Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2019; 11(3);205-213
doi: 10.25258/phyto.11.3.16

ISSN: 0975-4873

Research Article

Study of Biological Activities of Essential Oil and Extracts from The Hydrodistillation Residue of Anise (*Pimpinella Anisum*)

Lemjallad Lamiaa[1*], Sayah Mohamed Yassine[1], Zaid Yassir[1], Errachidi Faouzi[2], El Ghadraoui Lahsen[2], Chabir Rachida[3], Ouzzani Chahdi Fouad[1], Kandri Rodi Youssef[1]

1Department of Chemistry, Laboratory of Applied Organic Chemistry, Faculty of Sciences and Technologies, University Sidi Mohamed Ben Abdellah, B.P. 2202-route Imouzzer, Fez, Morocco.
[2]Laboratory of functional ecology and environment, Faculty of sciences and technologies, University Sidi Mohamed Ben Abdellah, B.P. 2202-route Imouzzer, Fez Morocco.
3Team of Nutrition, Agri-Food and Environment. Laboratory of Human Pathology, Biomedicine and Environment. Faculty of Medicine and Pharmacy, University Sidi Mohamed Ben Abdellah. P.B. 1893 Km 2200 Road Sidi Harazem. Fez. Morocco.

Received: 3rd Feb, 19; Revised 7th May, 19; Accepted 15th May, 19; Available Online:25th Jun, 19

ABSTARCT

Essential oil, methanol extract from the fresh aniseed and the extracts obtained by serial solvents from the residue recovered from the hydrodistillation of *Pimpinella anisum* were investigated for their scavenging activity using DPPH method. Results showed that highest radical scavenging effect was found in the essential oil and methanol extracts. The later contained the highest amount of phenolic and flavonoid compounds. Total phenolic contents was determined by using the Folin-Ciocalteu reagent with the values 10.71 - 28.34 – 1.06 and 0.93 (mg Gal eq /g of aniseed) respectively in aqueous, methanol, acetone and ethyl acetate extract. Total flavonoid content is evaluated using the method $AlCl_3$ with the values 8.89 - 25.92 - 0.74 and 0.77 (mg Qer eq /g of aniseed) respectively in aqueous extracts, methanol, acetone and ethyl acetate. As a comparison of the amount of phenolic and flavonoid compounds, we did a methanol extraction of the fresh aniseed. The total phenolic and flavonoid content of methanol extract of fresh aniseed respectively is 36.98 (mg Gal eq/g of aniseed) and 31.80(mg Qer eq /g of aniseed). The antimicrobial activities of aniseed essential oil was tested against Gram negative bacteria, Gram positive bacteria and fungi. The essential oil of the green anise had an inhibitory effect against all the strains tested.

Keywords: Pimpinella anisum, essential oil, phenolic compounds, flavonoids, scavenging activity, antimicrobial activity.

INTRODUCTION

Pimpinella anisum L. (Anise), member of Umbelliferae family, is an annual herb with flowers and small green to yellow seeds, which grows in Iraq, Turkey, Iran, India, Egypt[1], Morocco[2] and many other warm regions of the world. . This plant is primarily grown for its fruits (aniseeds) that harvested in August and September[3], according to Shojaii et al (2012), anise seed contain 1.5 - 5 % of volatile oil[4] which is characterized by the presence of the trans-anethole in concentrations varying from 75 to 90%. The trans-anethole is the characteristic compound responsible for the pleasant smell of the essential oil of anise[5]. In addition, it's largely used as a substrate for synthesis of various pharmaceutical substances[6].

The seeds of *Pimpinella anisum* are listed in British, German and European pharmacopoeia[7]. It is used in industry as flavoring and perfuming agent[8] (chewing-gums, alcoholic drinks, ice cream) and also used in medicinal purposes to treat dyspeptic complaints[9], gynecologic diseases[10], epilepsy[11], expectorant[12], diuretic and disinfectant[13].

Because the natural antioxidants extracted from medicinal plants are associated with lower risks of degenerative diseases[14] and the essential oil extraction avoid to contain a wide variety of plant secondary metabolites that can inhibit or slow the growth microorganisms[15]. The objective of this study is to investigate Moroccan aniseed essential oil and phenolic compounds recovered from the residue of hydrodistillation as bioactive molecules (antioxidant and antimicrobial activity) and to evaluate their potential usage in therapy and food technology. In the other hand, this study aims to evaluate the role of organic solvent in the extraction of bioactive molecules as phenolic compounds and flavonoids.

MATERIALS AND METHODS

Plant materials

The seeds of *Pimpinella anisum* were purchased from a local herbal store in Fez, Morocco. The identification was confirmed according to Flora of Morocco[16].

Essential oil extraction

Aniseeds (100g) were submitted to stem distillation in a Clevenger type apparatus for 3 hours. The essential oil

Author for Correspondence: Email: lamiaa.lem@gmail.com

obtained was separated from water and stored at 4°C until analysis and bioassays. The filtered residue of hydrodistillation was stored at 4°C until use.

GC-MS analysis

Pimpinella anisum essential oil identification and characterization was carried out by gas chromatography

