

Are the plants used in Algerian traditional medicine effective?

Assessment of the antibacterial, anti-inflammatory and anti-oxidative effects of three plants used in Algerian traditional medicine; *Olea europaea*, *Glycyrrhiza glabra* and *Ocimum basilicum*.

Type of article: Original

Elkolli Meriem, Elkolli Hayet

Department of microbiology, FSNV of Setif 1 university, Algeria

elkollim@yahoo.fr

Abstract

Background: Algeria has a very large vegetation biodiversity. Algerians use herbs in phytotherapy because of their easy, safe and inexpensive use. However, the consumption of these plants remains uncontrolled or regulated by the authorities, which lacks reassurances concerning their use.

Objective: The purpose of this work is to confirm or refute the empirical use of the plants as well as to start adjusting the dosages for each therapeutic purpose.

Methods: The present experimentation was done during the year 2017 (April-June) at the laboratory for the valorization of natural biological substances at the University of Setif 1. The *in vitro* anti-inflammatory effect of Olive and basil leaves and Licorice root aqueous extracts were evaluated against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 by the disc diffusion assay. The *in vitro* anti-inflammatory activity was realized by the estimation of the protein denaturation of the BSA, and the anti-oxidative test was done by the DPPH method. All experiments were done in triplicate results and were reported as mean \pm SD. Data were analyzed by GraphPad prism 5 software; the statistical analysis was done by the one way ANOVA.

Results: Olive leaf extract (OLE) and Licorice root extract (LRE) were active on both *E. coli* (13.5, 10mm) and *S. aureus* (14, 12mm) at 200 mg/ml. While the Basil leaf extract (BLE) was inactive against all strains. The percentage of BSA denaturation was concentration-dependent by both BLE and LRE and the maximum inhibition was recorded by the OLE at 250 μ g/ml, it was slightly different from BLE at $P \leq 0.05$, but not significantly different from LRE. The three extracts showed good values of IC_{50} with 0.65, 4.98 and 0.91 mg/ml OLE, LRE and BLE respectively, but they were inferior to that of BHT.

Conclusion: These results confirm the use of these plants but under control.

Keywords: traditional phytotherapy, Olive leaves, Licorice, Basil.

Corresponding author: Elkolli Meriem, Department of microbiology, FSNV of Sétif 1 university Algeria
elkollim@yahoo.fr

Received: 11 July, 2018, Accepted: 14 July, 2019, Published: 20 October, 2019.

Screened by iThenticate. ©2017-2019 KNOWLEDGE KINGDOM PUBLISHING.

1. Introduction

In Algeria, the pharmaceutical market is growing rapidly, as Algeria intends to develop local production and become a national production platform, knowing that a large share of the market is based on imports (close to 70 %) (Bouzabata, 2016). Often, the customer is attracted by the personality of the herbalist. Indeed, some herbalists express themselves perfectly, in the three languages, Arabic, Berber and French. They have the assurance of the therapist, do not hesitate to make reference to the international books (of Europe, America or the Middle East) and give examples lived by their clients, they give orally true prescriptions, with dosage, duration of

