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Phenolic compound profiles and antioxidant activity of *Ruta chalepensis L.* leaves, a spontaneous medicinal herb: influence of harvest zone (Western Algeria)

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ABSTRACT

Natural plant extracts contain a variety of phenolic compounds, to which various biological activities are attributed. Ruta chalepensis, known as "Fidjel", is widespread in the Algerian territory, which was selected in order to estimate its content of secondary metabolites (phenolic compounds, flavonoids, tannins), and its antioxidant activity (DPPH, FRAP). For this purpose, two methanolic extracts were prepared from the leaves of this plant, harvested from two different areas: Tessala mountains (Sidi-Bel-Abbes town) and Taougrite mountains (Chlef town), which crude extract yields are around of 12.4% and 20.1% respectively. The quantitative estimation of the different secondary metabolites showed that the methanolic extract of Tessala mountains (MER_{TES}) is the richest in polyphenols (10.65±0.67 mg GAE/g), flavonoids (31.16 ± 0.55 mg CE/g), hydrolyzable tannins (0.78 ± 0.02 mg CE / g), and in condensed tannins (2.75 ± 0.10 mg CE/g). However, an antioxidant activity evaluation of the two extracts showed that, both fractions are active with a maximum IC_{50} of 68.41±9.98 µg/ml in (MER_{TES}). On the other hand, the extract from that of the Taougrite Mountains (MER_{TAO}) shows a stronger reducing activity than (MER_{TES}); the difference remains not significant. In conclusion, the plant harvesting area, and the bioclimatic conditions might influence the Rutaceae leaf extracts content and quality of the same family.

Keywords: DPPH, FRAP, methanolic extract, *Ruta chalepensis*, secondary metabolites, yield.

INTRODUCTION

Since the beginning of humanity, people have been depending on plants to provide their vital necessities, thus the important role of plants as a good source of medicines (Sabitha Rani *et al.*, 2019). In modern medical aspects, various plants or plant-based materials have attracted a great focus on the potential drug candidate development/ extraction, particularly for the non-communicable diseases treatment such as diabetes mellitus and cancer, etc. (Bhowmik, 2019). The active principles of medicinal plants are often linked to the productions of secondary metabolites, which are widely used in therapy, such as preventive anti-inflammatory, antimicrobial, antiseptic, diuretic agents, essentially antioxidant agents which defend against oxidative stress (Bourgaud *et al.*, 2001; Kar, 2007). Phenolic compounds, essential oils and other secondary metabolites represent high value molecules, used in the pharmaceutical, cosmetic and food industries. The antioxidant activities of these products have been reported in numerous studies around the world (Bouzouita *et al.*, 2008). The *Ruta chalepensis* plant, belonging to the *Rutaceae* family is very rich in secondary metabolites, which explains its biological activities: antifungal, antioxidant and anti-inflammatory (Gonzalez-Trujano *et al.*, 2006; Raghav *et al.*,2006; Al-Said *et al.*,1990). This specie is spontaneous, widely distributed in North Africa, particularly in Algeria; where it is known as *Fidjel*.

The objective of our study is to evaluate *in vitro* the antioxidant activity, as well as, the content of total phenolic compounds, flavonoids,

hydrolyzable and condensed tannins of *Ruta Chalepensis methanolic extracts*, collected before the flowering period. Sampling was undertaken in two different western Algeria areas, (Tessala mountains in Sidi-Bel-Abbes region and Taougrite mountains in Chlef region) characterized by different bioclimatic conditions with the purpose to assess whether the different climatic conditions may influence the bioactive compound content.

MATERIALS AND METHODS

Plant material

Ruta Chalpensis was harvested in February 2018 in two different, areas: Tessala mount, which is located at 15 km northwest of the Sidi-Bel-Abbes city and the Taougrite mountain of the Chlef city. Bioclimatic data for the two regions are listed in (Table 1).

The leaves were washed in running water, and then dried at room temperature and away from light. The samples were then crushed and sieved to obtain a homogeneous granular structure, and stored in glass vials for further analysis (Fig. 1).

Preparation of *methanolic extracts*

The powdered *Ruta Chalepensis* leaves (10 g) were extracted with 100 ml of 80% methanol, under agitation for 24 hours at room temperature (Majheniè *et al.*, 2007). The mixture was filtered and then concentrated using a rotavapor (Heidolph instruments), to obtain two *extract*: MER_{TES} = *methanolic extract* of *Ruta* from Tessala mount; MER_{TAO} = *methanolic extract* of *Ruta* from Taougrite mount.

Determination of secondary metabolites

Total phenols

The total phenol content of the *extracts* was determined by the Folin-Ciocalteu method (Qusti *et al.*, 2010). 200 µl of the *extract* was mixed with 1 ml of freshly prepared Folin-Ciocalteu reagent (ten times diluted), and 0.8 ml of 7.5% sodium carbonate (Na_2CO_3) has been added. The mixture was incubated at room temperature for 30 minutes, and the reading was taken against a blank using a spectrophotometer at 765 nm. Phenol concentration in each sample was calculated against a calibration curve with gallic acid at different concentrations. The results are expressed as milligrams gallic acid equivalent per gram of dry matter (mg GAE/g d.w).