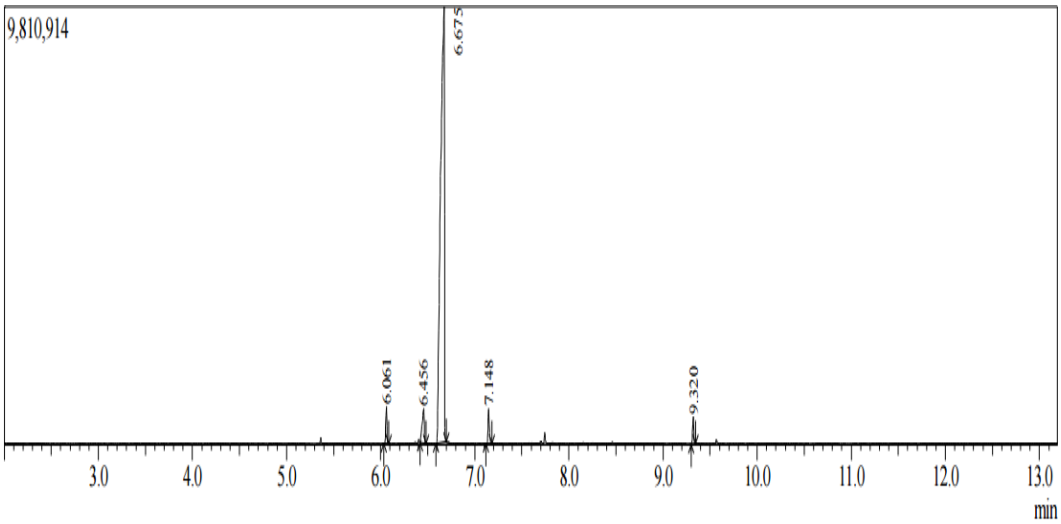

Figure 1 : GC Chromatogram analysis of the essential oil of *Pimpinella anisum.*

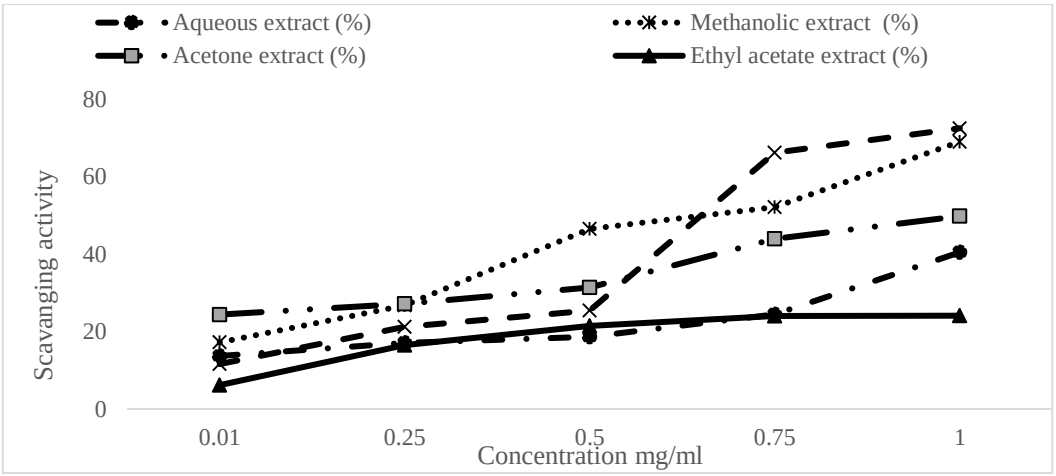

Figure 2 : Scavenging activity of *Pimpinella anisum* extracts on the DPPH radical

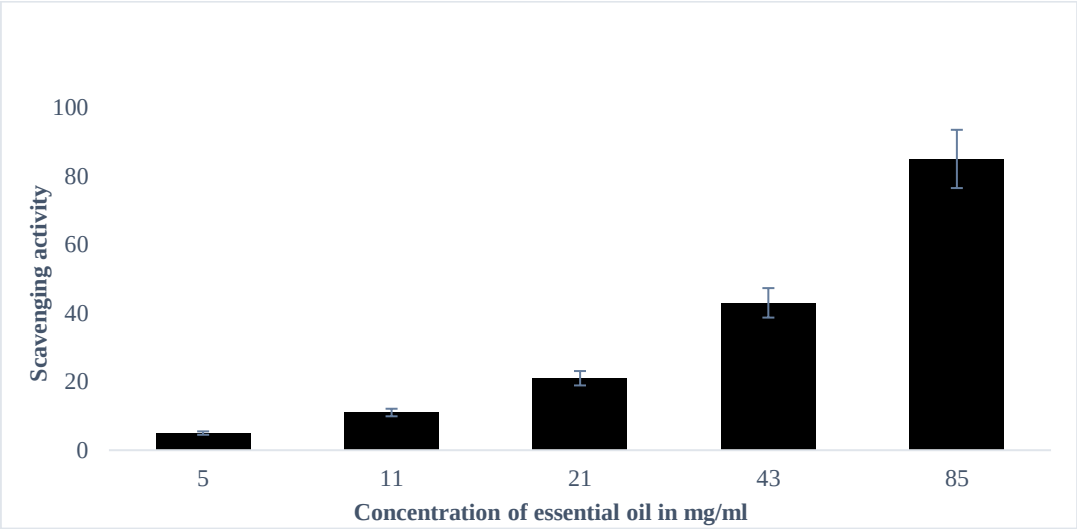

Figure 3 : The scavenging activity of the essential oil of *Pimpinella anisum* as a function of the concentration

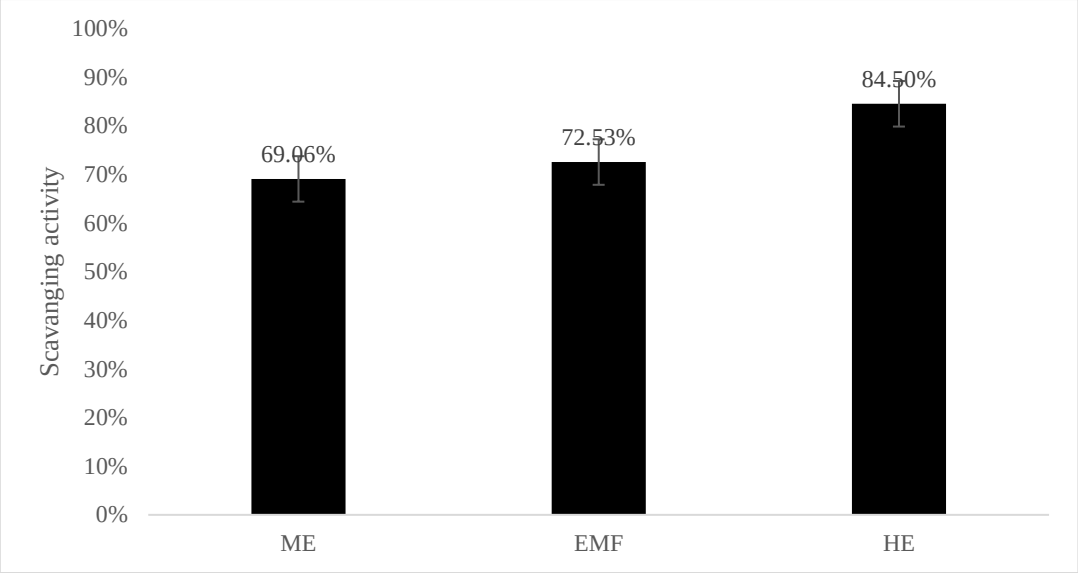

Figure 4 : Scavenging activity of the methanol extracts and essential oil.

