals are justified by their antioxidant and antimicrobial effects [28]. Our results revealed that the aqueous extracts are rich in polyphenols and flavonoids. The results were shown in Table 1.

***Table 1**:Yield, Total polyphenolflavonoids contents of the extract*

Extract	Yield %	Content of flavonoids (µg EQ/mg of extract)	Content of polyphenols (µg EAG/mg of Extract)
EGAE	8.63	37.28±0.19	280.63±0.11
TFAE	55.24	95.85±0.007	272.64±0.09

Zin *et al*. (2006) [29] reported that polyphenols are present in different parts of the plant studied but with varying levels from one part to another. The content was noted for the leaves of *E. globulus* (432.63 ± 4.59 µg EAG/mg dw). In our study, the leaf extraction rate was almost half that of the other study. This can be explained by the solvent as it is one of the parameters that can affect the extraction of polyphenols [30]. Studies of Spignon *et al* (2007) [31] showed that aqueous solvents give better extraction yields than absolute solvents. The acetone-water system is one of the most widely used systems for the extraction of polyphenols because on the one hand, it allows to extraction of considerable contents of polyphenols compared to water [30].

According to the results obtained, the flavonoids are present in the aerial part of the plant in a significant way as for the determination of total polyphenols, the leaves revealed content of (37.28±0.19 µg EQ/mgE). Our results are superior to those obtained by [32, 33], who reported that the leaf extract has a low content (1.16±0.03 mg EQ/g EB) which corresponds to a level of 31% of total flavonoids.

The quantitative assay of aqueous extract gave a very high content of total polyphenols and flavonoids compared to the methanolic extract of *Trigonella foenum-greacum* L with a recorded value of 5.75±0.002 mg/g of extract) and 607±3.6 µg/g of extract) respectively [26].

This large difference could be explained by the region in which the plant is grown, the dosage method, the sensitivity, and purity of the reagents used as well as the harvest period [27].

DPPH free radical scvenginging test

The anti-free radical activity of the extracts was evaluated by the DPPH test, this one is often used for the speed of the results as it is used for the screening of the molecules endowed with antioxidant activities present in the extracts of the plants [34].Table 2 shows IC_{50} of different extracts

***Table 2:**The IC_{50} values of aqueous extracts and BHT.*

Extract	IC_{50} (µg/ml)	BHT
EGAE	18.9±1.5	5.7±1.2
TFAE	3.4± 0.3	

The results of the anti-free radical action of the extracts showed an IC_{50} of the order of (18.9 ± 1.5 µg / ml) for EGAE and (3.4 ± 0.3 µg / ml) for TFAE. EGAE is less active than BHT (5.7 ± 1.2 µg/ml) but the strong activity is exerted by TFAE. These results suggest that the extracts contain free radical scavengers acting as primary antioxidants.

The action of these antioxidants is believed to be due to their ability to donate hydrogen atoms or electrons mainly derived from the A-ring hydroxyl of flavonoids [4].

Mishra *et al* (2010)[35] showed that the extract of *E. globulus* has an IC_{50} of (0.057mg/mL) and those of Pal Singh *et al* (2012) [25] which was (0.136 mg/mL) for the leaves. Antioxidant power results can be influenced not only by chemical composition but also by test conditions (reaction temperature, antioxidant/DPPH ratio, type of solvent, pH, sample concentration) [36, 37, 38].

The IC_{50} of TFAE (3.4 ± 0.3µg/ml) is lower when compared to the methanolic extract obtained by [39], which had a value of 68.96µg / ml (0.068 mg / ml). So the aqueous extract is richer in polyphenols than methanolic extract and its ability to scavenge DPPH radicals is higher. This shows that there is a correlation between the content of polyphenols and the antioxidant activity of the extracts.

The biological mechanisms of polyphenolic compounds have been attributed to their antioxidant properties through several possible mechanisms, such as their ability to scavenge free radicals, break down free radical chain reactions, directly reducing peroxides, and stimulate enzymatic activities of the antioxidant defense [40]. Unlike BHT (a pure radical), the extract is not made up of a single molecule but of several dozen compounds at varying concentrations. Strong antioxidant activity is indicated by a low IC_{50} value. This activity is determined by a decrease in the absorbance produced by the anti-free radical substances [41].

Antibacterial activity

The antibacterial activity of extracts is tested against three bacterial strains by the disk diffusion method. The results were shown in Figure 1 and Table 3.