Flavonoids

The flavonoid content of the *extracts* was determined using the aluminum trichloride colorimetric method (Kim *et al.*, 2003). An amount of 500 µl of the *extract* was mixed with 1.5 ml of distilled water, and subsequently with 0.3 ml of a 5% sodium nitrite solution $NaNO_2$. After five minutes, 3 ml of a 10% $AlCl_3$ solution was added. After 6 min, 1 ml of 4% NaOH was added. After five minutes the whole mixture was stirred with a vortex. The absorbance was measured at 510 nm. Quercetin was used as standard for the calibration curve. Total flavonoids contents are expressed as, mg quercetin equivalent/g of dry matter (mg CE/g d.w).

Condensed tannins

This determination rate method of the condensed tannins is based on the condensation of polyphenolic compounds with vanillin in an acidic medium, which will give a brown compound. For the determination of condensed tannins, 0.1 ml-0.5 ml *extracts* were placed in tubes and then 3 ml of 4% (w/v) vanillin in methanol are added. After vigorous stirring 1.5 ml of concentrated HCl was immediately added and stirred again. Absorbance was measured at 500 nm after 20 min of incubation (Julkunen-Titto,1985).The calibration curve was prepared under the same conditions, using catechin as standard and the results are expressed as mg catechin equivalent/g dry matter (mg CE/g).

We have adopted the vanillin method with HCl. Which depends on the vanillin reaction with the terminal flavonoid group of condensed tannins, with the red complexes formation, this is explained by the characteristic of tannins to transform into red colored anthocyanidols by the reaction with vanillin. The content of condensed tannins was determined by the vanillin method described by (Julkunen-Titto,1985). A volume of 50 ìl of each *extract* was added to 1500 ìl of the 4% vanillin/ methanol solution and mixed vigorously. Then, 750 ìl volume of concentrated hydrochloric acid (HCl) was added. The obtained mixture was allowed to react at room temperature for 20 min. The absorbance was measured at 550 nm against a control. Various concentrations between 0 and 1000 µg/ml prepared from a catechin stock solution, will be used to trace the calibration curve.

Hydrolyzable tannins

The hydrolyzable tannins determination was performed by the Mole and Watrman method (1987), based on a reaction with ferric chloride. The *tannic extract* mixture with the ferric chloride reagent produces a blue-black coloration in the presence of gallic tannins; and a green-brown coloration in the presence of catechic tannins, from which the (Fe3+) ions are formed. To accomplish this, 1 ml of the *extract* was added to 3.5 ml of a 0.01M Fe $C1_3$ solution in 0.001M HCl (V/V). The mixture was vigorously mixed, and the optical density was read at 660 nm with a spectrophotometer. The hydrolyzable tannins content in the *extracts* was calculated from a calibration curve, carried out with gallic acid, under the same experimental conditions as the tested samples

A 0.01 M Fe Cl_3 solution was mixed with a 0.001M (v/v) HCl solution. 3.5 ml of this solution was added to 1ml of *extract*. After 15 seconds, the absorbance was measured at 660 nm.

The hydrolyzable tannins are expressed by the following formula:

H T (%) = (Abs x M x V) / E mole x W

With: HT: hydrolysable tannins, Abs: absorbance, E mole: 2169 of gallic acid (constant expressed in mole), M: mass = 300, V: volume of the used *extract*, W: weight of the sample. The results are expressed as milligram gallic acid equivalent per gram of dry *extract* (mg GAE/g DE).

Antioxidant activity

DPPH Test

In the presence of free radical scavengers, the purple-colored DPPH (2,2-diphenyl-1-picrylhydrazyl) is reduced to yellow-colored 2,2-diphenyl-1-picrylhydrazine (Maataoui *et al.*, 2006 and Molyneux, 2004).

The free radical scavenging activity of plant *extracts* was determined according to (Benhammou *et al.,* 2009). A volume of 50 ìl of different concentrations of each *extract* was added to 1.95 ml of the freshly prepared DPPH methanolic solution (25 mg/l). The negative control, were prepared in parallel, by mixing 50 ìl methanol with 1.95 ml of a methanolic DPPH solution, at the same concentration. After incubation in the dark for 30 minutes at room temperature, the absorbances of each sample and negative control were read at 515 nm using a spectrophotometer. The reactions were repeated three times for each dilution, and then the value mean of the percentage inhibition of scavenging activity for DPPH was taken.

Calculation of inhibition percentages of DPPH

We calculate the inhibition percentages by the following formula :

I % = ((Ca - Ta) / Ca) × 100. With: Ca: the control absorbance; Ta: the test absorbance performed.

All tests were performed in triplicate. The *methanolic extracts* kinetics reactions and ascorbic acid with DPPH were recorded at each examined concentration. The concentrations of the different *extracts* and ascorbic acid, as a function of the percentages of inhibited DPPH, were graphed at the end of the reactions to obtain the IC_{50} index.

