Introduction

The growing awareness of consumers concerning the relation between industry and health is revolutionary for the pharmaceutical, food industries. Therefore, there is a growing interest in natural substances that exhibit antioxidant properties to reduce or eliminate chemically synthesized additives such as BHA, BHT or TBHQ in foods [1].

Medicinal plants and their extracts have been used for many centuries to treat different diseases. Furthermore, their essential oils, which obtained by hydro distillation or steam distillation, can be a source of alternative natural treatment of disease, because of their antioxidant [2], antimicrobial [3] and Pharmaceutical properties [4]. Most essential oils are classified as generally recognized as safe and have been approved for food and beverage consumption by US food and drug administration.

The plant kingdom produces a wide range of natural antioxidants including phenolic compounds, which are commonly found in various plants as secondary metabolites. Also, they have large variability of the physico-chemical properties and multiple effects such as antioxidant activity [5,6].

Syrian flora is well known for its diversity and richness and it consists of numerous species for medicinal uses. Among plants grown in Syria VAC and RO. VAC, Lamiaceae (placed in Verbenaceae, also), is native to Mediterranean, European and Asian regions. Also, it is grown for ornamental purposes in many countries. This plant has a wide range of biological activities, including Premenstrual syndrome (PMS) [7-9] and their activity against cancer cell lines [10,11]. In addition, it has been used as antifungal [12], antibacterial [13], antileishmanial [14,15] and antioxidants agents [16].

RO, Lamiaceae, is an aromatic, medicinal plant. It is widely spread in Syria and broadly used in traditional medicine. RO is well known as medicinal plants and it is commonly used in pharmaceutical, cosmetic, food industries, because of antioxidant, anti-carcinogen and antibacterial characteristics [17-19].

The objective of this research is to compare the chemical composition of the VAC and RO leaves essential oils cultivated in Syria using GC-MS system and to study their antioxidant properties.
Material and methods

Plant material and chemicals

VAC was collected in July 2013 from local park (Tishreen park: 33°30'59.0"N 36°16'08.9"E), Damascus, Syria. The plant was identified by Prof. Anwar alkhateeb (Taxonomy and Ecology, Faculty of science, Damascus University, Syria). The leaves of VAC were dried in the shade. Most chemicals were purchased from Sigma-Aldrich (USA).

Essential oil extraction

VAC leaves were grounded in an electric grinder, then the essential oil was isolated by hydro-distillation, according to the procedure of the European Pharmacopoeia 4 [20]. The obtained oil (0.464±0.056%) was dried over anhydrous sodium sulfate and stored at +4°C in the dark until analyzed. While RO essential oil was obtained from Bio-cham company (March, 2013-Batch number: B13009028).

Essentials oils analysis

VAC and RO essential oils were analyzed by GC-MS, using an Agilent 7890A Gas chromatography system coupled with quadruple mass spectrometer (model 5975C). An HP-5MS 5 % Phenyl Methyl Siloxane column (30 m x 250 µm x 0.25 µm thickness) was used with helium as the carrier gas (1 ml/min). Interface, ion source, selective mass detector and injector temperatures were maintained at 280°C, 230°C, 150°C and 260°C, respectively. The oven temperature was programmed from 60°C to 200°C at a rate of 4°C/min, then at a rate of 8°C/min up to 260°C, finally maintained constant at 260°C for 7.5 min. 1.0 µl of diluted oils in n-hexane (1/100, V/V) were injected with a split ratio 1:10.