treatment and administration mode (**Hammiche et al., 2013**). The problem is that, recently, there is a decrease in the knowledge of medicinal plants in young generations with a growth in the number of herbalists not specialized in this field. In order to establish the list of medicinal plants, which are sold at the local market and which really have biological effects that corroborate the use, we tested three of these plants for their antibacterial, anti-oxidative and anti-inflammatory activities: Olive and Basil leaves and Licorice roots. The Algerians consider the Olive tree (*Olea europaea*/ Oleaceae), known locally as “Zitoun”, as an almost sacred tree because of its multiple virtues; it is even evoked in the holy Quran. The Olive tree is one of the most typical and economically important tree in the Mediterranean countries and its leaves are one of the byproducts that can be found in high amount (**Orak et al., 2012**). Its leaves and barks have astringent, diuretic, febrifuge, hypoglycemic, tonic and hypotensive properties. Traditionally, Olive leaf infusion is used as a gargle against oral conditions (inflammation of the gums, canker sores and bad breath) (**Rebas et al., 2012**). Common Basil, named locally “H’bak”, (*Ocimum basilicum*/ Labiatae) is grown all over the world, in the warm and temperate zones, due to its great popularity. It is an important economic and medicinal herb. The fresh leaves are used as flavourings and have been used as an appetite stimulant, carminative, diuretic, mouth wash and astringent to cure inflammations in the mouth and throat (**Hiltunen and Holm, 1999**). Licorice (*Glycyrrhiza glabra*/ Fabaceae), locally called “Erg Essous”, is native to Eurasia, in central and south-western Asia and the Mediterranean. It is used fresh or dried as a mouth freshener, tooth cleaner, expectorant and carminative, flavoring agent, antimicrobial, antioxidant and anti-inflammatory (**Lim, 2016; Rohinishree and Negi, 2016**). This work is part of an ethnopharmacological survey that aims to standardize the sale of medicinal plants by herbalists. In this study, an assessment of biological activities of these three plants, sold for their anti-infectious and anti-inflammatory properties, was done.

2. Materials and Methods

2.1. Plant material

Olive (Fig.1,a) and basil leaves (Fig.1,b) and licorice roots (Fig.1,c) were used to prepare the aqueous extract (Aqe). The three plants were procured from a local herbalist in Sétif. The choice of plants was made according to a survey done beforehand.



Figure 1: Olive leaves (a), basil leaves (b) and licorice roots (c)

2.2. Bacterial strains, culture media and chemical reagents

The antibacterial activity was evaluated on three bacterial strains of the American Type Culture Collection (ATCC): *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. Muller-Hinton agar medium (MHA), in addition to various chemical reagents, namely methanol, 2,2'-diphenyl-1-picryl hydrazyl (DPPH), dimethyl sulfoxide (DMSO), Butylated hydroxytoluene (BHT), bovine serum albumin (BSA), Aspirin and Gentamicin (GM) discs. These reagents come from different sources; Sigma, Fluka, Prolabo and Sanofi Diagnostic.

2.3. Preparation of the aqueous extract

The choice of the aqueous extract was made in accordance with the use. Practically, 20g of dried Olive leaves 20g of dried leaves of Basil and 40g of root of Licorice were each placed in 500ml of cold water. The mixtures were boiled for 15, 5, and 25 min respectively. Subsequently, the mixtures were filtered and subjected to ambient air drying to have the aqueous extract. The extraction's yields are calculated as following:

$$\text{Yield} = \frac{\text{The weight of the obtained extract}}{\text{the weight of the initial plant material}} \times 100 \quad (1)$$

2.4. Agar Diffusion Method

Discs of six mm diameter filter paper, impregnated with different concentrations (50, 100, 150, 200 mg/ml) of the Aqe, are deposited on MHA inoculated with a standardized inoculum at 0.5 Mc Farland density. Gentamicin is tested in simultaneously. The Petri dishes are then incubated at 37 ° C. for 24 hours. After incubation, the diameters of the inhibition zones are measured around each disc (Rahal et al., 2008). To determine if there a bactericidal or bacteriostatic effect, a sample from the inhibition zone is transferred to a Petri dish containing MHA and is incubated at 37°C for 24 h and then examined with the naked eye. Bacterial growth

indicates a bacteriostatic effect, while an absence of growth indicates a bactericidal effect of the extract tested.

2.5. In-vitro anti-inflammatory activity

Inhibition of protein denaturation is evaluated by the method of (Reshma et al. 2014) with slight modification. 500 µl of 1% BSA are added to 100 µl of plant extract with different concentrations (250, 500, 1000 µg/ml). This mixture is kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution is cooled down to room temperature and absorbance is recorded at 660 nm. Acetyl salicylic acid is taken as a positive control. The experiment is carried out in triplicates and percent inhibition for protein denaturation is calculated as follows:

$$\% \text{ Inhibition} = 100 - \frac{(A_s - A_p)}{A_c} \times 100 \quad (2)$$

Where A_s is the absorbance of the sample, A_p is the absorbance of the product control and A_c is the absorbance of the positive control.