IC_{50} or 50% inhibitory concentration is the test sample concentration required to reduce 50% of the DPPH radical. The IC_{50} was graphically calculated by the linear regressions of the graphical representations of the inhibition percentages as a function of different concentrations of the fractions tested.

FRAP Test (Ferric Iron Reducing Power)

The principle is based on the ferric iron reduction reaction (Fe^{3+}), present in the $K_3Fe(CN)_6$ complex to ferrous iron (Fe^{2+}) by an antioxidant, the reaction is revealed by the change of the yellow color of ferric iron (Fe^{3+}) to the blue-green color of ferrous iron (Fe^{2+}).The intensity of this coloration is measured by spectrophotometry at 700 nm (Chung *et al.,* 2002).

The protocol established by (Oyaizu *et al.,*1986) was adopted, which consists of taking 0.5 ml of each *extract*, at different concentrations and mixing them with 1.25 ml of a 0.2M phosphate buffer solution (pH = 6.6) and 1.25 ml of a 1% potassium ferricyanide solution $K_3Fe(CN)_6$. The mixture was incubated at 50°C, for 20 min, and then cooled at room temperature. 2.5ml of 10% trichloroacetic acid was added to stop the reaction, and then the tubes are centrifuged at 3000 rpm for 10 min. Then 1.25ml of the supernatant added to 1.25 ml of distilled water and 250 ml of 0.1% (Fe Cl_3) solution. The absorbances read spectrophotometrically at a wavelength of 700 nm. The positive control was

represented by an antioxidant standard solution; the ascorbic acid, the absorbance of which was measured under the same conditions as the samples. The iron (Fe^{3+}) reducing activity determination was performed in triplicate.

Statistical analysis

The results are expressed in the form of the mean and their standard (X ± ES). Statistical analysis of the data is conducted using Microsoft Excel version 2010 software. The statistical analysis of the different groups data; was carried out by the Student test "t"; this parametric statistical test is suitable for a comparative analysis between the means of the experimental samples, and that of the control group. In all cases, a p value <0.05 was considered significant.

RESULTS AND DISCUSSION

Extractions yield

The *Ruta chalepensis* leaves *crude extract* yields changed according to the stations (Table 2). The highest yields were measured in the plant leaves of the Taougrite mountains MER_{TAO} (20.1%) whereas in Tessala MER_{TES} were 12.4 %.

The difference in yield rate obtained was due before hand to the solvent used on the one hand and on the other hand, to the richness of *Ruta chalepensis* in methanol-soluble substances. Indeed, studies have indicated that a ratio of methanol 70% is generally used in the flavonoids extraction, phenol acids and their derivatives; and a wide range of biomolecules (Al-Farsi and Lee, 2008). The results of (Al-Said *et al.*,1990) on *R. chalepensis*, gave a *crude extract* yield of the whole aerial parts with 3.75%, this yield is clearly much lower than that obtained in our study, this may be due to characteristics of each species and the harvest region (soil, temperature). Thus, for *Ruta chalepensis*, the *crude methanolic extracts* yields found was higher than those reported by (Merghache *et al.,* 2009) (0.82%), (Hnatyszyn *et al.,* 1974) (0.9%), (Mejri *et al.,* 2010) (5.51%) and (Fakhfakh *et al.*, 2012) (2.32 to 1.25%).

Total phenols and flavonoids content

The levels of total phenols and flavonoids are reported in (Fig. 2). According to our results, we noticed that MER_{TES} was richer in flavonoids ($p < 0.05$) in comparison with MER_{TAO}. While, the difference in polyphenol content in the two *extracts* remains statistically insignificant ($p > 0.05$).

The difference in total phenols and flavonoids content may be due to the climatic conditions, which differ from region to another. The polyphenol content variability in plant species is probably due to, the *extracts* phenolic composition (Hayouni *et al.,* 2007), to genotypic factors, biotic (species, organ and physiological stage), abiotic (edaphic factors) (Ksouri *et al.,* 2008), the soil nature and the microclimate type (Atmani *et al.*, 2009), and also to the bioclimatic stages where these plants grow. On the other hand, Bentabet *et al*.(2007) confirmed that the polyphenolic content varies both qualitatively and quantitatively from plant to another, which can be attributed to several factors such as climatic and environmental factors, genetic patrimony, the harvesting period and the stage of plant development, the *extract* concentrations, the extraction used method, etc.

The polyphenol content of tested *extracts* was lower compared to the study realized by Bettaieb *et al*.(2012), who reported that the total phenol content in the *Ruta chalepensis* aerial part obtained from *methanolic extract* was around 13.7 mg GAE/g of dry matter, while Ghazghazia *et al.* (2013) have reported that, the *R. chalepensis* leaves polyphenol contents are 12.82 mg GAE/g of dry matter. In another study carried out by Shuib *et al.* (2015), the *Ruta angustifolia* species registered a content of 18.89 mg GAE/g.

Table 1: Climatic features of the study areas

Characters	Longitude	Latitude	Altitude	Climate
Tessala	00:76408°	35:26978°	1061 m	Semi-arid dry and cold
Taougrite	0°552 222 2	36°142 392 2	528 m	Mediterranean with hot summer

Table 2: Crude *extracts* yields from *Ruta chalepensis* leaves.