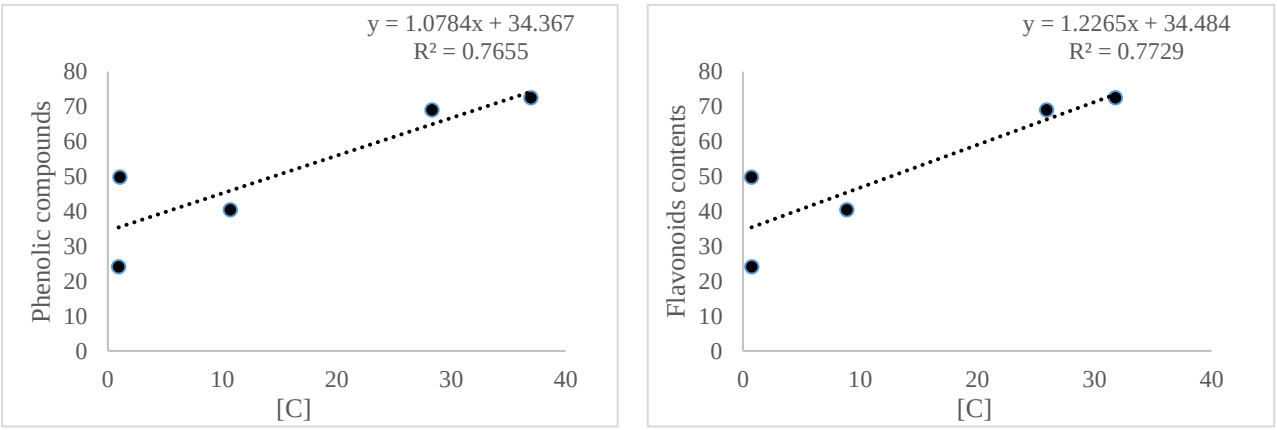

Figure 5 : Correlation between each of phenolic compounds (a), flavonoids(b) and antioxidant activity.

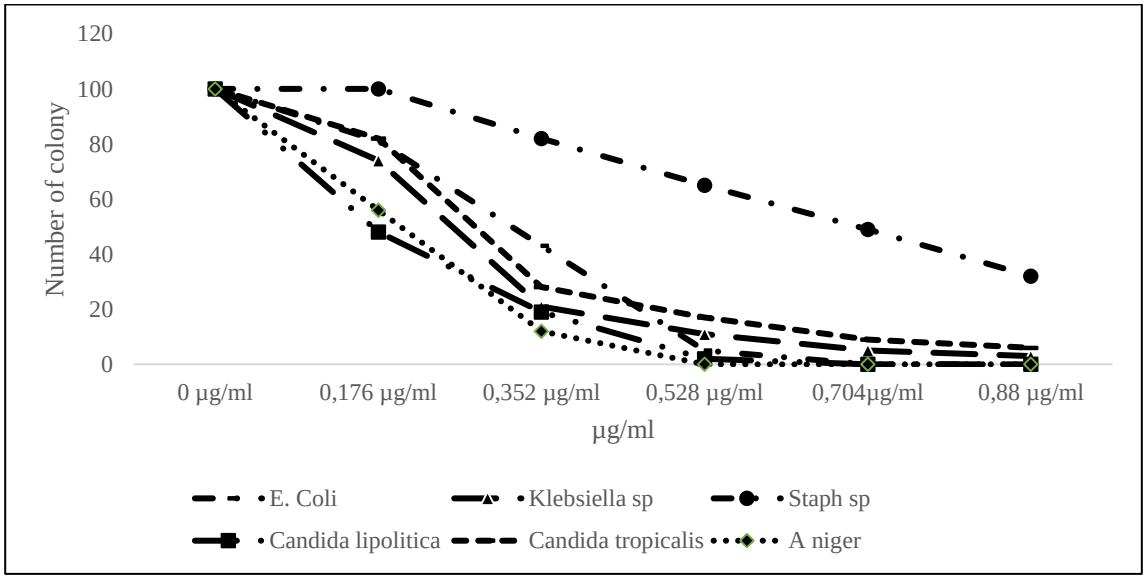

Figure 6 : Antimicrobial activity of *Pimpinella anisum* essential oil against different strains.

Agilent Technologies 6890 N coupled with an Agilent 5973 N mass spectrometry and an apolar HP5-MS capillary column (length 30 m, internal diameter 0.25 mm, 0.25 µm film thickness). The analysis was performed by injecting essential oil (1 µl). The carrier gas used is Helium (0.5 Bar). Under following conditions: the initial injection temperature of column is 50 ° C, then raised to 280 °. Volatile compounds were identified by their mass spectrum, mass spectrum libraries and their Kovat's retention indices calculated from the retention times of the separate compounds and linear alkanes.

Recovered seeds extracts

We have adopted a new method by using the filtrate and the residue recovered from the hydrodistillation. The filtrate was separated from the seeds by simple filtration with Whatman filter, and was then concentrated with a rotary evaporator and considered as the aqueous extract (AE).