2.6. DPPH free radical scavenging assay

The DPPH radical absorbs at 517nm and the antioxidative activity can be measured by monitoring the decrease in this absorbance. The method used is that of (Singh et al., 2006) slightly modified. Practically 50 µl aqueous extract of each plant with different concentrations are mixed with 1250 µl of 0.004% methanolic solution of DPPH. The absorbance is measured at 517 nm after 30 min of incubation in the dark. BHT is used as a standard and is subjected to the same treatment. The DPPH scavenging activity is calculated as following:

$$\% \text{ of DPPH scavenging effect} = \frac{(A_t - A_c)}{A_c} \quad (3)$$

Where A_t is the absorbance of the test, A_c is the absorbance of the control.

2.7. Statistical analysis

Values are expressed as mean \pm Sd. The results of the various tests are analyzed by the univariate ANOVA followed by the Dunnett and Tukey tests for multiple comparisons and determination of significance values. The statistics are made by Graphpad Prism 5

3. Results and discussion

3.1. The extraction yield

The yield obtained by decoction of Basil ($23.14 \pm 1.99\%$) was the highest compared to the other plants; Olive leaves ($5.82 \pm 0.09\%$) and Licorice ($5.29 \pm 4.17\%$) (Table1).

Table 1: The yield, color and appearance of the extracts obtained

	Yield (%)	Color	Consistency
Olive	5.82 ± 0.09	Dark brown	Sticky
Basil	23.14 ± 1.99	Black brown	Breakable Dryer
Licorice	5.29 ± 4.17	light brown	Very sticky

3.2. Antibacterial assay

Olive leaves (OLE) and Licorice roots extracts (LRE) were active on *E. coli* (13.5, 10mm) and *S. aureus* (14, 12mm) respectively at the highest concentration (200mg /

ml) with total resistance of *P. aeruginosa* (which is known to be resistant to many antibacterial agents) (Table 2).

Table 2: inhibition diameters in mm: each value represents 3 measures \pm SD

Plant extract	Strain	Concentration (mg/ml)				GM
		200	150	100	50	
Olive	<i>E.coli</i>	13.5 \pm 0,7	-	-	-	40
	<i>S. aureus</i>	14 \pm 2	-	-	-	37.5
	<i>P. aeruginosa</i>	-	-	-	-	26
Licorice	<i>E.coli</i>	10+	10 +	-	-	40
	<i>S. aureus</i>	12 \pm 1	8 \pm 0	10 +	7 +	37.5
	<i>P. aeruginosa</i>	-	-	-	-	26
Basil	<i>E.coli</i>	-	-	-	-	40
	<i>S. aureus</i>	-	-	-	-	37.5
	<i>P. aeruginosa</i>	-	-	-	-	26

-; no activity, +: decrease in bacterial density, GM: gentamycin.

3.3. Anti-inflammatory assay

The data of our study show that the maximum percentage of inhibition was observed from OLE, at 250 μ g/ml, slightly different ($P \leq 0.05$) from BLE, but not significantly different from LRE (Fig.2). There is also a concentration-dependent inhibition of protein (albumin) denaturation by both BLE and LRE throughout the concentration range of 250 to 1000 μ g/ml with a non significant difference at $P \leq 0.05$.