Samples	Yield (%)
MER_{TES}	12.4
MER_{TAO}	20.1

On the other hand, the tested plant flavonoid content remained higher compared to other researches performed by Khlifi *et al.* (2013) who reported the *Ruta chalepensis methanolic extract* aerial part flavonoid content to be around 12.78 ± 0.08mg EAG/g of dry matter. While Bettaieb *et al.* (2012) registered a content of 6.50 mg QE/g of *hydro-methanolic extrac*t (80:20) (V:V). This signifies that the flavonoid content varied according to the used extraction solvent.

Condensed and hydrolyzable tannins content

The condensed and hydrolyzable tannins quantification is shown in (Fig.3). Our results confirm that, the plant *extract* from the Tessala mountains is slightly rich in hydrolyzable ($p<0.05$) and condensed ($p<0.01$) tannins, compared to that from the Taougrite mountains. In general, the chemical families detected in our experimentation, confirmed the researches carried out on various origins species (Saudi Arabia, Turkey, Jordan, Algeria, India, Morocco and Oman) (El-Sayed *et al.,* 2000; Gunaydin and Savci, 2005; Shehadeh *et al.,* 2007; Haddouchi *et al.*,2013; Raaman *et al.,* 2014; Al-Brashdi *el al.,* 2016; Daoudi *et al.,* 2016).

Indeed, the presence of these secondary metabolites can explain various biological activities: protective against biotic and abiotic aggressions, antifungal, antioxidant, phytotoxic, abortive, anti-allergic, anti-tumoral, anticancer, neuroprotective, antispasmodic, cytotoxic, antibacterial, anti-inflammatory, antiviral and insecticides (Conti *et al.*,2013; Shuib *et al.,* 2015; Chaibeddra *et al.,* 2016; Daoudi *et al.*,2016).

Antioxidant activity

DPPH (2.2'-diphenyl-1-picrylhydrazyl)

The results shown in the figure 4, illustrate the percentages of the antiradical activity of the two *Ruta chalepensis L. extracts* shows that MER_{TES} exhibits the highest inhibition percentage of order 85.6% at maximum concentration (250µg/ml), followed by MER_{TAO} with a value of 84.35%.

Among the obtained different fractions, the MER_{TES} fraction represents the most active *extract*, its IC_{50} was 68.41 ± 9.98 µg /ml, followed by the MER_{TAO} fraction with an IC_{50} around 70.6 ±12.12 µg/ml. Compared to the standard antioxidant ascorbic acid, both fractions are more active. The difference between the IC_{50} of the tested *extracts* was not statistically significant (Fig.5).

Both *extracts* showed significant antioxidant capacity. According to Abou Zeid *et al.*(2014), phenolics such as flavonoids, phenolic acids, tannins and furocoumarins directly contribute to the plant antioxidant capacity. Turkmen *et al.* (2007) noted that polyphenols appear to be effective hydrogen donors to the DPPH radical, due to their chemical structure. Works carried out by Kang *et al.* (2003) suggested that, the polar molecules present in the plant *extracts* contribute to the antioxidant activity increase. This is generally due to the synergy between the different existing antioxidant compounds. Similarly, studies by Ouerghemm *et al.* (2016), which were performed on the *R. chalepensis* flowers *methanolic extract* from two different provenances (wild and cultivated), revealed that the antioxidant potential was quite high in the plant's aerial part with an IC_{50} in the range of 23.73ìg/ml for the wild flowers and 28.48ìg/ml for the cultivated *R. chalepensis* flowers. The study achieved by Ghazghazi *et al.* (2013) on the *Ruta chalepensis* antioxidant activity revealed a remarkable antioxidant capacity; IC_{50} of the leaves wich was estimated at 35ìg/ml. The antioxidant capacity seems to be influenced by the total polyphenols contents. The studies of Kacem *et al.* (2015) also showed that *R. chalepensis* possesses a fairly high antioxidant potential. Indeed, at a concentration of about 500 mg/ml, the *ethanolic extract* decolors the DPPH and scavenges the free radicals with an inhibition rate of about 84%.

Iron reducing (FRAP*)*

The reducing activity results clearly show that, MER_{TAO} exhibits the reduce power of Fe^{+3} ion more strongly in comparison with MRE_{TES}, the statistical analysis doesn't show a significant difference ($P> 0.05$) (Fig. 6). According to results obtained, the activity of our *extracts* is average, these results which synchronize with that of Djeridane *et al.* (2006), where they found that, the *Ruta sp. phenolic extract* activity was less important compared to the other ten plants studied at the same time, although the phenolic compounds content was important, this is generally due to the synergy between the different existing antioxidant compounds, which makes the activity not only concentration-dependent. The antioxidant activity depends on several factors, such

Fig. 1: *Ruta chalepensis* fresh plant (A) and after drying (B) (personal photo)