Residue recovered from hydrodistillation was subjected to different extraction with organic solvents. It was dried at room temperature then subjected to extraction with three organic solvent (methanol, acetone and ethyl acetate). 25 g of the dried residue was weighed and extracted with 150 ml of methanol using a Soxhlet extractor at 60°C for 3h continuously. The solvent was removed using a rotary vacuum evaporator at 40°C to give concentrated extracts and weighed to calculate yield extraction. The second residue was dried again and subjected to other extractions, under the same conditions, but with other solvents (acetone and ethyl acetate). This serial extraction provides three crude organic extracts: methanol extract (ME), acetone extract (ACE) and ethyl acetate extract (EAE), which were recovered in microtubes and stored at 4°C until use.

Fresh seeds extract

To assess the effect of extraction on the content of phenols and flavonoids, a direct extraction of the fresh seeds of anise was done using methanol as solvent. This extraction aimed the comparison of the methanol extract from fresh seeds of anise with that resulting of residual seed recovered from hydrodistillation in phenol, flavonoids content and the antiradical activity. The fresh seeds (25 g) were extracted using a Soxhlet type extractor with 150 mL of methanol at 60°C for 3 h. Thereafter, the extract was filtered and evaporated to dryness under vacuum at 40°C with a rotary evaporator. After the yield was determined, the extract was stored at 4°C until use.

Total phenolic compounds in the seeds extracts

Total phenolic content was determined according to the Folin–Ciocalteu method. Briefly, 1 ml of the investigated extracts were mixed with 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) in a test tube. Then 2.5 ml of sodium carbonate solution (20%) was added. The reaction mixture was mixed thoroughly and then allowed to stand for 40 min in the dark. The absorbance was measured with a spectrophotometer at a wavelength of 725 nm. The concentration of total phenolic compounds was calculated by comparison with the absorbance of Gallic acid (GA) at different concentrations (calibration curve). The amount of total phenolic compounds was expressed as Gallic acid equivalent (GA) in milligrams per gram dried extract (GA mg/g of dried extract) and Gallic acid equivalent in milligrams per gram dry aniseed (GA mg/g aniseed)[17].

Total flavonoids contents in the seeds extracts

Total flavonoid content was determined by a colorimetric assay using aluminum chloride. One ml of plant extract in DMSO (10mg/1ml) was mixed with 0.3 ml 5% $NaNO_2$. After 5 minutes 0.3 ml of $AlCl_3$ 10% was added. Once 6 minutes have expired, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled water [18]. The absorbance of the reaction mixture was measured at 510 nm with a spectrophotometer (UV 2300 II). The calibration curve was prepared by preparing quercetin solutions at concentrations from 0.05 to 1 mg/ml in DMSO under the same conditions as the sample. The results were expressed in milligrams of quercetin per gram of dried extract (mg Qer / g of dried extract).

DPPH radical scavenging activity

The free radical scavenging activity of *Pimpinella anisum* seeds extracts was measured by the 1, 1-diphenyl-2-picryl-2-picryl-hydrazil (DPPH) method proposed by Jang et al (2010). This method measures hydrogen atom- or electron-donating activity. DPPH is a stable free radical with a purple color which is reduced to yellow-colored diphenylpicrylhydrazine. In brief, 0.1 ml aliquot of extract dissolved in DMSO was mixed with 2 ml of 0.041 mM DPPH. After thirty minutes of incubation period at room temperature in the dark, the absorbance was measured at 517 nm using a spectrophotometer. Absorption of blank sample containing the same amount of DMSO and DPPH solution was prepared and measured. The scavenging activity of the extracts was calculated as a percentage inhibition and it was calculated by the following formula[19]:

$$DPPH.\ Scavenging\ Effects\ \% = \frac{\text{Abs (control)} - Abs\ \text{(sample)}}{\text{Abs (control)}} \times 100$$

Where Abs (control) is the absorbance of the control reaction and Abs (sample) is the absorbance in the presence of the sample of *Pimpinella anisum* seeds extracts. Gallic acid were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Antimicrobial activity

Antimicrobial activity of anise essential oil was assessed on three bacteria strain (*Escherichia coli, Klebsiella sp and Staphylococcus sp)* two yeasts (*Candida tropicalis and candida lipolytica) a*nd one molds *(Aspergillus niger*). Antimicrobial activity was carried out by mixing anise essential oil ranging from 0 to 0.88 µg/ml with culture medium favorable for the growth of tested micro-organism[20]. The mixture was vigorously stirred to ensure a homogeneous dispersion of essential oil before pouring it into Petri dishes. The inoculation was done by spreading 100 µl of microbial suspension in physiological water (NaCl 0.9%). Free essential oil culture medium were used as control. Petri dishes were incubated according to the nature of strain tested (37°C for bacteria during 24h, and 30°C for fungi during 48h)The death fraction of population (% Mortality) was evaluated by the following equation:

Table 1 : Major compounds of essential oils of *Pimpinella anisum*

Compound	Apex RT	Start RT	End RT	Area %	Formula
Cis-anethole	6.061	6.033	6.087	2.17	$C_{10}H_{12}O$
p-anisaldehyde	6.456	6.420	6.480	3.83	$C_8H_8O_2$
Trans-anethole	6.675	6.587	6.700	90.07	$C_{10}H_{12}O$
Eugenol	7.148	7.120	7.180	2.39	$C_{10}H_{12}O_2$
Trans-pseudoisoeugenyl 2-methylbutyrate	9.320	9.300	9.347	1.54	$C_{15}H_{20}O_3$
Total				100	

Table 2 : Yield of different extracts.

Extract	The yield (%)
aqueous extract	4.17 ± 0.07
Methanol extract	8.23 ± 0.28
Acetone extract	0.60 ± 0.06
Ethyl acetate extract	0.64 ± 0.12
Fresh Methanol extract	11.76 ± 0.56

Values are mean of three replicates ± standard deviation.

$$\%\, Mortality = 100 \times (1 - (X/100))$$

Control is considered as 100% of viability and X is the viability obtained from each essential oil concentration on culture medium.