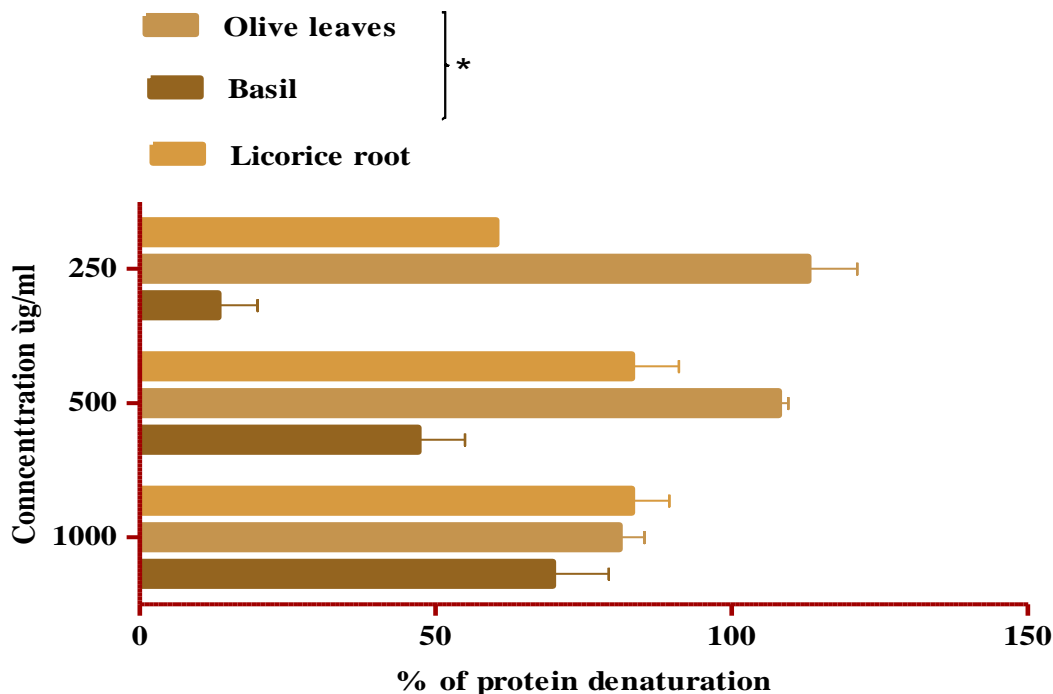


Figure 2: Inhibition of protein denaturation by the extracts and BHT
Values are means \pm SD of three replicates (n=3)

3.4. Anti-oxidative assay

The three extracts were less active than the BHT (0.08 mg/ml), OLE (0.65 mg/ml), BLE (0.91 mg/ml) and LRE (4.98 mg/ml) (Table 3).

Table 3: IC₅₀ values compared to BHT; Values are means ± SD of three replicates (n=3)

Extracts	IC ₅₀ (mg/ml)
BHT	0.08 ± 0.001
Olive leaves	0.65±0.01
Licorice roots	4.98 ±0.49
Basil leaves	0.91± 0.03

3.3.4. Discussion

Water is almost universally the solvent used to extract activity at home, dried plants can be ingested as teas (plants steeped in hot water) or, rarely, tinctures (plants in alcoholic solutions) (Cowan, 1999). The yield obtained by decoction of Basil was the highest compared to the other plants. This is due to the fact that the part used of the plant influences its extraction yield; Basil leaves are fine; those of the Olive are hard as far as for the Licorice root, which influences the exchange surface between the solvent (water) and the used part of the plant, despite the prolongation of the extraction time. According to (Dhanani et al., 2017), in conventional extraction, heat is transferred through convection and conduction from the surface, the extractability of solvents depends mainly on the solubility of the compound in the solvent, the mass transfer kinetics of the product and the strength of solute/matrix interaction with corresponding limitations on heat and mass diffusion rate. Decoction is the method of choice when working with tough and fibrous plants, barks and roots and with plants that have water-soluble chemicals (Handa et al., 2008). Despite all, the aqueous hot decoction remains effective, environmentally friendly in terms of solvent, safe and not expensive.