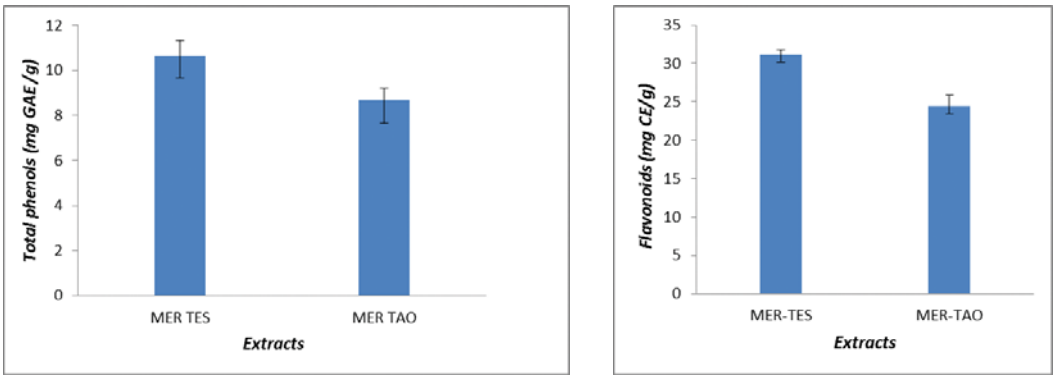

Fig. 2: Total phenols and flavonoids contents of *Ruta chalepensis* leaves *extracts*

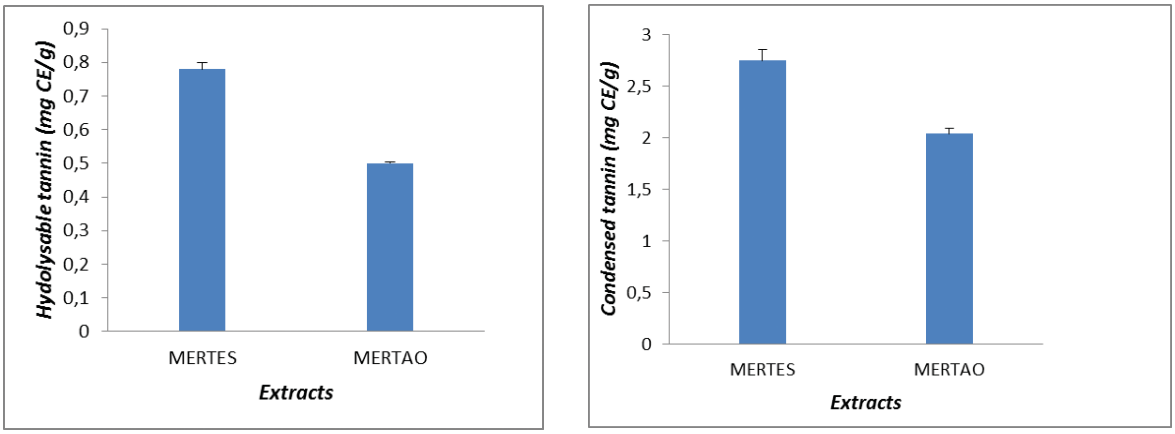

Fig. 3: Hydrolyzable and condensed tannin content of *Ruta chalepensis* leaves *extracts.*