Table 1: compositions of VAC leaves essential oil.

| No. | VAC constituents                      | RT (min) | RI <sub>cal</sub> | RI <sub>lit</sub> | %    | Identified methods |
|-----|--------------------------------------|----------|-------------------|-------------------|------|-------------------|
| 1   | α-Thujene                            | 4.838    | 925               | 924               | 0.77 | MS/RI             |
| 2   | α-Pinene                             | 5.002    | 932               | 932               | 4.75 | MS/RI/St.         |
| 3   | Sabinene                             | 5.907    | 975               | 969               | 12.50| MS/RI             |
| 4   | β-Pinene                             | 5.979    | 978               | 974               | 1.20 | MS/RI/St.         |
| 5   | β-Myrcene                            | 6.249    | 991               | 988               | 1.80 | MS/RI             |
| 6   | α-Terpinene                          | 6.942    | 1018              | 1014              | 1.19 | MS/RI             |
| 7   | p-Cymene                             | 7.159    | 1026              | 1020              | 0.67 | MS/RI             |
| 8   | 1,8 Cineoles                         | 7.409    | 1034              | 1028              | 19.34| MS/RI/St.         |
| 9   | γ-Terpinen                           | 8.098    | 1059              | 1054              | 1.96 | MS/RI             |
| 10  | Terpinen-4-ol                        | 11.762   | 1180              | 1174              | 3.61 | MS/RI             |
| 11  | (−)-α-Terpineol                     | 12.190   | 1193              | 1186              | 4.12 | MS/RI             |
| 12  | δ-Elemene                            | 16.871   | 1340              | 1335              | 1.50 | MS/RI             |
| 13  | Terpinyl acetate                     | 17.261   | 1353              | 1346              | 2.29 | MS/RI/St.         |
| 14  | α-Gurjunene                          | 19.168   | 1413              | 1409              | 0.77 | MS/RI             |
| 15  | β-Caryophyllene                      | 19.510   | 1424              | 1417              | 6.74 | MS/RI/St.         |
| 16  | (E)-β-Farnesene                      | 20.618   | 1461              | 1454              | 5.43 | MS/RI             |
| 17  | Aromadendrene <dehydro->             | 20.748   | 1464              | 1460              | 1.68 | MS/RI             |
| 18  | Germacrone D                         | 21.364   | 1485              | 1484              | 1.16 | MS/RI             |
| 19  | Bicyclogermacrone                    | 21.855   | 1502              | 1500              | 5.30 | MS/RI             |
| 20  | (−)-Spathulenol                      | 24.205   | 1582              | 1577              | 1.38 | MS/RI             |
| 21  | Caryophyllene oxide                  | 24.369   | 1587              | 1582              | 0.82 | MS/RI             |
| 22  | Ledol                                | 24.942   | 1607              | 1602              | 0.82 | MS/RI             |
| 23  | α-Cadinol                            | 25.992   | 1646              | 1652              | 2.03 | MS/RI             |
| 24  | Unknown                              | 31.881   | 1878              | -                 | 4.25 | -                 |
| 25  | Bifemorne                            | 32.348   | 1899              | 1931              | 0.86 | MS                |
| 26  | (Z,Z)-Geranylinalool                 | 34.038   | 1961              | 1960              | 0.99 | MS/RI             |
| 27  | 5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate | 34.900 | 1993 | n/a | 0.86 | MS |
| 28  | phyllocladene                        | 35.651   | 2021              | 2016              | 1.54 | MS/RI             |
| 29  | 7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene | 36.364 | 2047 | n/a | 0.99 | MS |

| Total identified | 90.34 |

*Compounds listed in order to their elution on the HP-5MS column, RT retention times, RI <sub>cal</sub> Retention indices on the HP-5MS column relative to C8-C22 n-alkanes, RI <sub>lit</sub> Retention indices from the literatures, St. standard terpenoids, n/a=not available.

Citation: Al Saka F, Daghestani M and Karabet F. Composition and Antioxidant Activity of Vitex agnus-castus L. and Rosmarinus Officinalis L. Leaves Essential Oils Cultivated in Syria. SM Anal Bioanal Technique. 2017; 2(1): 1010.
Identification of constituents

Individual constituents were identified using mass spectrum and matching them with mass spectral library (NIST), along with the retention data from analytical standards of available terpenoids (Sigma-Aldrich). As well, the retention indices determined using a homologous series of n-alkanes C8–C22 and confirmation was done by comparing their calculated retention indices with literature [21].

