ORIGINAL ARTICLE

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Phytochemical, antioxidant and antibacterial activities of the aqueous and ethanol extracts of *Pinus halepensis*

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ABSTRACT

The present study was designed to evaluate the phytochemical screening of different extracts(Aqueous and ethanol) of the needles of *Pinus halepensis*; to explore their antioxidant activities with the different tests and to determine their antibacterial proprieties. Our results revealed that these extracts contain polyphenols, flavonoids and tannins. Then, the same extracts(Aqueous and ethanol)present the highest content of total phenol and tannins which were of order respectively(735.41 ± 0.09 µgGAE/ mg of extract and 49.60 ± 0.012 µg CE /mg of extract compared with other extracts. The results showed alsothat the different extracts had an important antioxidant andantibacterial activity. Overall, the richness of the different extract of *Pinus halepensis* with total polyphenols, flavonoids and tannins explained the biologic propriety of this plant.

Keywords: Antibacterial activity, Antioxidant activity,*Pinus halepensis*, phytochemical study.

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INTRODUCTION

The phytotherapy still as the most discipline that treats some functional disorders. Therefore, the most molecules and the principal bioactive constituents were discovered and exploited by the medicinal plant. Among the bioactive, there are the tannins, flavonoids, carbohydrates, vitamins, anthocyanins, coumarins, saponins, alkaloids, glucosides, proteins, lipids and essential oils.

All these active ingredients make the interest of numerous scientific works for phytotherapeutic applications. In the context, the present work has been undertaken to evaluate the antioxidant and antibacterial activities of the extracts of the aerial part of *Pinushalepensis*, a plant known to the name "Aleppo pine", belongs to the family Pinaceae (Pinaceae). This plant is widely used in traditional medicine.[1] Previous studies had shown that it presented the antioxidant, antibacterial, analgesic, antifungal, anticancer, anti-inflammatory and antiviral, properties.[2,3,4] Based on these beneficial properties cited for *Pinushalepensis* and published by our previous studybo.[5,6] It is interesting to complete this study by exploring other biological and antioxidant activities for the same plant, for that we aimed to (a) evaluate the phytochemical activity of various extracts of *Pinus halepensis*; (b) to explore antioxidant activities and antibacterial of the different extracts of *Pinus halepensis*.

METHODOLOGY

Chemicals and reagents

All chemicals used were purchased from Sigma-Aldrich, Mo, USA including quercetin, catechin, butylatedhydroxytoluene (BHT), 2-thiobarbituric acid, methanol, ethanol, sodium hydroxide, gallic acid, aluminiumtrichloride, hydroxyde of sodium, sodium phosphat buffer, potassium ferricyanide, ferric chloride. Commercials Kit obtained from Spinreact, Spin.

Collection of plant material

The needles of *Pinushalepensis* were collected during October and November 2018 from the region of Sidi Aich (Gafsa, Tunisia). The region is characterized by Longitude: 8.8E Latitude: 34.683N Altitude: 522 m, rainfall: 150 mm/year. The needles were washed with distilled water and air dried at room temperature of 25C° for 5 days in shaded and ventilated space.

Preparation of extracts

The different extracts of the needles of *Pinus halepensis* were obtained with the follow steps; firstly, the needles were ground to fine powder by a mortar. Then, the powder dissolved in 10 mL of distilled water, methanol and ethanol respectively, and they were macered for 24 h for threetimes at room temperature with magnetic stirring. After

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filtrating, the recovered extracts were centrifuged at 3000 rpm / 10 min. The filtrates were entirely evaporated under vacuum at 40°C usinga Büchi rotary evaporator to obtain the dry extract (AEP, EEP).

Yields of extracts

The yields of different extracts were calculated as follows: R= (P h/P v) × 100

With R= Yield by%. P h = Weight of extract (g). P v = Weight of biomass vegetal (g)

Phytochemical screening

The following phytochemicals in each extract were identified: polyphenols, flavonoids and tannins.

Test for phenolic compoundsAccording with the method of Rosine&Momo,[7] a small amount of each extract was dissolved in 5 mL of distilled water, and a few drops of 1% (w/v) ferric chloride were added. The appearance of a dark green color indicates the presence of phenolic compounds.

