Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

# Investigation of the volatile oils, lipid constituents and biological activity of *Ballota andreuzziana, Teucrium zanonii* and *Verbena tenuisecta* in Libya

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ARTICLE INFO

*Article history:*
Received 29 March 2010
Received in revised form 2 June 2010
Accepted 10 July 2010
Available online 20 August 2010

*Keywords:*
*Ballota andreuzziana*
*Teucrium zanonii*
*Verbena tenuisecta*
Volatile oil
Lipid constituents
Biological activity

ABSTRACT

**Objective:** To determine the chemical composition of essential oils and lipid constituents of *Ballota andreuzziana (B. andreuzziana), Teucrium zanonii (T. zanonii)* and *Verbena tenuisecta (V. tenuisecta)* growing in Libya, and to test the antibacterial activity of different extracts of *Teucrium zanonii*. **Methods:** The volatile oils of all plants were extracted by hydrodistillation method and analyzed by GC/MS method. The lipid constituents of plants were obtained by extraction with petroleum ether and fractionated into fatty alcohols, fatty acids and unsapoinfiable matters. Antibacterial activity of *T. zanonii* extracts and antioxidant activity of different extracts of *T. zanonii* were also studied. **Results:** The volate oil of *B. andreuzziana* was found to consists of 20 compounds in which caryophyllin is the main one (63.1%), the volatile oil of *T. zanonii* consists of 74 compounds in which germacrene-D was the main compound, while the volatile oil of *V. tenuisecta* consists of 13 compounds with 1-octen-3- ol as a major constituent (52.87%). The study of antimicrobial activity of different extracts of *V. tenuisecta* showed that, both methanol and butanol extracts exhibited the highest activity against *Mycobactirium phlei (M. phlei)* and *Candida albicans (C. albicans)* respectively, while petroleum ether, fatty alcohols and unsaponifiable fractions had no antimicrobial activity against all the tested microorganisms. Investigation of the antioxidant activity of different extracts of *T. zanonii* using DPPH method proved that, the ethyl acetate and butanol fractions showed the highest activity where the inhibition percentage (I%) are 93.6 and 92.1 respectively. **Conclusions:** This is the first report about the volatile oils of these plants where *T. zanonii* have the highest content and the highest number of the identified compounds. The study of antioxidant *T. zanonii* extracts proved that, the ethyl acetate, butanol and aqueous extracts have the highest antioxidant activity. Methanol and butanol extracts of *V. tenuisecta* exhibited the highest activity against *M. phlei* and *C. albicans* respectively.

## 1. Introduction

The genus *Ballota* and *Teucrium* belongs to family *Labiateae (Lamiaceae)*, it comprises thirty five species in the world and is represented by four species in Libya[1]. Many compounds like volatile oils, diterpenoids and various polyphenols including phenylpropanoid derivatives and natural phenolics (flavonoids and phenolic acids) have been isolated and identified from *Ballota* genus[2,3]. Plants of this genus possess antiulcer, antispasmodic, diuretic, antihemorrhoidal, antioxidant, antiemetic, antimicrobial, anti-inflammatory and hepatoprotective properties, and have been used traditionally and in modern medicine for treatment of wounds, burns, suppress cough, upper respiratory system inflammation, neurosedative[4-11].

*Teucrium zanonii* (*T. zanonii)* belongs to the same family as *Ballota* genus, and is represented by 13 species in the flora of Libya. Both of the two plants are endemic Libyaian plants. *Teucrium* species are rich source of volatile oils and neoclerodane diterpenoids, in addition to furanoid diterpenoids and flavonoids. The genus *Teucrium* is the most abundant natural source for these compounds; therefore

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*Teucrium* species are accepted as chemotaxonomic markers for neoclerodanes. Chemical investigation of this genus showed that some of species also contain sesquiterpenes, triterpenes, sterols, flavonoids, iridoids, phenolic acids and some alkaloids. Many *Teucrium* species have been used for more than 2 000 years as medicinal plants. They exhibited some interesting biological activities like diuretic, diaphoretic, antiseptic, antipyretic, antispasmodic, antiulcer, antirheumatic, antibacterial, antioxidant, hypoglycemic, antifeedant. Besides, some *Teucrium* extracts are used in folk medicine to treat various ailments such as stomach and intestinal troubles, rheumatism, hemorrhoids and renal inflammatory, asthma, an infusion of the leaves and flowers is used as a hop substitute for flavouring beer[12-16].

*Verbena tenuisecta(V. tenuisecta)* is introduced from South America[17] as an ornamental plant growing in many region of Libya. The phytochemical information on *Verbena* genus has been published and various constituents *vis.* volatile oil, sterol and triterpenoids , some lipid classes, iridoids and many phenolic compounds[18-25]. In course of our studies on *Verbena* genus, the plant has been extensively used as popular herb in folk for the treatment of rheumatism, also widely used to cure fever, tonsillitis, gastrointestinal disorders and some sexually transmitted diseases in South America; and in modern medicine as antimicrobial, antioxidant, anti-inflammation, neurosedative and diuretic agents[26-29]. In this paper we aimed to investigate some chemical constituents of the three plants like (volatile oils, lipid constituents) in addition to their biological activity.