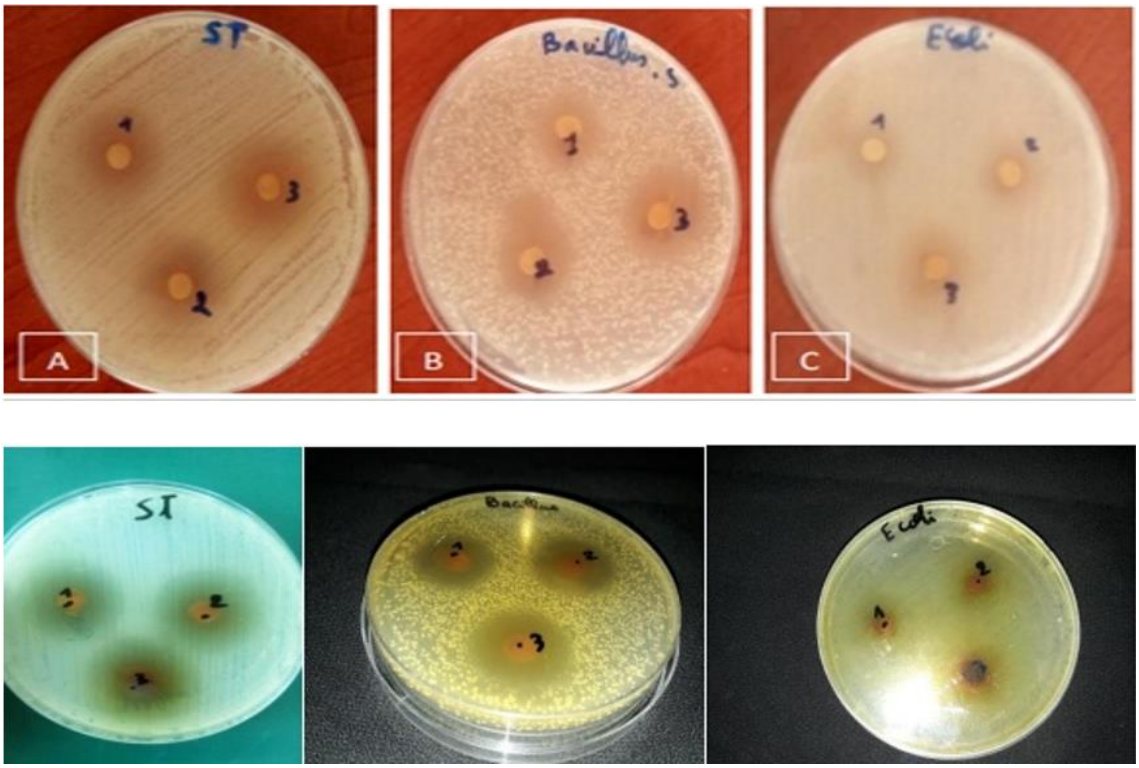

Fig. 1: *Growth inhibition zone of the three bacteria towards two different extracts EGAE and TFAE respectively***.**

The diameters of the zone of inhibition are determined around the disks containing the extracts tested at different concentrations. These measurements were interpreted according to [42, with the following categories (+++): IZ >20 mm as high activity, (++): 10 mm >IZ >20 mm as moderate activity and (+): IZ<10 mm as low activity [20].

These results showed that the extracts are active on two strains studied Gram + with an inhibition diameter varying between (19 to 23 mm for EGAE and 25 to 29.5mm for TFAE) for *Staphylococcus aureus*, (16.5 to 21.5mm for EGAE and 26 to 28.5mm for TFAE) for *Bacillus subtilis*, there is, therefore, a dose-effect relationship. However, no effect on the Gram- strains. Interpretations are made regarding the antimicrobial activity rating scale given by[43]. From this scale, we can classify the bacteria from the most sensitive to the most resistant as follows: *S. aureus*>*B. subtilis*>*E. coli*. These results agree with those of [24] on the sensitivity of *S. aureus* to the extract of *E. globulus* leaves.