3.2. Antibacterial assay

Olive leaves and Licorice roots extracts were active on *E. coli* and *S. aureus* at the highest concentration with total resistance of *P. aeruginosa*. The same, the OLE, tested by (Aliabadi et al. 2012), showed inhibitory effects at 50mg/ml on *S. aureus* (9mm) and *E. coli* (8.2mm). There was even inhibition of biofilm formation of methicillin susceptible *S.aureus* and a decrease in expression and production of exotoxins (hemolysins) and enterotoxins (Rohinishree and Negi, 2016; Alhamd et al., 2015). Indeed, there is compelling scientific evidence that Olive leaf polyphenols are bioactive (antiviral and antimicrobial). The polyphenol content in the olive leaf could range from 1.5 to 7.0 g/100g. Oleuropein and other secoiridoids are the principal (De Leonardis et al., 2008). The water-soluble compounds, such as polysaccharides and polypeptides, are commonly more effective as inhibitors of pathogen adsorption (Cowan, 1999). While BLE was completely inactive on the three strains tested. In a similar study, (Hiltunen and Holm (1999) have found that the aqueous extracts or infusions of *O. gratissimum* showed no activity against *Salmonella* spp., *Shigella* sp. *S. aureus* and *E. coli*. This indicates that the antibacterial principles of this species are not water soluble. By testing several types of solvents, Bacon et al., (2016) found that the extracts from the hot water extraction method showed no inhibition compared with the ethanolic and methanolic extracts. Furthermore, fractionation confirmed the relative hydrophobic nature of active compounds. Contrariwise, antimicrobial activity of Basil has been found against such organisms as *B. cereus*, *S. aureus* (10mm), *E. coli* (7.5 mm) (Udochukwu et al., 2015), because Basil contains antimicrobial compounds, one of them being eugenol. these differences in antimicrobial properties of a plant extract is attributable to the age of the plant used, the freshness of plant materials, physical factors (temperature,

light water), contamination by field microbes, adulteration and substitution of plants, incorrect preparation and dosage (**Okigbo and Mmeka, 2008**). It should be noted that the recorded activities are all bacteriostatic.

3.3. Anti-inflammatory assay

Denaturation of proteins is a well-documented cause of inflammation because non-steroidal anti-inflammatory drugs (NSAIDs) are reported to possess prevention of the denaturation of proteins, which act as auto-antigens and leads to auto-immune diseases or arthritis diseases (**Karthik et al., 2013; Chatterjee et al., 2012**). In this survey, the maximum percentage of inhibition was observed from OLE. Phenolic compounds, in Olive leaves, including flavones, flavonols (rutin), flavan-3-ols (catechin), substituted phenols (tyrosol, vanillin and caffeic acid) and oleuropein can be responsible of its activity (**Abaza et al., 2015**). In a close case, the aqueous suspension of *Ocimum sanctum* leaves inhibited acute as well as chronic inflammation in rats in a way dose dependent effect (**Hiltunen and Holm, 1999**). Study of *Ocimum basilicum* crude methanolic extracts exhibited anti-inflammatory activity as evidenced by the inhibition of the key pro-inflammatory cytokines and mediators (**Marwat et al., 2011**). The compounds of Licorice root, the glycyrrhizin, glycyrrhetic acid and glabridin were confirmed to have anti-inflammatory properties (**Ghannad et al., 2014; Tian et al., 2008**). The anti-inflammatory mechanism of Licorice implies the exhibition of a steroid like anti-inflammatory activity, similar to the action of hydrocortisone; this is due in part, to inhibition of phospholipase A2 activity, inhibition of cyclooxygenase activity and prostaglandin formation and inhibition of platelet aggregation (**Zadeh et al., 2013**).

3.4. Anti-oxidative assay

The three extracts were less active than the BHT. Despite this, the IC₅₀ values remain good by comparing them to other studies; OLE from Turkey 59µg/ml (**Orak et al., 2012**), from Iran 121.05 µg/ml (**Rafiee et al., 2012**), BLE from Egypt 53 mg/ml and LRE from Egypt 81% at 100 µg/ ml (**Lawal et al., 2016**). Actually, there are many substances responsible for the anti-oxidative effect such as β-carotene, tocopherol, eugenol, rosmarinic and caffeic acids and linalool in Basil (**Hiltunen and Holm, 1999; Gbadegesin and Odunola, 2010**). In Licorice, glabridin has been reported to affect protection of mitochondrial functions from oxidative stresses (**Tian et al., 2008**). Experiments revealed that water extracts have anti-oxidative effects which are concentration-dependent (**Fig. 3**) which corroborates the results found by (**Marwat et al. 2011**). According to (**Cowan 1999**), most active components are not water soluble, this may be due to ability of organic solvents to extract the maximum of bioactive compounds.