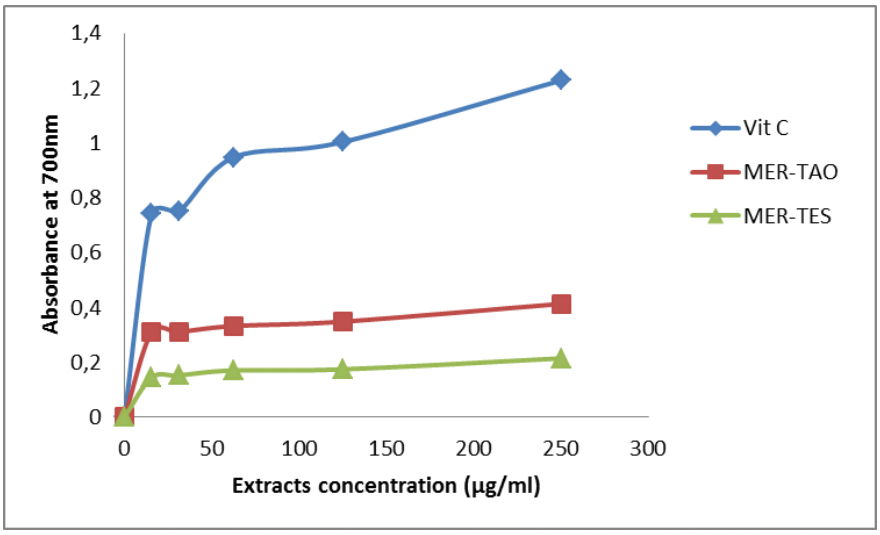

Fig. 4: Antiradical activity of *Ruta chalepensis methanolic extracts*

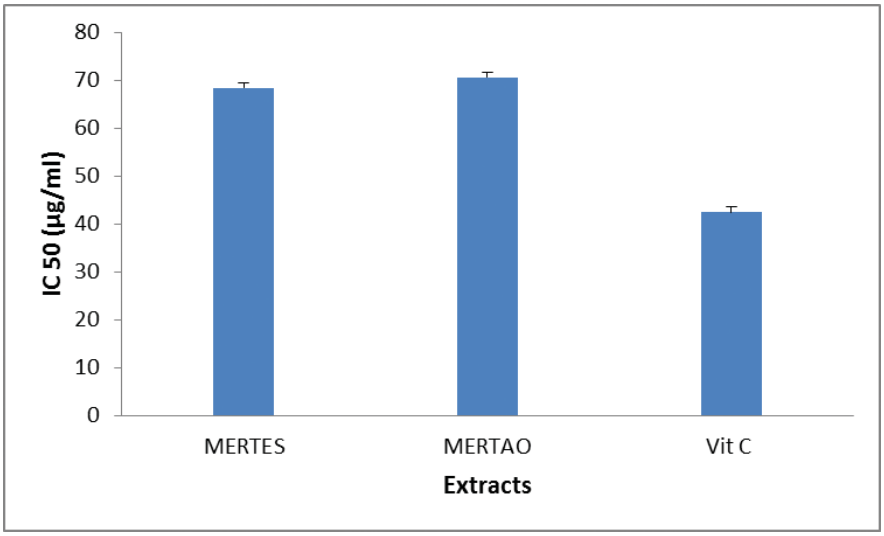

Fig. 5: Median inhibitory concentration of *Ruta chalepensis extracts*

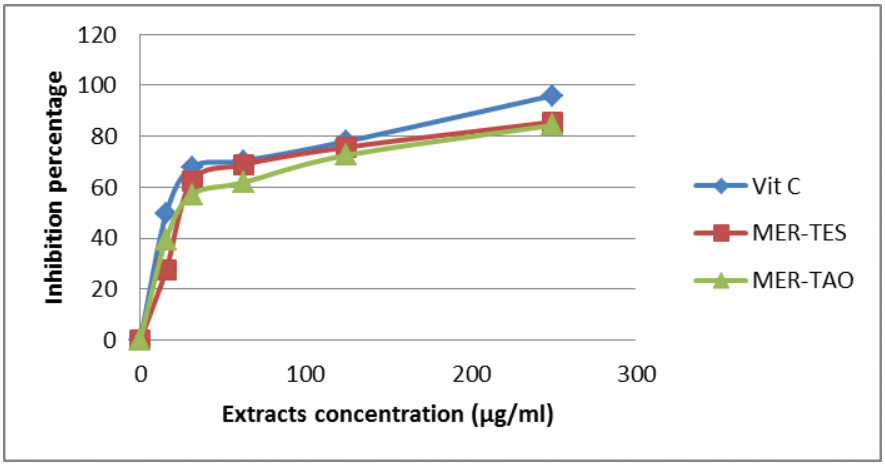

Fig .6: Antiradical activity potency of *Ruta chalepensis extract*

as: the *extract* concentration, the evaluation method, the antioxidant sensitivity to the test temperature, the antioxidant's water -soluble or lipid-soluble nature (Kadri, 2011 and Pukalskas, 2012).

CONCLUSION

From *in vitro* data it was concluded that the *Ruta Chalepensis extract* from the Tessala mountains was slightly rich in certain secondary metabolites and antioxidant elements, than that of the Taougrite mountains. The study has highlighted the influence of area harvest factor of plant on the *extracts* content and quality. In addition, an evaluation of the antioxidant activity of the *Ruta chalepensis* essences against DPPH and FRAP, and the spectral assay of the bioactive substances (polyphenols, flavonoids and tannins), showed that this *Rutaceae* exhibit an interesting antioxidant power suggesting their use in the food, in cosmetics and in the pharmaceutical industry.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with respect to the publication of this document.

REFERENCES :

Abou-Zeid, H.M. Bidak, L.M. and Goh, Y.M. 2014. Phytochemical screening and antimicrobial activities of some wild medicinal plants of the western Mediterranean coastal region Egypt. *Int. J. Pharm. Sci. Res.*,**5** (10): 4445-4455.

Al-Brashdi, A.S. Al-Ariymi, H., Al Hashmi, M. and Khan, S.A. 2016. Evaluation of antioxidant potential, total phenolic content and phytochemical screening of aerial parts of a folkloric medicine, *Haplophyllum tuberculatum* (Forssk) A Juss. *J. Coast. Life Med*., **4**(4): 315-319.

Al-Farsi, M.A and Lee ,C.Y. 2008. Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chem.,* **108** (3): 977-985.

Al-Said, M.S., Tariq, M.M., Al-Yahya, A., Rafatullah, S., Ginnawi, O.T. and Ageel, A.M .1990. Studies on *Ruta chalepensis*, an ancient medicinal herb still used in traditional medicine. *J Ethnopharmacol*., **28**(3):305-312.

Atmani, D., Chaher, N., Barboucha, M., Ayouni, K., Lounis, H., Boudaoud, H., Debbache, N. and Atmani, D. 2009. Antioxydant capacity and phenol content of selected Algerian medicinal plants. *Food Chem.,* **112** (2):303-309.

Benhammou, N., Atik Bekkara, F. and Kadifkova, P.T. 2009.Antioxidant activity of *methanolic extracts* and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus Chimie*., **12** (12): 1259-1266.