***Table 3**: The antimicrobial activity ofEGAE and TFAE.*

	Diameter of the zone of inhibition		
Extract(C)	***S. aureus***	***B. subtilis***	***E. coli***
EGAE			
50 mg/ml	19	16.5	-
75 mg/ml	20.5	19.5	-
100 mg/ml	23	21.5	-
TFAE			
200mg/ml	25	26	-
600mg/ml	28	28	-
900mg/ml	29.5	28.5	-
Gentamicin (GEN)	16	22	16
Oxacillin (OX)	-	-	-
Ampicillin (AM)	14	14	-

Using the agar diffusion method, we determined the antibiotics inhibited visible growth of the bacteria investigated under the same conditions as the extract. The results of the zone of inhibition were shown and Table 3.

Comparative analysis of the effects of EGAE, TFAE extracts, and antibiotics revealed: Resistance of the three strains to Oxacillin, and *E. Coli* against Ampicillin. The sensitivity of the bacteria studied to Gentamicin with diameters of the zones of inhibition between 16 and 23mm. A significant difference between the effect of extracts and antibiotics on the same strain of bacteria. The similarity of antibacterial action of Gentamicin and leaf extract on *B. subtilis* between 18 and 22mm. The sensitivity of *S. aureurs* to extracts and Ampicillin, which can be explained by the fact that the extracts had the same mode of action as Ampicillin on Gram+ bacteria. Further study by [44] showed a similar sensitivity to Gentamycin for *E. coli* could be due to the same mode of action of Gentamycin on Gram- bacteria.

The study of Mawahib *et al* (2015) [45], showed that *E.coli*, *B.subtilus*, *S.aureus* strains sensitive to Gentamicin (17mm, 25mm, 17mm) and to Ampicillin (20mm, 20mm, 11mm), respectively.

The variation in the antimicrobial activity of antibacterial agents could be explained by structural differences between bacteria. DMSO is used for dissolving the extract, it has been tested as a solvent, the results showed that the solvent was suitable and had no effect on the normal growth of microbial strains. Because of the results obtained for the antibiogram and taking into account the concentrations of the antibiotic disks, the inhibitory power of *E. globulus* on *Staphylococcus aureus* and *Bacillus subtilis* are very satisfactory compared to Gentamicin. This is interpreted by the fact that plants produce a huge variety of small antibiotic molecules having a broad spectrum of structures such as flavonoids and polyphenols. However, most of these small molecules have low antibiotic activity compared to common antibiotics produced by bacteria.

CONCLUSION

The evaluation of medicinal plants for their biological activities has increased considerably in the World. This shows that the molecules isolated from plants' medicinal products are certainly of interest to be used as an alternative therapy or as a model for the synthesis of new substances.

The estimation of the anti-free radical potential of the extract by the DPPH test showed that the phenolic compounds from the plant studied were exhibited an effective antioxidant activity. The results also indicate that the extracts had antimicrobial activity on strains: Gram- and Gram+ tested while Gram-negative strains show resistance.

Following these results, it would therefore be interesting to extend the range of tests antioxidants and antimicrobials as well as the isolation and characterization of active compounds in the extracts to identify the different molecules responsible for the different biological activities of these plants.

Conflict of interest. The authors declare no conflict of interest

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REFERENCES

[1] Dahmoune, F., Nayak, B., Moussi, K., Remini, H., Madani, K. (2015): Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. Food Chemistry 166: 585-595.

[2] Boyd, B., Ford, C., Koepke, M, Gary, K., Horn, E. (2003): Etude pilote ouverte de l'effet antioxydant d'Ambrotose sur des personnes en bonne santé. Glycoscience et Nutrition 4(6):70-77.

[3] Dastmalchi, K., Damien Dorman, H., Oinonen, P., Darwisj, Y. (2008): Chemical composition and *in vitro* antioxidative activity of *Lemon balm*. Food Science and Technology 41: 391-400.

[4] Lee, B., Lee, S., Lee, H., Sim, G., Kim, J., Cho, Y., and Hong, J. (2007): Anti-oxidative and Photo-protective effects of coumarins isolated from *Fraxinus chinensis*. Arch Pharm Res 30: 1293-1301.

[5] Gutiérrez, M., Garcia, A., Sagrista, M., Casado, F., Mora, M. (2003): Interaction of tocopherols and phenolic compounds with membrane lipid components: Evaluation of their antioxidant activity in a liposomal model system. Life Science 72: 2337-2360.

[6] 6.Daker, M., Abdullah, N., Vikineswary, S., Goh, P., Kuppusamy, U. (2008): Antioxidant from maize and maize fermented by *Marasmiellus sp*. as stabilizer of lipidrich foods. Food Chemistry 107: 1092-1098.