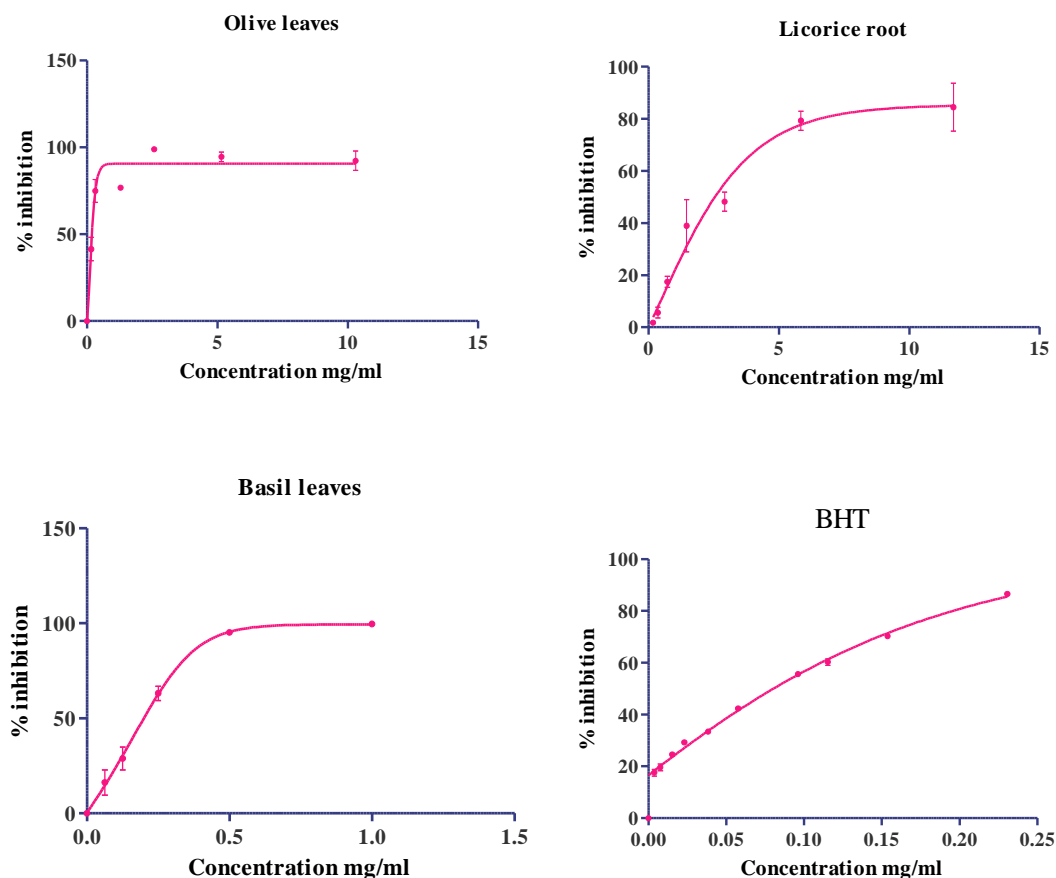


Figure 3: DPPH scavenging effect of the extract and this of BHT
Values are means \pm SD of three replicates (n=3),

4. Conclusions

Our study supports the use of active in treating infections and inflammation, which could be further evaluated by other methods. However, further studies, including the analysis of the extracts may result in the development of potent bioactive agents with the minimum of toxicity. To confirm the use and to compare the results found with previous works, it will first be necessary to standardize the conditions of cultivation of the plants as well as the techniques of extraction, the conditions of picking, packing, drying and storage conditions in addition to the sale by herbalists which should be controlled by the local authorities.