Test for flavonoidsThe test was carried outaccording to the method of Karumi et al.[8] In Brief, a few drops of sodium hydroxide were added to dissolve in distilled water, the appearance of an intense yellow color indicates the presence of flavonoids. Disappearance of the color after the addition of diluted hydrochloric acid confirms the presence of flavonoids.

Test for tanninsThe method consists of mixing a solution of ferric chloride 1% (w/v) was added with 1 mL of extract. The appearance of green color indicates the presence of tannins.[8]

Test for coumarinsThe test consists to dissolve an amount of each crude extract in distilled water. Then, 3 mL of NaOH 10% (w/v) were added. The appearance of yellow color indicates the presence of coumarins.

Determination of Total Phenolic Compounds

The polyphenols of each extract were determined by the Folin-Ciocalteu method.9 50 µL of diluted solution of each extract was mixed with 400 µL of FolinCiocalteu reagent. This mixture incubated for 8 min at room temperature and 500 µL of sodium carbonate solution(7.5%) was added. After 1 h of incubation, the absorbance was measured at 725 nm against water blank. A standard calibration curve was plotted using gallic acid (50–200 µg/L). The results were expressed as µg of gallic acid equivalent (GAE)/mg of extract.

Total Flavonoids Determination

According to method of Zhishen et al,[10] the total flavonoids were estimated. Briefly, 500 µL of diluted solution of each extract was mixed with 0.75 mL of 5% sodium nitrite solution. 10% aluminum chloridesolution was added and the mixture was left standingfor 5 min, and then 0.5 ml of 1 M sodium hydroxidewas added to the solution. The volume of the mixture wasadjusted to 2.5 mL with distilled water and mixed well.

After 15 min, the absorbance was read at 510 nm against a blank sample. A standard calibration curve was plotted using quercetin (50–200 µg/L). The results were expressed as µg of quercetin equivalent (QE) /mg of extract.

Condensed Tannin Content

The content of Tannin was determined according to the method of Ba et al.[11] 400 µL of diluted solution or standard was added to 3 mL of solution of vanillin and 1.5 mL of hydrochloric acid. The solution incubated for 15 min. Then, the absorbance was read at 500 nm. The concentrations were determined by a standard calibration curve was plotted using catéchin (0–300 µg/mL). The results were expressed as µg catechin equivalent (CE) /mg of extract.

Antioxidative capacity in vitro

DPPH radical scavenging assay

This activity was assessed according to the method of Blois[12] with a little modification. In brief, 25 µL of various concentrations (50-200 µg/mL) were mixed with 975 µL of DPPH solution. Then the mixture was shaken and incubated for 30 min in dark. The control used contained with 1 mL of methanol. BHT was used as the standard antioxidant. The absorbance was measured at 515 nm, and the results were expressed by the percent of inhibition (I%) and calculated according to the following equation:

$$I\% = \frac{(DOA\text{-}DOB)}{(DOA)} \times 100$$

With: DOA is the absorbance of control, and DOB is the absorbance in the presence of the extract or standard sample. All determinations were performed in triplicate.

Ferric Reducing Antioxidant Power (FRAP)

The capacity of the extracts to transform Fe^{3+} to Fe^{2+} was according to the method of Yen and Chen.[13] In brief, 2.5 mL of various concentrations of each extract (100-500 µg/mL) was dissolved in 1 mL with distilled and mixed with 2.5 mL of sodium phosphate buffer (0.2 M,pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v in distilled water). The solution was incubated in a water bath at 50C° for 20 min. After incubation, 2.5 mL of trichloroacetic acid solution (10%) was added to stop the reaction.

Then, the mixture was centrifuged at 3000 rpm for 10 min. 2.5 mL of aliquot of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of a 0.1% (w/v) solution of ferric chloride ($FeCl_3$). The absorbance was read at 700 nm and BHT was used as an authentic standard.

Antibacterial activity

Test microorganisms

The antibacterial activity of each extract of *Pinus halepensis* (1mg/mL) was tested against four strains of bacteria: Gram-positive bacteria including Bacillus cereus and Gram-negative bacteria including *Pseudomonaaeroginosa*, *Escherichia coli*, *klebsellapneumaria*. The bacteria are identified and obtained from the hospital of region of Gafsa.