This method give qualitative and quantitative on essential oil lethality evaluation. 100% of mortality refers to the minimal inhibition concentration (MIC). Therefore, this methodology give dynamical evaluation of essential oil toxicity and tendency to resistance or sensibility of the microorganism tested and that is according to the mathematical model of inhibition.

RESULTS AND DISCUSSIONS

Aniseed essential oil identification and characterization

The steam distillation of 100 g of seeds of anise (*Pimpinella anisum*) had achieved the following yield of the essential oil: 1.2; 1.5 and 1.1%. The average yield is 1.26 ± 0.15%. Aniseed essential oil has a mobile liquid appearance at room temperature with a pale yellow color and a sweet taste.

Pimpinella anisum essential oil GC chromatogram (Fig.1) reveals the presence of five majority components. The peak of trans-anethole, located at 6.675 min retention time was identified as the most abundant compound (90.07%) in *Pimpinella anisum* essential oil. Trans-anethole belonged to the family of phenylpropene. In this group, we also found cis-anethole (2.17%) and eugenol (2.39%). Aside the compounds of the class phenylpropanoid which represented 94.63%, there was one sesquiterpene Trans-pseudoisoeugenyl 2-methylbutyrate which was present in *Pimpinella anisum* essential oil constituting the 1.54% of the essential oil.

A detailed description of all of the components detected by GC-MS found in the essential oil in the Moroccan seeds of *Pimpinella anisum* (Table 1), which included the RI values of each component. The major constituents of investigated essential oil were respectively *trans*-anethole (90.07%), p-anisaldehyde (3.83%), eugenol (2.39%), cis-anethole (2.17%) and trans-pseudoisoeugenyl 2-methylbutyrate (1.54%). It was confirmed that trans-anethole was the major compound in the Brazilian *Pimpinella anisum* essential oil with a high percentage (90.88%)[21]. The second most abundant compound was p-anisaldehyde (3.83%). According to Skuhrovec et al (2016), P-anisaldehyde is a constituent of the essential oil of aniseed by 7.74%[22]. Besides, the study of Lee (2004) has shown that p-anisaldehyde is identified in the aniseed essential oil with 2.9%[23]. The other principal compounds in our aniseed oil were cis-anethole and eugenol. The cis anethole is usually present in the essential oil of *P. anisum*[24 25 26]. The presence of eugenol is demonstrated by many studies like Besharati-[27], [1] and [10]. The minor compound of our essential oil was the trans-pseudoisoeugenyl 2-methylbutyrate with a percentage of 1.5. Our contribution entente to that of Orav et al (2008) which confirms that trans-pseudoisoeugenyl 2-methylbutyrate exist in the essential oil of *Pimpinella anisum* located in different geographical areas of Europe with a percentage varying between 0.4 and 6.4% [5].

Yield of extraction

The yield of the organic solvent extraction process for each 25 g of the seed of *P. anisum* was represented in table 2. Extraction of the fresh aniseed by methanol gave us a higher yield (11.76 ± 0.56%) than those extractions by organic solvent of the seeds of *Pimpinella anisum* recovered from hydrodistillation. For the extraction of the seeds recovered from the hydrodistillation, we notice that methanol was the solvent which could extract more plant material aniseed (8.23 ± 0.28%), followed by aqueous extract (4.17 ± 0.07), ethyl acetate (0.64 ± 0.12%), and the acetone solvent with a yield of (0.6 ± 0.06%).

Methanol was the solvent that could extract more plant material. This can be attributed to the fact that the vast majority of the compounds of this plant are soluble in methanol. We notice that the amount of material removed by the organic solvent from the seeds recovered from hydrodistillation (13.64%) was greater than that extracted by methanol alone on the fresh seed.

Total phenolic compounds and flavonoid contents

Total phenolic compounds of the various extracts is represented in Table 3 where contents has been reported in milligram equivalents of gallic acid per gram of weight of the dried extract (GA mg / g dried extract) and in milligram equivalents of gallic acid per gram of aniseed (mg GA / g aniseed). Hydrodistillation residue of aniseed has undergone a serial extraction by organic solvents (methanol, ethyl acetate and acetone). The filtrates was concentrated and considered as aqueous extract. The total flavonoid content of the different extracts were represented in Table 3.

Table 3 : Total phenolic and flavonoid content in the organics solvent extracts of the seeds of *Pimpinella anisum.*

Extract	Phenolic content mg GA / g dried extract	Phenolic content mg GA / g aniseed	Content of flavonoids mg Qer / g dried extract	Content of flavonoids mg Qer / g aniseed
Aqueous	256.72	10.71	213.31	8.89
Methanol	344.10	28.34	314.68	25.92
Acetone	174.60	1.06	122.00	0.74
Ethyl acetate	143.58	0.93	118.65	0.77
Total	919.00	41.04	768.64	36.31
Fresh ethanol	404.94	36.98	348.21	31.80

It was shown that the four extracts recovered from the hydrodistillation contain abundant phenolic compounds (Table 3). The reduction of phenolic compounds appeared clearly in the values obtained after evaluating extracts. It was clear that methanol was the solvent that could extract maximum phenolic compounds with a value of 28.34 mg / g of anise, followed by water (10.71 mg / g aniseed), acetone (1.06 mg / g of aniseed) and ethyl acetate (0.93 mg / g of aniseed). The same for the flavonoids, the highest value was that of the methanol extract (25.92 mg / g of aniseed), followed by the aqueous extract (8.89 mg / g of aniseed), ethyl acetate extract (0.77 mg / g of aniseed) and acetone extract (0.74 mg / g of aniseed). We noticed that the flavonoid content changes with the solvent used in the extraction. This confirms that the flavonoids are the majority of phenolic compounds in *P. anisum.*

We extracted fresh aniseed with methanol and compare its total phenolic and flavonoid content to the residual seed recovered from the hydrodistillation. We found that the content of phenolic compounds in fresh seeds was 344.10 (mg/ g dried extract) and 36.98 (mg / g aniseed). Total flavonoid content in the methanol extract of the fresh aniseed was 31.80 (mg/g aniseed) (Table 3). This content was lower than those extracted from the aniseed hydrodistillation residue with the different solvent.