Antioxidant Activity of the Essential Oils

Scavenging effect on DPPH radical

One of the quick methods to evaluate antioxidant activity is the scavenging activity, a stable free radical and widely used index [22]. 3 mL of freshly prepared ethanol DPPH solution (45µg/ml) was mixed with 300 µl of the samples at varying concentrations (0.2-0.5-1 mg/ml). The mixture was shaken vigorously and allowed standing for 30 min in the dark at room temperature. The decrease in absorbance (A) was measured at 517 nm with a spectrophotometer (Optizen 2120 UV Plus, Mecasys Co., Ltd, Korea). The inhibition percentage of the radicals (I %) was calculated according to the following formula:

\[
I\% = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100
\]

Where: A control is the absorbance of the control reaction (containing all reagents except the sample) and A Sample is the absorbance of the sample. 50µl of 0.2 mg/ml solution of vitamin C, which was used as a control, treated as the sample and at the same condition.

Assay for total phenolic contents

Total phenolic contents of the essential oils were determined by employing the methods given in the literature [23, 24], involving Folin–Ciocalteu reagent and Gallic acid (Sigma) as standard. The absorbance was measured at 760 nm (λ max) using the previous spectrophotometer against a blank. A calibration curve of Gallic acid standard solutions were prepared in 70 % ethanol (0-125 mg/L and R2 = 0.9997) and the data were expressed as Gallic acid equivalents.

Assay for total flavonoids contents

Total flavonoids contents of the essential oils were determined according to the aluminum chloride colorimetric method as described by [23,25].The absorbance of the reaction mixture was measured at 440 nm (λ max) using the previous spectrophotometer against a blank. A calibration curve of quercetin solutions was measured at 440 nm (λ max) using the previous spectrophotometer. The inhibition percentage of the radicals (I %) was calculated according to the following formula:

\[
I\% = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100
\]

Where: A control is the absorbance of the control reaction (containing all reagents except the sample) and A Sample is the absorbance of the sample. 50µl of 0.2 mg/ml solution of vitamin C, which was used as a control, treated as the sample and at the same condition.

Statistical analysis

Statistical Package for the Social Science (SPSS, 20) was used for statistical analysis. Data were expressed as mean ± SD of three different experiences. Comparisons were performed by One-way ANOVA, the significance level was < 0.05.

Results and Discussion

Chemical composition of the essential oil

Tables 1 and 2 demonstrate the GC-MS results which proved that 29 constituents represent 90.34 % of VAC essential oil and 20 constituents represent 93.41 % of RO essential oil. The major constituents of VAC essential oil were 1,8-Cineole (19.34 %) and Sabine (12.50%). These compounds were the main constituents of other VAC essential oils in various places [10,12, 26-27]. RO essential oil consisted mainly of 1,8-Cineole (28.03 %) and α-Pinene (14.70%), which is in agreement with some researches [28]. Whereas, 1,8-Cineole and Camphor were the major compounds in RO essential oil [29, 30]. Also, α-Pinene and Camphor were the main compounds in other reports [31]. 1,8-cineole and α-pinene have very high antimicrobial potency as shown in literature [12]. It is necessary to mention, that the composition of these volatile oils varies according to the countries, or the places in the same country. These differences seem to depend on climate changes and other factors like the method and the time of extraction, which can influence essential oil composition [30,32] (Tables 1 and 2).

Antioxidant activity of the essential oils

Antioxidant activity of VAC and RO essential oils was determined by three different tests systems DPPH radical scavenging effect, total phenol and flavonoids contents, as shown in Tables 3 and 4. The scavenging effects of VAC and RO essential oils on DPPH radical increased with concentration and the scavenging activity of VAC essential oil was more effective than RO. However, in the current study, none of the evaluated samples showed antioxidant activity as