Bentabet, N., Boucherit-Otmani, Z. and Boucherit, K. 2007. Composition chimique et activité antioxydante d'extraits organiques des racines de *Fredolia aretioides* de la région de Béchar en Algérie. *Phytotérapi* .,**12** :364-371.

Bettaieb, A. Moujahed, N. and Ksouri, R. 2012.Secondary compounds characterization in some autochtonous species from a North-Eastern region of Tunisia. Options mèditerranèennes: New approaches for grassland research in a context of climate and socio-economic changes Series A. Séminaires Méditerranéens., n° **102**, Samsun (Turkey).

Bhowmik, D.2019. Aloe vera-Gift to mankind. *International Journal of Minor Fruits, Medicinal and Aromatic Plants,* **5** (1): 01-06.

Bourgaud, F., Gravot, A., Milesi, S. and Gontier, E. 2001. Production of plant secondary metabolites: A historical perspective. *Plant Sci*., **161**(5): 839-851.

Bouzouita, N., Kachouri, F., Ben Halima, M.K. and Chaabouni, M.M. 2008. Composition chimique et activités antioxydante, antimicrobienne et insecticide de l'huile essentielle de *Juniperus phoenicea*. *Journal de la Société Chimique de Tunisie.,* **10** : 119-125.

Chaibeddra, Z., Baaziz, N. and Zellagui, A. 2016. *Ruta tuberculata* Forsk: Phytochemical screening, total phenolic contents and antioxidant activity of crude coumarins and alkaloids extracts. *Der Pharm Lett*., **8** (6):212-216.

Chung, Y.C., Chang, C.T., Chao, W.W., Lin, C.F. and Chou, S.T. 2002. Antioxidative activity and safety of the 50 *ethanolic extract* from red bean fermented by *Bacillus subtilis* IMR-NK1. *J Agric food chem*., **50** (8):2454-2458.

Conti, B., Leonardi, M., Pistelli, L., Profeti, R., Ouerghemmi, I. and Benelli, G . 2013. Larvicidal and repellent activity of essential oils from wild and cultivated *Ruta chalepensis L. (Rutaceae)* against *Aedes albopictus Skuse (Diptera: Culicidae*), an arbovirus vector. *Parasitol Res*., **112**(3) :991-999.

Daoudi, A., Hrouk, H., Belaidi, R., Slimani, I., Ibijbijen, J. and Nassiri, L. 2016. Valorization of *Ruta montana* and *Ruta chalepensis*: Ethnobotanical study, phytochemical screening and Antibacterial activity. *J Mater Environ Sci.*, **7** (3):926-935.

Djeridane, A., Yous, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*., **97** (4): 654-660.

El Sayed, K., Al-Said, M. S., El-Feraly, F. S. and Ross, S. A. 2000. New Quinoline Alkaloids from *Ruta chalepensis. J. Nat. Prod.*, **63**(7):995-997.

Fakhfakh, N., Zouari, S., Zouari, M.M., Loussayef, C. and Zouari, N. 2012. Chemical composition of volatile compounds and antioxidant activities of essential oil, *aqueous* and *ethanol extracts* of wild Tunisian *Ruta chalepensis L. (Rutaceae). J Med Plant Res*., **6**(4): 593-600.

Ghazghazia, H. Chediab, A., Abderrazakb, M. and Brahima, H. 2013. Comparaison des contenus en polyphénols et de l'activité antioxydante des extraits méthanoliques de quatre plantes collectées du nord de Tunisie. *Microbiol Hyg Alim*., Vol **25**, N° 73 (4ème Journée GEDIV).

Gonzalez-Trujano, M.E. Carrera, D. Ventura-Martinez, R. Cedillo-Portugal, E and Navarrete, A.2006. Neuropharmacological profile of an *ethanol extract* of *Ruta chalepensis* L. in mice. *J Ethnopharmacol*., **106** (1): 129-135.

Gunaydin, K. and Savci, S. 2005. Phytochemical studies on *Ruta Chalepens* (LAM.) LAMARCK. *Nat. Prod. Res.*, **19**(3): 203-210.

Haddouchi, F., Chaouche, T.M., Zaouali, Y., Ksouri, R., Attou, A. and Benmansour, A. 2013. Chemical composition and antimicrobial activity of the essential oils from four *Ruta* species growing in Algeria. *Food Chem.*, **141** (1):253-258.

Hnatyszyn, O., Arenas, P., Moreno, A.R., Rondina, R. and Coussio, J.D. 1974. Plantas reguladoras de la fecundidad segun la medicina folklorica. *Revista de la Sociedad Cientifica*., **14**: 37.

Hayouni, E., Abedrabba, M., Bouix, M. and Hamdi, M. 2007. The effects of solvent and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quecus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chem*., **105** (3): 1126-1134.

Julkunen-Tiitto, R. 1985. Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics .*J Agric food chem*., **33** (2): 213-217.

Kacem, M., Kacem, I., Simon, G., Ben Mansour, A., Chaabouni, S., Elfeki, A. and Bouaziz, M. 2015. Phytochemicals and biological activities of *Ruta chalepensis* L. growing in Tunisia. *Food Biosci*., **12** (1):73-83.