[7] De Billerbeck, G. (2007) : Huiles essentielles et bactéries résistantes aux antibiotiques. Phytotherapie 5: 249-253.

[8] Fisher, K., Philip, C. (2008): Potential antimicrobial uses of essential oils in food: is citrus the answer. Trends in Food Science & Technology 19:156-164.

[9] Traore, N., Sidibe, L., Bouare S., Harama, D., Somboro, A., Fofana, B., Diallo, D., Figueredo , G., et Chalchat, J. (2013) : Activités antimicrobiennes des huiles essentielles de Eucalyptus citriodoraHook et Eucalyptus houseanaW.Fitzg. ex Maiden. Int. J. Biol. Chem.Sci 7(2): 800-804.

[10] Ghedira, K., Goetz, P., Jeune, R., (2008). Eucalyptus GlobulusLabill. Phytothérapie , 6: 197- 200p.

[11] Amakura, Y.,Umino ,Y.,Tsuji,S. (2002) : Constituents and their antioxydative effects in eucalypyus leaf extract used as a natural food additive. Food Chemistry 77(1): 47-56.

[12] Song, A., Wang, Y., Liu, Y. (2009): Study on the chemical constituents of the essential oil of the leaves of Eucalyptus globules Labill from China. Asian Journal of Traditional Medicines 4(4): 134-140.

[13] Mabrouk, B., D'Araujo, M. E. M., Gouia, H., Bettaieb, B. K. L. (2017): Effets de l'acide salicylique sur l'activité antioxydante de Fenugrec (*Trigonella foenum-graecum*L). Cultivé en présence d'Arsenic et de Zinc. Revue des Régions Arides 43 (3): 381-387

[14] Ghédira, K, Oueslati, H.A. (2015) : Notes ethnobotanique et phytopharmacologique sur *Trigonellafoenumgraecum*. Phytothérapie 13: 234-238.

[15] 15.Fellah, H., Ksouri, K., Chaieb, K., Karray, N., Trabelsi, N., Boulaaba, M. (2008): Phenolic composition of E, Globulus. Organs, and their biological activities. Compte rendu de biologie 331: 372-379.

[16] Hadouchi, F., Gaouche, T., Halla, N. (2016): Screening phytochimique, activités antioxydantes et pouvoir hémolytique de quatre plantes sahariennes d'Algérie. Phytothérapie 1-9.

[17] Benslama, A., Harrar, A. (2016): Free radicals scavenging activity and reducing power of two Algerian Sahara medicinal plants extracts. International Journal of Herbal Medicine 4(6): 158-161.

[18] Brand-Williams, W., Cuvelier, M., Berset, C. (1995): Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissens-chaft-und-Technologie 28: 25-30.

[19] Tyagi, A., Malik, A. (2011): Antimicrobial potential and chemical composition of Eucalyptus globulus oil in liquid and vapour phase against food spoilage microorganisms. Food Chemistry 126: 228–235.

[20] Benslama, A., Harrar, A., Gül, F., Demirtaş, I. (2019): *In vitro* antioxidant, antibacterial activities and HPLC-TOF/MS analysis of *Anvillea radiata* (Asteraceae) extracts. Current Nutrition & Food Science 15(4): 376-383.

[21] Su, X., Duan, J., Jian, Y., Shi, J., Kakuda, Y. (2006): Effect of soaking conditions on the antioxidant potenials of oolong tea. Journal of Food Composition and Analysis: 19:348-353.

[22] Albano, S., Miguel, M. (2010): Biological activities of extracts of plants grown in Portugal. Industrial Crops and Products 33(2*):*338-343.

[23] Seidel, V. (2005): Initial and Bulk Extraction. *In*: Sarker, S and Gray, A. Natural products isolation. Ed, Totowa, Humana Press 27-37.

[24] Raho, B., Ghalem, M., Benali, M. (2008): Antibacterial activity of leaf essential oils of Eucalyptus globulus and Eucalyptus camaldulensis. African Journal of Pharmacy and Pharmacology 2(10): 211-215.

[25] Pal Singh, H., Kaur, S., Negi, K., Kumari, S., Saini, V., Batish, R. (2012): Assessment of *in vitro* antioxidant activity of essential oil of Eucalyptus citriodora (lemonscented Eucalypt; Myrtaceae) and its major constituents. Food Science and Technology 48: 23-241.