## 2. Material and methods

### 2.1. Plants material

*Ballota andreuzziana (B. andreuzziana)* was collected in April 2006 during the flowering stage from Wadi El-Husaien, along the coastal of Ras El-Hilal to Shahat city road, Gebel Akhdar city, *Teucrium zanonii (T. zanonii)* was collected in April 2008 during the flowering stage from Abo- fakhra region about 25 km from Benghazi city. The two plants were kindly identified by Dr. Mohamed Alsharif at Botany Department, Faculty of Science, Gariuones University, Benghazi city, Libya. While *Verbena tenuisecta (V. tenuisecta)* Briq. was collected from central garden of Sirte city, Libya in August 2007 during the flowering stage, the plant was kindly identified and authenticated by Dr. Mohamed N. Abohadra Prof. of taxonomy at Botany Department, Faculty of Science, Al Fatih University, Tripoli, Libya. All the species specimens were deposited at the herbarium of Biology Department, Faculty of Scienle, Altahady University, Sirt, Libya.

### 2.2. Extraction of the volatile oils

About 350 g of fresh plant material (flower buds) of *B. andreuzziana*, *T. zanonii* and *V. tenuisecta* respectively was subjected to hydrodistillation method in all-glass apparatus for about three hours according to Gunther method[30]. The trapped oil in the side arm was removed separately and kept in refrigerator to GC/MS analysis after complete distillation and dry over anhydrous sodium sulphate to give a pale yellow oil (0.20%, 20.00% and 0.13% v/w respectively). Constituents of *T. zanonii* were extracted using a solvent of n-hexane:ether (50:50).

### 2.3. Extraction of lipids and related substances

About 800 g, 1.40 kg and 1.75 kg of the dried powdered plant of *B. andreuzziana, T. zanonii* and *V. tenuisecta* were extracted with petroleum ether (b.r. 40-60 ℃) respectively in a continuous extractor (Soxhlet apparatus). The combined petroleum ether extract was passed through fuller's earth to remove coloured pigments, filtered, dried over anhydrous sodium sulphate and evaporated in vacuo at 40 ℃ till dryness to give a pale yellow residue (6.0 g, 12.3 g and 10.1 g). These residues were dissolved in boiling acetone (250 mL) and left overnight at room temperature. Amorphous precipitates were filtered, washed with cold acetone and recrystallized from chloroform/methanol to give bright white crystals (1.95 g, 2.80 g and 1.27 g) of acetone insoluble fraction (Fatty Alcohols mixture). The filtrates (Acetone soluble fraction) were evaporated till dryness (3.85 g, 7.50 g, 8.02 g) and subjected to saponification process.

### 2.4. Saponification of acetone soluble fraction[31]

The acetone soluble fractions of the three plants (3.85 g, 7.50 g and 8.00 g) respectively were saponified (as in reference 31) to give a yellowish brown semisolid residue of unsaponified matter (0.84 g, 4.10 g and 2.96 g) and 0.44 g, 0.70 g and 1.31 g of the total fatty acids which were methylated and subjected to GLC.

### 2.5. Analysis and identification of the volatile oil and lipids constituents

The volatile oil and fatty alcohols mixtures were subjected to GC/MS using the same conditions while the unsaponifiable fraction and the fatty acids mixture were subjected to GLC as mentioned before[31].

### 2.6. Antimicrobial activity study of V. tenuisecta

The study of the antimicrobial activity of different extracts of *V. tenuisecta* was carried out using disc-diffusion modified- Kirby- Bauer and Streaking methods[32,33] against some selected microorganisms.

### 2.7. Tested microorganism

*Staphylococcus aureus (S. aureus), Bacillus subtillis (B. subtillis), Mycobactirium phlei (M. phlei)* and *Candida albicans (C. albicans)* were cultured and they were supplied by the Unit of the Chemistry of Natural and Microbial Products, Department of National Research Center, Cairo, Egypt and Biotech Center, Tripoli, Libya. The control agents used for antimicrobial and antifungl activity are tetracycline and metradinazol as antifungl. The microbiological media are Muller Hanton-Nutrient broth and Nutrient agar (Oxid, UK) and Sabouraud dextrose (Oxid, UK).

### 2.8. Antioxidant activity of different extracts of T. zanonii [34]

The DPPH test is a commonly employed assay in antioxidant studies because it offers a rapid technique to screen the RSA of pure synthetic compound, isolated natural compound, crude plant extracts and food.