5. Conflict of interest statement

We certify that there is no conflict of interest with any financial organization in the subject matter or materials discussed in this manuscript.

6. Authors' biography

No Biography

7. References

- [1] Abaza L. Taamalli A. Nsir H and Zarrouk M. Olive Tree (*Olea europaeae* L.) Leaves: Importance and Advances in the Analysis of Phenolic Compounds. *Antioxidants*, 2015. 4:682-698. doi: 10.3390/antiox4040682. PMID:26783953. PMCID: PMC4712944.
- [2] Alhamd AKJ. Improvement extraction of crude compounds from iraqi olive leaves by applying water-base and alcoholic-base extraction and their biological application. 2015. *World J Pharm Sci*, 4; 1 :183-200. doi: 10.3390/antiox6020034. PMCID: PMC5488014. PMID: 28513570.
- [3] Aliabadi MA. Darsanaki RK. Rokhi ML. Nourbakhsh M. and Raeisi G. Antimicrobial activity of olive leaf aqueous extract. *Ann Biol Res*, 2012. 3(8):4189-4191. Available online at: www.scholarsresearchlibrary.com.
- [4] Bacon K. Boyer R. Denbow C. O'Keefe S. Neilson A. and Williams R. Evaluation of different solvents to extract antibacterial compounds from jalapeno peppers. 2016. *Food Sci Nutr*, 1-7. doi: 10.1002/fsn3.423. PMCID: PMC5448369. PMID: 28572934.
- [5] Bouzabata A. Les médicaments à base de plantes en Algérie: réglementation et enregistrement. 2016. *Rev Phytother*. 1-8. DOI 10.1007/s10298-016-1089-5.
- [6] Chatterjee P. Chandra S. Dey P. and Bhattacharya S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative *in vitro* study. *J Adv Pharm Technol Res*, 2012. 3(2): 136-138. doi: 10.4103/2231-4040.97298. PMCID: PMC3401676 PMID: 22837963
- [7] Cowan MM. 1999. Plant product as antimicrobial agents. *Clin. Microbiol. Rev*, 12 (4), 564-582. DOI: 10.1128/CMR.12.4.564. PMID: 10515903 PMCID: PMC88925
- [8] De Leonardis A. Aretini A. Alfano G. and Giancarlo R. Isolation of a hydroxytyrosol-rich extract from olive leaves (*Olea Europaea* L.) and evaluation of its antioxidant properties and bioactivity. 2008. *EUR FOOD RES TECHNOL*. 226(4):653-659. DOI: 10.1007/s00217-007-0574-3.
- [9] Dhanani T. Shah S. Gajbhiye NA. and Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *ARAB J CHEM*, 2017. 10(1): S1193–S1199. doi.org/10.1016/j.arabjc.2013.02.015.
- [10] Gbadegesin MA. and Odunola OA. Aqueous and ethanolic leaf extracts of *Ocimum basilicum* (sweet basil) protect against sodium arsenite-induced hepatotoxicity in Wistar rats. 2010. *Nig. J. Physiol. Sci*, 25 : 29- 36. PMID: 22314900.
- [11] Ghannad MS. Mohammadi A. Safiallahy S. Faradmal J. Azizi M. and Ahmadvand Z. The Effect of Aqueous Extract of *Glycyrrhiza glabra* on *Herpes Simplex Virus 1*. *Jundishapur J Microbiol*, 2014. 7(7): e11616. doi: 10.5812/jjm.11616. PMCID: PMC4216581. PMID: 25368801.
- [12] Karthik K. Kumar BR. Priya and V. KumarS. Evaluation of anti-inflammatory activity of *Canthium parviflorum* by in-vitro method. *Indian Journal of Research in Pharmacy and Biotechnology*, 2013. 729-730.
- [13] Orak HO. Isbilir SS. and Yagar H. Determination of antioxidant properties of lyophilized olive water extracts obtained from 21 different cultivars. *Food Sci Biotechnol*. 2012. 1065-1074. DOI 10.1007/s10068-012-0138-6.
- [14] Lawal B. Shittu OK. Oibiokpa FI. Berinyuy EB. and Mohammed H. African natural products with potential antioxidants and hepatoprotectives properties: a review. *Clinical Phytoscience*, 2016. 2 ; (23):1-66. DOI: 10.1186/s40816-016-0037-0.
- [15] Zadeh JB. Kor ZM. And Goftar MK. Licorice (*Glycyrrhiza glabra* Linn) As a Valuable Medicinal Plant. 2013. *IJABBR*, 1(10): 1281-1288.
- [16] Singh G. Marimuthu P. De Heluani CS. and Catalan CAN. Antioxidant and biocidal activities of *Carum nigrum* (seed) essential oil, oleoresin, and their selected components. *J Agric Food Chem*, 2006. 54: 174-181. DOI: 10.1021/jf0518610. PMID:16390196.