Kadri, A., Zarai, A., Bekir, A., Gharsallah, N., Damak, M. and Gdoura, R. 2011. Chemical composition and antioxidant activity of *Marrubium vulgare L.* essential oil from Tunisia. *Afr J Biotechnol*., **10**(9): 3908-3914.

Kang, D.G., Yun, C.K. and Lee, H.S. 2003. Screening and comparison of antioxidant activity of extracts of herbal medicines used in Korea. *J. Ethnopharmacol*., **87** (2-3): 231-236.

Kar, A. 2007. Pharmaocgnosy and Pharmacobiotechnology. Revised-Expanded Second Edition. New Age International Limted Publishres, New Delhi. pp. 332-600.

Khlifi, D., Sghaier, R.M., Amouri, S., Laouini, D., Hamdi, M. and Bouajila, J. 2013. Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of *Artemisiaherba-alba, Ruta chalpensis L.* and *Peganum harmala L. Food Chem Toxicol*., **55**: 202-208.

Kim, D.O., Chun, O.K., Kim, Y.J., Moon, H.Y. and Lee, C.Y. 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J Agric Food Chem*., **51** (22):6509-6515.

Ksouri, R., Megdiche, W., Falleh, H., Trabelsi, N., Boulaaba, M., Smaoui, A. and Abdelly, C. 2008. Influence of biological, environmental and technical factors on phenolic content and

antioxidant activities of Tunisian halophytes. *C R Biol*., **331**(11) :865-873.

Maataoui, B.S.,Hmyene, A. and Hilali, S. 2006.Activités anti-radicalaires d'extraits de jus de fruits du figuier de barbarie (*Opuntia ficus indica*). *Leban Sci J*., **7**(1):3-8.

Majheniè, L. Škerget, M. and Knez, Ž 2007. Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chemistry,* **104**(3);1258-1268.

Mejri , J., Abderrabba, M. and Mejri, M. 2010. Chemical composition of the essential oil of *Ruta chalepensis* L: influence of drying, hydro-distillation duration and plant parts. *Ind. Crops Prod*., **32**(3) : 671-673.

Merghache, S., Hamza, M. and Tabti, B. 2009. Etude phytochimique de l'huile essentielle de *Ruta chalepensis* L. de Tlemcen, Algérie. *Afr Sci*., **5**(1): 67-81.

Mole, S. and Waterman, P.G. 1987. A critical analysis of techniques for measuring tannins in ecological studies: I. Techniques for chemically defining tannins. *Oecologia.,* **72,** (1):137-147.

Molyneux, P. 2004. The use of the stable free radical diphenyl picryl hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol.*,**26** (2):211-219.

Ouerghemmi, I., Rebey, I. B., Rahali, F.Z., Bourgou, S., Pistelli, L., Ksouri, R., Marzouk, B. and Tounsi, M.S. 2016. Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*. *J Food Drug Anal*., **25**(2):350-359.

Oyaizu, M. 1986. Studies on products of browning reactions: Antioxidative activities of product of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition and Dietetics*, **44**(6): 307-315.

Pukalskas, A., Venskutonis, P.R., Salido, S., Waard, P. and Van Beek, T.A. 2012. Isolation, identification and activity of natural antioxidants from horehound (*Marrubium vulgare* L) cultivated in Lithuania. *Food Chem.,* **130** (3):695-701.

Qusti, S., Abo-Khatwa, A.N. and Lahwa, M. A. 2010. Screening of antioxidant activity and phenolic content of selected food items cited in the holly Quran. *Eur J Biol Sci*., **2** (1):40-51.

Raaman, N., Natarajan, P., Swaminathan, A., Balan, L. and Laxmanan, H. 2014. *In vitro* propagation, qualitative phytochemical analysis and antioxidant activity of *Ruta chalapensis* L. *Med Plants*, **6** (2):87-93.

Raghav, S.K., Gupta, B., Agrawal, C., Goswami, K. and Das, H.R. 2006.Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells. *J Ethnopharmocol*., **104** (1-2):234-239.

Sabitha Rani, A., Hajera Sana, G., Sulakshana, E., Shravya, P. and Keerti, M. 2019. *Spilanthes acmella*-an important medicinal plant. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **5** (2): 15-26.

Shehadeh, M.B., Afifi, F.U. and Abu-Hamdah, S.M. 2007. Platelet aggregation inhibitors from aerial parts of *Ruta chalepensis* grown in Jordan. *Integr Med Insights,* **2** (1): 35-39.

Shuib, N.A., Iqbal, A., Akmal Sulaiman, F., Razak, I. and Susanti, D. 2015. Antioxidant and antibacterial activities of *Ruta angustifolia extract*. *J Teknol (Sciences and Engineering),* **77**(25):101-105.

Turkmen, N., Velioglu, Y. S., Sari, F. and Polat, G. 2007. Effect of extraction conditions were total polyphenol content and antioxidant Measured and antibacterial activities of black tea. *Molecules*, **12** (3):484-496.