Paper disc diffusion method

The bacterial strains were first grown on Muller Hinton medium to obtain isolated colonies. After 24 h of incubation at 37°C, four or five colonies were isolated and transferred into a tube contained distilled water and adjusted to the 0.5 McFarland turbidity standard. According to the method of Ngameni et al,[14] the sterile filter discs with 6 mm in diameter were dropped with 10 µL of each extract were placed on the infusion agar seeded with bacteria. Standards disc contained*Inepenum*served as positive antibiotic controls. The petri dishes were kept for 1h and incubated at 37°C for 24 h. The results were expressed by measuring the zone of growth inhibition surrounding the discs.

Analyse statistique

In vitro results are expressed as mean ± SD. IC50 values (50% inhibitory concentration) are calculated using the linear regression method from of the curve [% inhibition= f (concentrations)].

Table 1 **Yields of different extracts of *Pinus halpensis*. Values are the mean ± standard deviations of three independent experiments**

	AEP	EEP
Yields (%)	5.13±1.02	32.66±3.78

AEP: Aqueous extract, EEP: Ethanol extract

Table 2 **Phytochemical composition of different extracts of needles of *Pinus halepensis***

Phytochemicals	*Polyphénols*	*Flavonoids*	*Tannins*	*Coumarines*
EEP	+ +	+	+	+ +
AEP	+	+ +	+ +	+

+ presence
AEP: Aqueous extract, MEP: methanolic extract, EEP: Ethanolic extract

RESULTS AND DISCUSSION

The medicinal plants are still the source of active compoundsknown by their therapeutic proprieties. Based on the context, the present study evaluated the biological activities of the two extracts of *Pinus halepensis* based on the phytochemical analyses.

In the present work, the yields of various extracts of the needles of *Pinus halepensis* were determined (Table 1). Indeed, the highest yield was found in the ethanol extract (32.66±3.78%) compared with aqueous extractwhich were in the order of (5.13±1.02%). These results confirmed with the study of Benaziza[15] who revealed that the yield of aqueous extract of *Pinus halepensis* gowned in Algeria which was in the order 3%. However, the other yields of EEP and MEP were stronger compared to other solvent of *Pinus halepensis* such us 3.16 and 1.07% for the n-butanol and water acetone extracts.[15] Indeed, the variety of the yields in the different extract was due to the solvent of extraction used, which the solvent the most polar show the important yield of extraction compared to less polar solvent.Then, the differences are due to several factors including the origin geography, the climatic factors, the plant species, the organ of plant, the stade of growth, the time of collecting, the conservation of the plant and method of extraction method.[16]

According to the present study, to explain the richness of the various extracts with the bioactive compounds, the study *in vitro* was conducted to report the presence of phenolic compounds, the flavonoids and tannins in the various extracts (Table 2). In fact, the total polyphenol content of the different extracts of *P. halepensis* showed that the highest content was obtained in the AEP (735.41 ± 0.09 µg GAE/mg of extract), whereas the highest Flavonoid content was found in the EEP (69.82±0.016 µg QE/mg of extract). The table 3 showed also that the AEP contained the highest amount of condensed tannins (52.64±7.05 EQ/ mg of extract). Then, previous studies found that the distilled water is the most solvent recommendedforextracting the maximum of phenolic compound.[17]

In addition,the content of polyphenol, flavonoids and tannins inducted an important antioxidant that can scavenge the radicals and enhanced the status defense of the antioxidant. Indeed, the antioxidant activity of the various extract of *Pinus halepensis* were evaluated using two methods includingtest DPPH and the reducing power (FRAP).

Table 3 **The phytochemical compositions in each extract of *Pinus halepensis***

Extract	APE	EEP
Total Phenolics (µg GAE/mg of extract)	735.41 ± 0.09	597.95 ±0.14
Flavanoids (QE/mg of extract)	49.60± 0.012	69.82±0.016
Condensed Tannin (µg E C/ mg of extract)	52,64±7.05	43,52 ± 4.9

Values are expressed as mean ± standard deviation (n =3).