- [17] Rebbas K. Bounar R. Gharzouli R. Ramdani M. Djellouli Y. and Alatou D. Plantes d'intérêt médicinaux et écologique dans la région d'Ouanougha (M'Sila, Algérie). *Rev Phytother*, 2012. 10:131-142. DOI: 10.1007/s10298-012-0701-6.
- [18] Marwat SK. Rehman FU. And Khan MS. Ghulam S. Anwar N. Mustafa G. et al. Phytochemical constituents and pharmacological activities of sweet basil *Ocimum basilicum* L. (Lamiaceae). *Asian J. Chem*, 2011. 23(9): 3773-3782.
- [19] Rohinishree YS. and Negi PS. Effect of extract on cell viability, biofilm formation and exotoxin production by *Staphylococcus aureus*. *J Food Sci Technol*, 2016. 53(2):1092-1100. DOI:10.1007/s13197-015-2131-6. PMID:27162389b PMCID: PMC4837708.
- [20] Tian M. Yan H. and Row KH. Extraction of glycyrrhizic acid and glabridin from licorice. *Int J Mol Sci*, 2008. 9(4):571-7. PMCID: PMC2635700. PMID: 19325770.
- [21] Udochukwu U. Omeje FI. Uloma IS. And Oseiwe FD. Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. 2015. *Am. J. Res.* 3(5):225-235.
- [22] Okigbo RN. and Mmeka EC. Antimicrobial effects of three tropical plant extracts on *Staphylococcus Aureus*, *Escherichia Coli* and *Candida Albicans*. *Afr J Tradit Complement Altern Med*, 2008. 5(3). PMID: 20161941. PMCID: PMC2816559. DOI: 10.4314/ajtcam.v5i3.31277.
- [23] Rafiee Z. Jafari SM. Alami M. and Khomeiri M. Antioxidant effect of microwave-assisted extracts of Olive leaves on Sunflower oil. *J. Agr. Sci. Tech*, 2012. 14: 1497-1509.
- [24] Reshma. Arun KP. and Brindha P. *In vitro* anti-inflammatory, antioxidant and nephroprotective studies on leaves of aegle Marmelos and *Ocimum sanctum*. *Asian J Pharm Clin Res*, 2014. 7(4) : 121-129.
- [25] Hammiche V. Merad R. and Azzouz M. Plantes toxiques à usage médicinaux du pourtour méditerranéen, 2013 Springer-Verlag France, Paris.
- [26] Handa SS. Singh SP. Longo KG. and Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. International Centre for Science and High Technology, 2008. Italy.
- [27] Rahal K. Standardisation de L'antibiogramme en Médecine Humaine à l'Echelle Nationale selon les recommandations de l'OMS, fourth ed. Ministère de la Santé, de la Population et de la Réforme Hospitalière, 2005. Algiers.
- [28] Lim TK. Edible medicinal and non-medicinal plants. 2016. Springer. Vol 10.
- [29] Hiltunen R. and Y Holm. Basil The Genus *Ocimum*. Tenth volume, harwood academic publishers, 1999. Amsterdam.