| No. | RO constituents | RT  | RI_{cal} | RI_{lit} | %      | Identified methods |
|-----|-----------------|-----|----------|---------|--------|--------------------|
| 1   | α-Pinene        | 5.031 | 932 | 932 | 14.70 | MS/RI/St.          |
| 2   | Camphene        | 5.344 | 949 | 946 | 8.08  | MS/RI              |
| 3   | β-Pinene        | 5.965 | 978 | 974 | 1.29  | MS/RI/St.          |
| 4   | 3-Octanone      | 6.128 | 986 | 979 | 0.53  | MS/RI              |
| 5   | β-Myrcene       | 6.244 | 991 | 988 | 1.36  | MS/RI              |
| 6   | α-Terpine       | 6.942 | 1018 | 1014 | 1.08  | MS/RI              |
| 7   | p-Cymene        | 7.164 | 1026 | 1020 | 2.65  | MS/RI              |
| 8   | 1,8 Cineole     | 7.429 | 1034 | 1028 | 28.03 | MS/RI/St.          |
| 9   | γ-Terpine       | 8.093 | 1059 | 1054 | 0.91  | MS/RI              |
| 10  | α- Terpinolen   | 8.965 | 1090 | 1086 | 0.57  | MS/RI              |
| 11  | β-Linalool      | 9.292 | 1102 | 1095 | 2.22  | MS/RI              |
| 12  | (-)-Camphor     | 10.770 | 1149 | 1141 | 12.95 | MS/RI              |
| 13  | Borneol         | 11.387 | 1168 | 1165 | 2.67  | MS/RI              |
| 14  | Terpinen-4-ol   | 11.743 | 1180 | 1174 | 0.82  | MS/RI              |
| 15  | (-)-α-Terpineol | 12.181 | 1193 | 1186 | 2.84  | MS/RI              |
| 16  | cis-Verbenene   | 12.812 | 1213 | 1204 | 2.92  | MS/RI              |
| 17  | (-)-Bomyl acetate | 15.239 | 1288 | 1284 | 3.38  | MS/RI              |
| 18  | α-Ylangene      | 17.955 | 1374 | 1373 | 0.70  | MS/RI              |
| 19  | β-Caryophyllene | 19.491 | 1424 | 1417 | 4.71  | MS/RI/St.          |
| 20  | α-Caryophyllene | 20.507 | 1457 | 1456 | 0.82  | MS/RI              |

*Compounds listed in order to their elution on the HP-SMS column, RT retention times, RI_{cal} Retention indices on the HP-SMS column relative to C8-C22 n-alkanes, RI_{lit} Retention indices from the literatures, St. stander terpenoids, n/a=not available.

Table 2: compositions of RO leaves essential oil.

Citation: Al Saka F, Daghestani M and Karabet F. Composition and Antioxidant Activity of Vitex agnus-castus L. and Rosmarinus Officinalis L. Leaves Essential Oils Cultivated in Syria. SM Anal Bioanal Technique. 2017; 2(1): 1010.
strong as vitamin C, which is known by its radical scavenging activity. Total phenolic contents of VAC essential oil was about 3 times higher than RO essential oil, while total flavonoids contents were not detected in both essential oils. Beside, the values of DPPH radical scavenging effect and total phenols showed a significant difference (P<0.05) between VAC and RO essential oils. The result shows that the VAC essential oil has higher antioxidant activity than that of RO, because phenolics constitute one of the major groups of compounds acting as primary antioxidant free radical terminators.

It appears that the antioxidant activity depends on the presence of some compounds in the essential oils. The main role of such compounds as reducing free radicals is highlighted in several reports [33] like α-Pinene and sabinene etc. Furthermore, it is not only the major compounds of essential oils that are responsible for the antioxidant activity, but there may be also other minor compounds that can interact synergistically or antagonistically to create an effective system against free radicals [26,34]. However, VAC and RO extracts have an excellent antioxidant activity in comparison with its essential oil [16,27,35] (Tables 3 and 4).

### Table 3: I % of VAC leaves and RO leaves essential oils at different concentrations

| Concentrations | 0.2mg/ml | 0.5 mg/ml | 1 mg/ml |
|----------------|---------|---------|---------|
| VAC            | 0.56±0.13  | 1.07±0.19  | 2.09±0.24  |
| RO             | 0.25±0.14  | 0.52±0.17  | 1.02±0.10  |
| Vitamin C      | 33.24±0.60* | -        | -        |

*Values are expressed as means ± SD of three parallel measurements, the significance showed by *; P < 0.05.