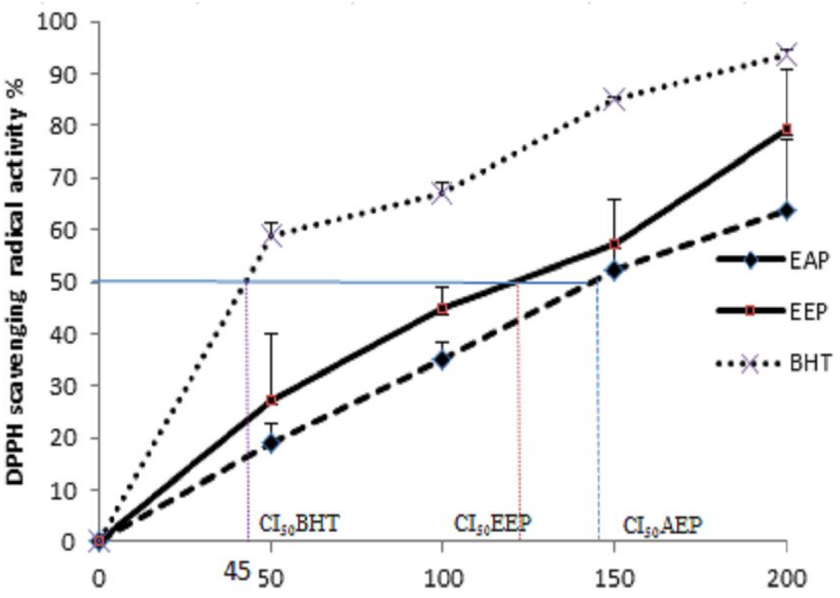

Figure 1 DPPH free radical scavenging activity of various extracts of ***Pinushalepensis.*** Values are means of three replications ± SD

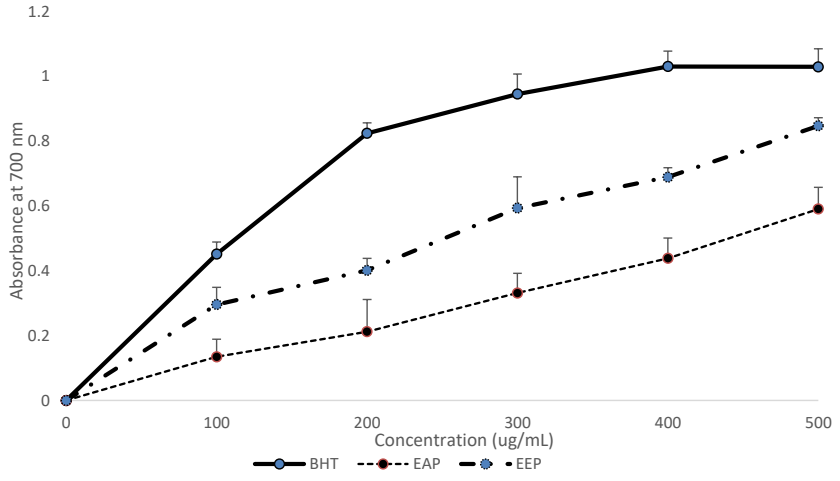

Figure 2 The reducing power of ***Pinus halepensis*** and BHT by the FRAP assay. Values are expressed as mean ± standard deviation (***n*** = 3)

Based on the DPPH test, The results were expressed by a percentage of inhibition of the radical DDPH or can also be express by the parameter IC_{50}, which defined as the concentration that caused a 50% loss of DPPH activity that has been determined from the curve. Therefore, the results showed that the percent of inhibition of DPPH increased with the concentration of the different extracts. The IC_{50} were: 120 µg / mL, 145 µg / mL for aqueous and ethanol extracts showing antioxidant activities lower than that of BHT which was order 45 µg / mL) (Figure 1). The obtained result showed that these extracts presented an important antioxidant capacity for the DPPH radical scavenging compared to the results obtained by Nigro[18] which revealed the IC_{50} in order to 61.46 µg/mL for the aqueous extract of the needles.

Indeed, the richness of the extracts of the various bioactive compounds neutralized the radicals and reinforced the statute of the antioxidant defense system. It has been known that the antioxidants molecules including ascorbic acid, tocopherol, flavonoids and tannins reduced and discolored the DPPH due to their ability to give up hydrogen.[19]

Inaddition, to evaluate the antioxidant activities of *Pinushalepensis*, the reducingpower has been determined. The presence of the reductants caused a reduction in the complex Fe^{3+} to Fe^{2+}. The total of extract of our study presented lowerantioxidant activity compared to the molecules of the reference BHT which have the absorbance 00.84 and 0.95 at the concentration 500 µg/mL respectively for AEP and EEP (Figure 2). Therefore, the reducer potential is due to the presence of group hydroxyl in the phenolic compounds which can intervene as donors of the electron.