The advantage of the extraction to the residual seeds of *Pimpinella anisum* recovered from the hydrodistillation was that besides the possibility of having rich extracts in phenolic 41.04 (mg Gal / g aniseed) and flavonoids compounds 36.31 (mg Qer / g of aniseed), we can extract also the essential oil. This without bringing a significant change in the effectiveness of antioxidant activity as based on the IC_{50} obtained, the methanol extract of fresh seeds and the residual seeds were equally effective in reducing free radical DPPH. According to Albayrak et al. (2011), we found that the concentration in phenolic compounds in the aniseed is 32.93 ± 0.6 (mg Gal / g d'anis). This showed us that *Pimpinella anisum* on which we have done the analysis was rich in phenolic compounds[28]. That was confirmed by the H. Womeni et al (2013), which determined that total phenolic contents of methanol extracts of aniseed is 29.6±0.17 (mg GAE/g d'extract)[29]. The polarity of solvents used in the extraction varies the extracted phenolic and flavonoids compounds, that was confirmed by numerous studies such as Mokrani et al. (2016),[30] and [31].

Free radical scavenging activity of essential oil and extracts

Free radical scavenging activity of seed extracts

The results representing in the Fig.2, illustrate the percentage of the antiradical activity of the extracts towards the free radical DPPH. The value of the antiradical activity increased in linear model with the concentration of the extracts. This allows us to demonstrate a significant decrease in the concentration of DPPH radical due to the scavenging capacity of free radicals by the aniseed extracts. And the scavenging effect of the different extracts on the DPPH decreased in the concentration of 1 mg / ml in the following descending order (Fig.2) : methanol extract from the fresh aniseed > methanol extract recovered from the residue of the hydrodistillation > acetone extract recovered from the residue of the hydrodistillation > water extract recovered from the filtrate of the hydrodistillation > ethyl acetate extract recovered from the residue of the hydrodistillation.

According to Fig.2, we notice that; only the methanol extracts that could reduce DPPH at over 50%; value known as the IC_{50}. The methanol extract from the fresh seeds of *Pimpinella anisum* reached the IC_{50} value the first with a concentration of 0.648 mg/ml followed by the methanol extract from the residue of steam distillation with a concentration of 0.658 mg/ml. Both values were close and they had a difference of 0.01 mg / ml, this means that our extracts were both rich in phenolic compounds and flavonoids (the good donors of the proton H), and even we can see that the content of both extracts polyphenols were almost equal. These results indicate clearly that the methanol extracts of aniseed was a reel free-radical scavengers and powerful antioxidants. This activity was largely related to the composition of the extracts and richness in phenolic compounds and flavonoids. These properties were cited in numerous scientific publications and report among them Albayrak et al (2011), which compares the anti-radical activity of aniseed (78.98%) with the BHT (92.15%) at a concentration of 2 mg / ml. This results confirm that aniseed were rich in antioxidant[28]. In order to compare the antioxidant activity of the hydrodistillation residue we enlist that the polarity of the used solvent for the extraction of residue from celery influences the antioxidant activity. In this study the methanol and ethanol (91.5% and91.2%) have the higher scavenging activity followed by acetone (56.2%)[32].

Free radical scavenging activity of essential oil

The anti-radical activity *Pimpinella anisum* essential oil was illustrated in Fig.3. This result indicates that the essential oil have a noticeable effect on scavenging free

Table 4 : Correlation between total antioxidant activity of the seed extract of *Pimpinella anisum* in the concentration 1 mg/ml with polyphenol and flavonoid content

	Polyphenol content mg Gal/g aniseed	Content of flavonoids mg Qer/g aniseed
Total antioxidant activity of the extracts of aniseed in the concentration of 1 mg/ml	0.87	0.88

radical. We notice that the scavenging activity increased in exponential model with the concentration of the essential oil.

Comparative study has done for the radical scavenging activity of the methanol extract recovered from the residue of the hydrodistillation, the methanol extract from the fresh aniseed and the essential oil. Fig.4 illustrated the difference of the radical scavenging. We saw that the essential oil of *Pimpinella anisum* showed an excellent radical scavenging activity and was high compared with the both methanol extracts. The essential oil managed to reduce (84.50 ± 0.95%) of the DPPH, while the methanol extract of fresh aniseed reduced (72.53 ± 0.87%), and the methanol extract of the seeds recovered from the residual hydrodistillation reduced only (69.06 ± 0.35%).

Correlation study

Correlation analysis was used to investigate the relationship between the antioxidant activity with the phenolic and flavonoid content of aniseed. From the Fig.5 and Table 4, we notice that the antioxidant activity was highly correlated with flavonoids and phenolic compounds, 0.88 and 0.87 respectively. We concluded that flavonoid presented the major constituents of phenolic compounds and the responsible for the antioxidant activity of *Pimpinella anisum* seeds.

Antimicrobial activity

From the Fig 6 we notice that *Pimpinella anisum* essential oil had antimicrobial activity against all the microorganism tested. At the concentration 0.88 µg/ml, the essential oil has completely inhibit a Gram-negative bacteria respectively *E.Coli* (100%) and *Klebsiella sp* (97%) which mean that MIC of the bacteria *E. Coli* was 0.704 µg/ml. But it wasn't a good inhibitor (68%) for the Gram positive bacteria (*Staphylococcus aureus)* which presented some resistance. In the opposite, anise oil had a maximal antifungal activity which inhibit 100% of both C*andida lipolitica, and Aspergillus niger* but only 94% of *Candida tropicalis*. As shown in Fig.6, we notice that *Pimpinella anisum* essential oil was inhibiting for the tested yeasts *Candida Lipolytica* and mold *Aspergillus niger* at a concentration of 0.528 µg/ml and 0.704 µg/ml respectively and considered as MIC. This proves that fungi strains especially the yeast *Candida lipolytica* and the molds *Aspergillus niger* were sensible for *Pimpinella anisum* essential oil. While bacterial species present some heterogeneity in the mathematical model of inhibition. The resistant strain *Staphlicoccus sp*. had linear model but the strains sensible to anise oil presented logarithmic mathematical model.