### Table 4: Total phenolic and flavonoids contents of VAC leaves and RO leaves essential oils

| Essential oils | total phenolic contents (µg GAEs/mg essential oil) | total flavonoids contents (µg QEs/mg essential oil) |
|----------------|-----------------------------------------------|-----------------------------------------------|
| VAC            | 32.12±0.615*                                 | -                                             |
| RO             | 12.52±0.193*                                 | -                                             |

*Values are expressed as means ± SD of three parallel measurements, the significance showed by *; P < 0.05.

1. Li H-B, Wong C-C, Cheng K-W, Chen F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT-Food Science and Technology. 2008; 41:385-390.
2. Amiri H. Essential oils composition and antioxidant properties of three thymus species. Evidence-based complementary and alternative medicine. eCAM. 2012; 2012: 7220056.
3. Al-Mariri A, Swied G, Oda A, Al Hallab L. Antibacterial activity of thymus syriacus bois essential oil and its components against some Syrian gram-negative bacteria isolates. Iran J Med Sci. 2013; 38:180-186.
4. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother res. 2007; 21:308-323.
5. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A. 2004; 1054: 95-111.
6. Proestos C, Sereli D, Komalits M. Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS. Food Chem. 2006; 95: 44-52.
7. Zahid H, Rizwani GH, Ishaque S. Phytopharmacological Review on Vitex agnus-castus: A Potential Medicinal Plant. Chinese Herbal Medicines. 2016; 8: 24-29.
8. Hogner C, Sturm S, Seger C, Stuppern H. Development and validation of a rapid ultra-high performance liquid chromatography diode array detector method for Vitex agnus-castus. J Chromatogr B Analyt technol in the biomed life sci. 2013; 927: 181-190.
9. Wutkew W, Jarry H, Christoffel V, Spengler B, Seidiova-Wutkew D. Chaste tree (Vitex agnus-castus)—pharmacology and clinical indications. Phyto medicine: international journal of phytotherapy and phytopharmacology. 2003; 10: 348-357.
10. Dymuog HG, Çiçfiy GA, Yıldirim ŞU, Demirci B, Kirner N. The cytotoxic activity of Vitex agnus castus L. essential oils and their biochemical mechanisms. Industrial Crops and Products. 2014; 55:33-42.
11. Daniele C, Coon JT, Pittler MH, Ernst E. Vitex agnus castus: a systematic review of adverse events. Drug Saf 2005; 28: 319-332.
12. Stojkovic D, Sokovic M, Glamočlija J, Džanić A, Ćičić A, Ristić M, Grubišić D. Chemical composition and antimicrobial activity of Vitex agnus-castus L. fruits and leaves essential oils. Food Chem. 2011, 128: 1017-1022.
13. Choudhary M. Antibacterial, phytoxic, insecticidal and cytotoxic potential of Vitex agnus-castus. Journal of Medicinal Plants Research. 2011; 5: 5642-5645.
14. Al Saka F, Karabet F, Daghestani M, Soukkarieh C. Composition, in Vitro Antioxidant and Antileishmanial activities of Vitex agnus-castus L. and Thymus syriacus Boiss. Essential Oils. International Journal of ChemTech Research. 2015; 8: 53-60.
15. Khanjani Jafroodi S, Farazmand A, Amin M, Doroodgar A, Shirzadi M, Razavi M. Methanolic Extract’s Activity of Artemisia absinthium, Vitex agnus-castus and Phytolaca americana Against Leishmania major; in vitro and in vivo. International Archives of Health Sciences. 2015; 2: 69-74.
16. Gökbulut A, Özhan O, Karacaoğlu M, Şarer E. Radical Scavenging Activity and Vitexin Content of Vitex agnus-castus Leaves and Fruits. FABAD J Pharm Sci. 2010; 35: 85-91.
17. Sienkiewicz M, Lysakowska M, Pastuszka M, Bienias W, Kowalczyk E. The potential of use basil and rosemary essential oils as effective antibacterial agents. Molecules. 2013; 18: 9334-9351.
18. Faixova Z, Faix S. Biological effects of rosemary (Rosmarinus officinalis L) essential oil (A Review). Folia veterinaria. 2008; 3-4.
19. Genena AK, Hense H, Smáňa Junior A, Souza SMd. Rosemary (Rosmarinus officinalis): a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. Food Science and Technology (Campinas). 2008; 28: 463-469.
20. Council of Europe, European Pharmacopoeia Commission European Directorate for the Quality of Medicines & Healthcare: European Pharmacopoeia. 4th edn. Strasbourg: Council of Europe; 2002.
21. Adams R. Identification of Essential Oil Components by Gas chromatography/ Mass Spectroscopy. Journal of the American Society for Mass Spectrometry. 1997; 6(8):671-672.
22. Brand-Williams W, Cuvelier M-E, Beret C. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995; 28: 25-30.
23. Al Hafez M, Kheder F, Al Joubbeh M. Polyphenols, flavonoids and (-)-epigallocatechin gallate in tea leaves and in their infusions under various conditions. Nutrition & Food Science. 2014; 44: 455-463.