[26] Bukhari, S. B., Bhanger, M. I. and Memon, S. (2008): Antioxidative activity of extracts from a fenugreek seeds (*Trigonellafoenum-graecum*). Pak. J. Anal. Environ. Chem 9 (2): 78 – 83.

[27] Zrira, S., El khiran, F., Benjllal, B. (1994): Huiles essentielles de six espèces xérophyles d'Eucalyptus. Effet du milieu sur les rendements et la composition-chimique 14 (l): 5-9.

[28] Li, B., Cheng, W., Wong, W., Jiand, Y. (2003): Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food chemistry 102 :771-776.

[29] Zin, Z., Abdul Hamid, A., Osman, A., Saari, N. (2006): Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.). Food Chemistry 94: 169-178.

[30] Troszyńska, A., Estrella, I., López-Amóres, L., Hernández, T. (2002): Antioxidant Activity of Pea Seed Coat Acetone Extract. Lebensm.-Wiss. u.-Technol 35: 158-164.

[31] Spignon, G., Tramelli, L., Faveri, M. (2007): Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering 81: 200-208.

[32] Chira, K., Suh, H., Teissède, L. (2008): Les polyphénols du raisin. Phytothérapie 6 :75- 82.

[33] Pietta, G. (2000): Flavonoids as antioxidants. J.Nat.Prod 63: 1035-1042.

[34] Yi, Z., Yan, Y., Liang, Y., Zeng, B. (2008): *In vitro* antioxidant and antimicrobial activities of Pericarpium Citri Reticulatae of a new Citrus Cultivar and its main flavonoid LWT 41:597 603.

[35] Mishra, K., Sahu, N., Mishra, A., Ashoke, K., Chattopadhyay, P. (2010): Phytochemical Screening and Antioxidant Activity of essential oil of Eucalyptus leaf. Pharmacognosy Journal 16 :25-28.

[36] Popovici, C., Saykova, I., Tylkowski, B. (2009): Evaluation de l'activité antioxydante des composés phénoliques par laréactivité avec le radical libre DPPH. Revue de Génie industriel 4: 25-39.

[37] Noipa, T., Supalax, S., Thawatchai, T., Wittaya, N. (2011): New approach for evaluation of the antioxidant capacity based on scavenging DPPH free radical in micelle systems. Food search International 44: 798-806.

[38] Costa, P., Goncalves, S., Valentao, P., Andrade, B., Anabela, R. (2012): *Thymus lotocephalus* wild plants and *in vitro* culture produce different profoles of phenolic compound with antioxidant activity. Food Chemistry 135: 1253-1260.

[39] Seasotiyal., Siwachp., Bais., Malika., Bharti1p., Sunita, D. (2014): Free radical scavenging activity, phenolic contents and phytochemical analysis of seeds of *trigonella foenumgraecum*. *Asian Pac.* J. Health Sci 1(3): 219-226.

[40] Ghedadba, N., Bousselsla, H., Hambaba, L., Mouloud, Y. (2014): Evaluation de l'activité antioxydant et antimicrobienne des feuilles et des sommités fleuries de *Marrubiumvulgare* L. Phytothérapie 12(1):15-24.

[41] Talbi, H., Boumaza, A., El-mostafa, K., Hilali, A. (2015): Evaluation de l'activité antioxydant et la composition physico-chimique des extraits méthanoliques et aqueux de la *Nigellasativa* L. Sci 6(4): 1111-1117.

[42] Chirane, A., Merzoud, Y., Benslama, A. (2020): Diversity and antibacterial activity of endophytic fungi associated with the Algerian medicinal plant *Artemisia herba alba*. Journal of Applied Biological Sciences 14(2): 145-154.

[43] Ponce, G., Fritz, R., Del Valle, E., Roura, I. (2003): Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensmittel-Wissenschaft and Technologie (36): 679–684.

[44] Rather, A., Dar, A., Wani, A., Shah, S., Bhat, A., Ganai, A., Bhat, A., Anand, R., Qurishi, A. (2012): Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of *Juglansregia* L. and its constituents. Phytomedicine 19: 11856-1190.

[45] Mawahib, E.M.E., Ammar, M.A.A. and BadrEldin A.E.S. (2015): Antimicrobial activities and phytochemical screening of callus and Seeds extracts of Fenugreek (*Trigonella foenum-graecum*). International Journal of Current Microbiology and Applied Sciences 4(2): 147-157.