Several studies have reported the antimicrobial activity of *Pimpinella anisum* essential oil. A study by Hammer and al. (1999) found that the MIC of *Pimpinella anisum* essential oil on *E.Coli* is 0.5µg/ml, 0.25 µg/ml on S*taphylococcus aureus* and even though at a concentration of 2 µg/ml of the essential oil of the total volume of the medium don't inhibit *Klebsiella sp*[33]. An antifungal activity was reported by Kosalec et al (2005) who mention that *Candida tropicalis* is a sensitive species with MIC 0.158µg/ml[34]. Also this essential oil have a highly inhibitory effect with the MIC and minimal fungicidal concentration (MFC) values of 35.2 µg/ml against *A. niger*[35]. The *Pimpinella anisum* essential oil have an antibacterial activity against *S. aureus* (MIC : 5.54µg/ml) and *E. Coli* (MIC 11 µg/ml) [36], this essential oil have the trans anethole as major compound constituting 86%. A higher essential oil antibacterial activity was observed in the study of Abdel-reheem et al (2015) against *S. aureus* and *E. Coli* with a MIC 3 and 2.5 µg/ml, respectively[37]. The composition of the later essential oil is caracterized by the trans anethole as major compounds (82.1%) followed by cis anethole (5.8%)[37]. *Pimpinella anisum* essential oil is know by trans anethole as major compounds but its percentages and its synergy with the other compounds infleunce the essential oil activity. By comparising the essential oil composition, we noticed from the study of abdel reheem et al (2015) that even if the major compounds (trans anethole) has a lower percentge from the Moroccan essential oil but the presence of cis anethole (5.8%) as a second compounds in common made it antibacterial activity against *E. Coli* and *S aureus* better than the one in the study of Bazargani et al (2016) (absence of cis anethole). And that explain the effecacity of the Morrocan *Pimpinella anisum* essential oil and that two molecules (trans-anethole and cis-anethole) contribute by synergy to improve the lethality of the essential oil.

Based on the work of G. Singh et al. (2002), it has been noticed that *Pimpinella anisum* essential oil have a very good antibacterial activity and can be used against *S. aureus, S. haemolyticus and B. subtilis*. These oils can also be used to control *P. aeruginosa*, *E. coli* [38]. These observations suggest that the essential oil can be used as an alternative to synthetic antimicrobial agents. The essential oil can be exploited as a source of natural antibacterial agent. Besides that, according to the mathematical models we notice that our study concord with that of Tahri et al. (2001)[39]. The latest study confirms that the linear model explain the resistance of and the logarithmic model demonstrate the sensibility of the tested microorganism studied[39].

CONCLUSION

The Moroccan essential oil of aniseed was characterized by high amount of trans-anethole (82.93 %). The results show that the essential oil of anise proved very interesting

by its antibacterial and scavenging activities. Besides the essential oil, the submission anise to successive extractions from the same substrate has allowed us to have other rich extracts of phenolic compound whose activity antiradical was very interesting.

The antioxidant activity of the various extracts and essential oil Anise was evaluated by the method of free radical DPPH. The scavenging activity was interesting in both methanol extracts (from the residue of hydrodistillation and the fresh aniseed), by against it was low for the other two extracts (acetone and ethyl acetate extract). The higher scavenging activity is observed in the essential oil of anise. Antimicrobial activity was determined on different bacteria and fungi. The results indicate that the essential oil of anise has an interesting antimicrobial activity on all strains tested.

In light of these results, we conclude that the anise seeds are rich in bioactive compound and the application of these extracts in the food industry (agri-food) can be very interesting to replace synthetic products.