24. Shagaghi M, Manzoori J, Jouyban A. Determination of total phenols in tea infusions, tomato and apple juice by terbium sensitized fluorescence method as an alternative approach to the Folin–Ciochaltu spectrophotometric method. Food chem. 2007; 108: 695-701.

25. Shagaghi M, Manzoori J, Afshar D, Jouyban A. Determination of flavonoids in pharmaceutical preparations using Terbium sensitized fluorescence method. DARU Journal of Pharmaceutical Sciences. 2009; 17: 264-268.

26. Asdadi A, Hamdouch A, Ouakcha A, Moutaj R, Gharby S, Harhar H, et al. Study on chemical analysis, antioxidant and in vitro antifungal activities of essential oil from wild Vitex agnus-castus L. seeds growing in area of Argan Tree of Morocco against clinical strains of Candida responsible for nosocomial infections. J mycol med. 2015; 25: 118-127.

27. Sarikurkcu C, Arisoy K, Tepe B, Cakir A, Abali G, Meta E. Studies on the antioxidant activity of essential oil and different solvent extracts of Vitexagnuscatus L. fruits from Turkey. Food Chem. Toxicol.: an international journal published for the British Industrial Biological Research Association. 2009; 47: 2479-2483.

28. Jamshidi R, Aftahi Z, Aftahi D. Chemical composition of hydrodistillation essential oil of rosemary in different origins in Iran and comparison with other countries. American-Eurasian J of Agric Environ Sc. 2009; 5: 78-81.

29. Rašković A, Milanović I, Pavlović N, Čebić T, Vukmirović S, Miko M. Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential. BMC complement alter med. 2014; 14: 1.

30. Boutekedjiret C, Bentahar F, Belabbes R, Bessiere JM. Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour Fragr J. 2003; 18: 481-484.

31. Derwich E, Benziane Z, Chabir R. Aromatic and medicinal plants of morocco: chemical composition of essential oils of Rosmarinus officinalis and Juniperus Phoenixia. International Journal of Applied Biology and Pharmaceutical Technology. 2011; 2: 145-153.

32. Taziki S, Hamedeyazdan S, Pasandi A. Variations in essential oils of Vitex agnus castus fruits growing in Qum, Khorasan and Tehran in Iran. Annals of Biological Research. 2013; 4: 308-312.

33. Villano D, Fernández-Pachón M, Moyá M, Troncoso A, Garcia-Parrilla M. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta. 2007; 71: 230-235.

34. Singh G, Marimuthu P, de Heluani CS, Catalan CA. Antioxidant and biocidal activities of Carum nigrum (seed) essential oil, oleoresin, and their selected components. J agric food chem. 2006; 54: 174-181.

35. Celiktas OY, Bedir E, Sukan FV. In vitro antioxidant activities of Rosmarinus officinalis extracts treated with supercritical carbon dioxide. Food Chem. 2007; 101: 1457-1464.