Concerned to the antibacterial activity, the results of the present study showed that the various extracts of *Pinushalepensis* had antibacterial activities against the strains *Esherchiia coli*, *Bacilus cereus* and*Klebsella pneumari* (Figure 3). These results confirmed with the study of Feng et al.[20] who reported that the aqueous extracts of *Pinus halepensis* exerted an activity antibacterial against the strains *E.coli*, *B. cereus, B. subtilis, S. aureus, M. luteus.* However, the results of our study showed that the various extract of *Pinushalepensis*don't exert an antibacterial activity against *Pseudomonasaeruginosa.* The results confirmed with the study of Fekih[21] which found that the oil of *Pinus halepensis* was inactive against the type of bacteria using the method of diffusion of the disc. The deductions indicated the

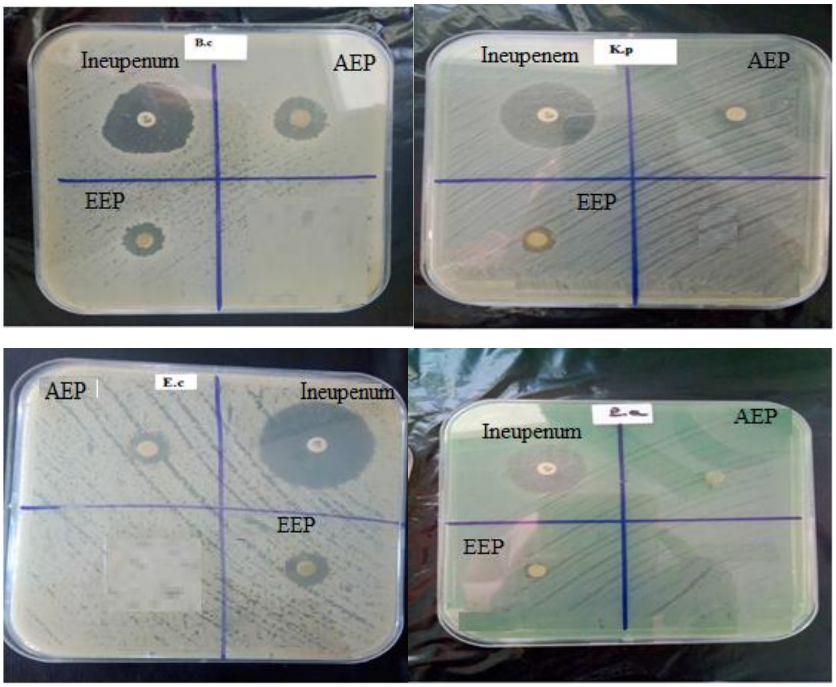

Figure 3 Anti-microbial activity of various extracts of needles of ***Pinus halepensis*** against tested microorganisms. (a): ***Escherichia coli;*** (b): ***Bacillus cereus;*** (c): ***Klebsella pneumaria;*** (d): ***Pseudomona aeroginosa***

strong capacity of antioxidant of the various extract and their richness with various principle actives.

CONCLUSION

Our findings found that the various extract *Pinus halepensis* exerted *in vitro* antioxidant activities and antibacterial activity. It could be considered as a source of polyphenols and as an extract with potential antioxidant activity.The same study encourages us to explore others findings in cells to evaluated the beneficial effects of these extracts against prooxydant induced damage.

DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

REFERENCES

1. Dob, R.I., Szachowicz-Petelska, B., Skrzydlewska, E., Figaszewski, Z. (2008).Effects of green tea on physicochemical properties of liver cell membranes of rats intoxicated with ethanol. Polish J. Environ. 17 (3), 327–333.
2. Sadou, N., Seridi, R., Djahoudi, A., Youcef, H. (2015). Chemical composition and antibacterial activity of the needls essential oil of Pinushalepensis Mill.from north east of Algeria. Rev. Sci. Technol. Synthèse.30, 33-39.
3. Ghedira, K. (2005). Les flavonoïdes: structure, propriétés biologiques, rôle prophylactique et emplois en thérapeutique. *Phytothérapie*IEC-6 cells. Archives of Physiology and Biochemistry. 364-370.
4. Kabera, JN., Semana E., Mussa AR., He X. (2014).Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. Journal of Pharmacy and Pharmacology. 2, 377-392.
5. Bouzenna, H, Samout, N, Dhibi, S, Rjeibi, I, Elfeki, A, Hfaiedh, N.(2016). Protective Effects of *Pinushalepensis L.* Essential Oil on Aspirin-induced Acute Liver and Kidney Damage in Female Wistar Albino Rats.J OleoSci.65(8), 701-12.
6. Bouzenna,H.,Hfaiedh,N.,Bouaziz,M.,Giroux-Metges,M-A., Elfeki, A., Talarmin, H. (2017). Cytoprotective effects of essential oil of *Pinushalepensis*L. against aspirin inducedtoxicity in in rats. Biomed Pharmacother. 2016; 83: 1327-1334.
7. Rosine, C., Momo, D. (2009). Évaluation de l'activité antidermatophytique des extraits auméthanol et fractions d'Acalyphammahirtum (melastomatacees). Université de Dschang –Master en biochimie clinique et pharmacologie.
8. Karumi, Y., Onyeyili, PA., Ogugbuaja., VO. (2004). Identification of active principales of M. balsamina (Balsam apple) leaf extract. J Med Scien.4, 179 – 182.
9. Wong, CC.,Li, HB., Cheng, KW.,et al. (2006). Systematic survey of antioxidant activity of 30chinese medicinal plants using the ferric reducing antioxydant power assay. Food Chem. 97, 705 – 11.
10. Zhishen, J., Mengcheng, T.,Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem.64, 555 – 559.
11. Ba, K., Tine, E., Destin, J., Cissé, N., Thonart P. (2010). Etude comparative descomposés phénoliques, du pouvoir antioxydant de différentes variétés de sorghosénégalais et des enzymes amylolytiques de leur malt. Biotechnol. Agron. Soc. Environ.14(1), 131-139.
12. Blois, M.S. (1958).Antioxydant determinations by the use of a stable free radical.Nature.26, 1199 – 1200.
13. Yen, GC.,Chen HY. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem.43, 27 – 37.
14. Ngameni, B., Kuete, V., Simo, I.K, Mbaveng, A.T, AwoussongP.K, PatnamR., Roy R., Ngadjui B.T. (2009). Antibacterial and antifungal activities of the crude extract and compounds from Dorsteniaturbinata (Moraceae). South African J Botany.75, 256-261.
15. Benaziza. (2017). Contribution à l'étude phytochimiqueet l'activité antioxydante des extraits de feuilles et de cônes de *Pinushalpensis*(Pin d'Alep).
16. Bourgou, S., Pichette, A., Lavoie, S., Marzouk, B., Legault, J. (2012). Terpenoids isolated from Tunisian *Nigella sativa* L. essential oil with antioxidant activity and the ability to inhibit nitric oxide production. FlavourFragrJ. 27, 69–74.
17. Trabelsi, N., Megdiche, W., Ksouri, R., Falleh, H., Oueslati, S., Soumaya, B., Abdelly, C. (2010). Solvent effects on phenolic contents and biological activities ofthe halophyte *Limoniastrummonopetalum*leaves. LWT-Food Science and Technology.43(4), 632-639.
18. Nigro, H. (2016). Contribution à l'étude phytochimique et à l'effet antioxydant des extraitsde Pinushalepensis. Mémoire En vue de l'obtention du Diplôme de MASTER.78p.
19. Talbi, H., Boumaza, KM., Talbi, J., Hilalai A. (2015). Evaluation de l'activitéantioxydante et la composition physic-chimique des extraits méthanolique et aqueuxde la Nigellasativa L. Mate-Environ. Sciences.6(4), 1111-1117.
20. Feng, S., Zeng, W., Luo, F, Zhao., J, Yang, Z., Sun, Q. (2010). Food Sci.Biotechnol.19(1), 35-41.
21. Fekih, N. (2014). Proprietes chimiques et biologiques des huiles essentielles de trois espèces du genre pinus poussant en algerie. Thèse en vue de l'obtention du diplôme de Doctorat Es-Sciences en Chimie.Option: Chimie Organique Appliquée.