REFERENCES

1. Al-Bayati, F. A. Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts. J. Ethnopharmacol. 116, 403–406 (2008).
2. Al maofari, a. Et al. Chemical composition and antibacterial properties of essential oils of pimpinella anisum l . Growing in morocco and yemen. Sci. Study Res. Chem. Chem. Eng. Biotechnol. Food Ind. 14, 11–16 (2013).
3. Foroughi, A. et al. Assessment of Chemical Composition and Antibacterial Effects of Anethole-Rich Hydroalcoholic Extract of Pimpinella anisum. Int. J. Pharm. Clin. Res. 2016; 8, 1459–1463 (2016).
4. Shojaii, A. & Mehri, A. Review of Pharmacological Properties and Chemical Constituents of Pimpinella anisum. ISRN Pharm. 2012, 1–8 (2012).
5. Orav, A., Raal, A. & Arak, E. Essential oil composition of Pimpinella anisum L. fruits from various European countries. Nat. Prod. Res. 22, 227–232 (2008).
6. Rather, M. A., Dar, B. A., Sofi, S. N., Bhat, B. A. & Qurishi, M. A. Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arab. J. Chem. 9, S1574–S1583 (2016).
7. Fujimatu, E., Ishikawa, T. & Kitajima, J. Aromatic compound glucosides, alkyl glucoside and glucide from the fruit of anise. Phytochemistry 63, 609–616 (2003).
8. Koriem, K. M. M. Approach to pharmacological and clinical applications of Anisi aetheroleum. Asian Pac. J. Trop. Biomed. 5, 60–67 (2015).
9. Tepe, B., Akpulat, H. A., Sokmen, M. & Daferera, D. Screening of the antioxidative and antimicrobial properties of the essential oils of Pimpinella anisetum and Pimpinella flabellifolia from Turkey. Food Chem. 97, 719–724 (2006).
10. Gülçin, I., Oktay, M., Kireçci, E. & Küfrevioğlu, Ö. I. Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. Food Chem. 83, 371–382 (2003).
11. Janahmadi, M., Farajnia, S., Vatanparast, J., Abbasipour, H. & Kamalinejad, M. The fruit essential oil of Pimpinella anisum L. (Umblliferae) induces neuronal hyperexcitability in snail partly through attenuation of after-hyperpolarization. J. Ethnopharmacol. 120, 360–365 (2008).
12. Cengiz, N., Ozbek, H. & Him, A. Hepatoprotective Effects of Pimpinella anisum Seed Extract in Rats. pharmacologyonline 870–874 (2008).
13. Al-Daihan, S. et al. Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. J. King Saud Univ. - Sci. 25, 115–120 (2013).
14. Do, Q. D. et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. J. Food Drug Anal. 22, 296–302 (2014).
15. Semeniuc, C. A., Pop, C. R. & Rotar, A. M. Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J. Food Drug Anal. 5, 403–408 (2017).
16. Mohammed, F., Ibn Tattou, M., Mathez, J., Ouyahya, A. & EL Oualidi, J. Flore Pratique du Maroc. (1999). doi:10.13140/RG.2.1.3227.8889
17. Sowndhararajan, K., Siddhuraju, P. & Manian, S. Antioxidant and free radical scavenging capacity of the underutilized legume, Vigna vexillata (L.) A. Rich. J. Food Compos. Anal. 24, 160–165 (2011).
18. K.Sarabjot & M.Poonam. Study of Total Phenolic and Flavonoid Content , Antioxidant Activity and Antimicrobial Properties of Medicinal Plants. J. Microbiol. Exp. 1, 1–6 (2014).
19. Jang, I. C. et al. Antioxidant and antigenotoxic activities of different parts of persimmon (Diospyros kaki cv. Fuyu) fruit. J. Med. Plants Res. 4, 155–160 (2010).
20. Feng, W. & Zheng, X. Essential oils to control Alternaria alternata in vitro and in vivo. Food Control 18, 1126–1130 (2007).
21. Rodrigues, V. M., Rosa, P. T. V, Marques, M. O. M., Petenate, A. J. & Meireles, M. A. A. Supercritical extraction of essential oil from aniseed (Pimpinella anisum L) using CO2: Solubility, kinetics, and composition data. J. Agric. Food Chem. 51, 1518–1523 (2003).
22. Skuhrovec, J. et al. The Effects of Pimpinella anisum Essential Oils on Young Larvae Leptinotarsa decemlineata Say (Coleoptera : Chrysomelidae). Am. J. Potato Res. 1–6 (2016). doi:10.1007/s12230-016-9549-x
23. Lee, H. p -Anisaldehyde : Acaricidal Component of Pimpinella anisum Seed Oil against the House Dust Mites Dermatophagoides farinae and Dermatophagoides pteronyssinus. Planta Med. 70, 279–281 (2004).
24. Ullah, H. & Honermeier, B. Fruit yield, essential oil

concentration and composition of three anise cultivars (Pimpinella anisum L.) in relation to sowing date, sowing rate and locations. Ind. Crops Prod. 42, 489–499 (2013).
25. Özcan, M. M. & Chalchat, J. C. Chemical composition and antifungal effect of anise (Pimpinella anisum L.) fruit oil at ripening stage. Ann. Microbiol. 56, 353–358 (2006).
26. Khalid, A. K. Quality and quantity of Pimpinella anisum L . essential oil treated with macro and micronutrients under desert conditions. 22, 2396–2402 (2015).
27. Besharati-seidani, A., Jabbari, A. & Yamini, Y. Headspace solvent microextraction : a very rapid method for identification of volatile components of Iranian Pimpinella anisum seed. Anal. Chim. Acta 530, 155–161 (2005).
28. Albayrak, S., Aksoy, A., Sagdic, O. & Albayrak, S. Antioxidant and antimicrobial activities of different extracts of some medicinal herbs consumed as tea and spices in turkey. J. Food Biochem. 36, 1–8 (2011).
29. Womeni, H. M., Djikeng, F. T., Tiencheu, B. & Linder, M. Antioxidant potential of methanolic extracts and powders of some Cameroonian spices during accelerated storage of soybean oil. Adv. Biol. Chem. 3, 304–313 (2013).
30. Amzad Hossain, M. & Shah, M. D. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant Merremia borneensis. Arab. J. Chem. 8, 66–71 (2011).
31. Li, H. et al. Comparison of the antioxidant properties of various solvent extracts from Dipsacus asperoides and identi fi cation of phenolic compounds by LC-ESI-QTOF-MS – MS. South African J. Bot. 109, 1–8 (2017).
32. Zhan-guo, L., Wei, L. & Peng-jun, W. Chemical Composition and Ability of Scavenging DPPH Radical of Essential Oil and Residue from The Celery Seed. Adv. Mater. Res. 185, 18–21 (2011).
33. Hammer, K. A., Carson, C. F. & Riley, T. V. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86, 985–990 (1999).
34. Kosalec, I., Pepeljnjak, S. & Kustrak, D. Antifungal activity of fluid extract and essential oil from anise fruits (Pimpinella anisum L., Apiaceae). Acta Pharm. 55, 377–385 (2005).
35. Matan, N. & Matan, N. Antifungal activities of anise oil , lime oil , and tangerine oil against molds on rubberwood (Hevea brasiliensis). Int. Biodeterior. Biodegtadation 62, 75–78 (2008).
36. Mohammadi Bazargani, M. & Rohloff, J. Antibiofilm activity of essential oils and plant extracts against Staphylococcus aureus and Escherichia coli biofilms. Food Control 61, 156–164 (2016).
37. Abdel-reheem, M. A. T. & Oraby, M. M. Anti-microbial, cytotoxicity, and necrotic ripostes of Pimpinella anisum essential oil. Ann. Agric. Sci. 60, 335–340 (2015).
38. Singh, G., Kapoor, I. P. S., Pandey, S. K., Singh, U. K. & Singh, R. K. Studies on essential oils: Part 10; antibacterial activity of volatile oils of some spices. Phyther. Res. 16, 680–682 (2002).
39. Jamai, L. et al. Physiological diffierence during ethanol fermentation between calcium alginate-immobilized Candida tropicalis and Saccharomyces cerevisiae. FEMS Microbiol. Lett. 204, 375–379 (2001).