## 3. Results

The obtained pale yellow oil of *B. andreuzziana*, *T. zanonii*, *V. tenuisecta* (0.20% v/w, 0.25 %v/w, 0.13% v/w) was analyzed by GC/MS (Table 1). The GC/MS analysis of fatty alcohols mixtures obtained from *B. andreuzziana*, *T. zanonii*, *V. tenuisecta* were shown in Table 2. Study of unsaponifiable fraction and the total fatty acids obtained from the studied species were shown in Table 3 & 4, Antibacterial activity of *T. zanonii* extracts against *S. aureus*, *B. subtillis*, *M. phlei*, *C.*

**Table 1**
GC/MS data of volatile oil of *B. andreuzziana*, *T. zanonii*, *V. tenuisecta*.

| No. | Components | $R_t$ (min) | Plants relative (%) | | |
|---|---|---|---|---|---|
| | | | *B. andreuzzianz* | *T. zanonii* | *V. tenuisecta* |
| 1 | Toluene | 4.18 | – | – | 1.25 |
| 2 | β –Myrcene | 5.39 | – | 1.13 | – |
| 3 | β –Pinene | 9.45 | 0.09 | 14.13 | – |
| 4 | Linalool | 9.72 | – | 11.00 | – |
| 5 | Octen–1–ol, acetate | 9.89 | – | t | – |
| 6 | E–Pinocarveol | 10.99 | – | 2.22 | – |
| 7 | 2(10)–Pinen–3–one | 11.92 | – | 1.20 | – |
| 8 | Borneol | 12.09 | – | t | – |
| 9 | D–Limonene | 12.74 | 0.08 | 3.48 | 9.33 |
| 10 | 3–Cyclohexene–1–methanol,– α , α ,4–trimethyl | 13.14 | – | t | – |
| 11 | 1–Octen–3–ol. | 13.29 | – | – | 52.87 |
| 12 | α –Terpineol | 13.44 | – | 5.56 | – |
| 13 | Myrtenol | 13.64 | – | 1.67 | – |
| 14 | Geraniol | 15.09 | – | 1.00 | – |
| 15 | Linalyl acetate | 16.32 | – | 11.10 | – |
| 16 | Linalyl acetate | 16.32 | – | 11.10 | – |
| 17 | 2–Nonen–1–ol | 17.74 | – | – | 1.32 |
| 18 | 3–Cyclohexene–1–methanol,4,5,5–trimethyl acetate | 20.02 | – | 1.15 | – |
| 19 | Geranyl acetate | 20.78 | – | 1.53 | – |
| 20 | Dihydrocarveol | 20.98 | t | – | – |
| 21 | 3(7)–Carene, 4–hydroxymethyl | 21.23 | – | – | 1.89 |
| 22 | 1,10–Decanediol | 24.84 | – | – | 5.03 |
| 23 | Germacrene–D | 25.58 | – | 8.81 | – |
| 24 | γ –Elemene | 26.21 | – | 7.79 | – |
| 25 | Spathulenol | 29.22 | – | 2.30 | – |
| 26 | Caryophyllene oxide | 40.37 | 2.00 | 0.28 | – |
| 27 | τ –Cadinol | 31.56 | – | 1.27 | – |
| 28 | Caryophyllene | 31.87 | 63.10 | 2.20 | – |
| 29 | Dodecane, 3–methyl | 31.90 | t | – | – |
| 30 | β –Eudesmol | 31.92 | – | 1.33 | – |
| 31 | α –Cadinol | 32.14 | – | 1.56 | – |
| 32 | Selinene | 35.81 | 5.03 | 1.80 | – |
| 33 | Cis– ɣ –Bisabolene | 42.91 | 26.30 | – | – |
| 34 | Cyclododecene, 1–methyl | 43.98 | – | – | 2.62 |
| 35 | 8, 10– dodecadienal | 44.66 | – | – | 3.25 |
| 36 | 1, 12–dodecandiol | 44.97 | – | – | 1.75 |
| 37 | 2,4,6–triisopropylphenol | 46.81 | | – | 5.44 |
| 38 | Caryophyllene oxide | 50.48 | 2.00 | – | – |
| 39 | Bicyclo[3.2.0]heptan–2–one, 6–hydroxy–5–methyl–6–vinyl | 56.91 | – | – | 13.89 |
| | Total (%) | | 98.5 | 93.61 | 98.64 |

Rt (min) = Retention time (min) .

**Table 2**
GC/MS data of fatty alcohols mixture obtained from the three studies plants.

| | Chemical formula | Compounds | $R_t$ (min) | Plants relative (%) | | |
|---|---|---|---|---|---|---|
| | | | | *B. andreuzzianz* | *T. zanonii* | *V. tenuisecta* |
| 1 | $C_{17}H_{36}O$ | Heptadecanol | 7.63 | – | – | 46.07 |
| 2 | $C_{18}H_{38}O$ | Octadecanol | 13.01 | 46.07 | – | – |
| 3 | $C_{23}H_{48}O$ | Tricosanol | 13.20 | – | 5.10 | – |
| 4 | $C_{24}H_{50}O$ | Tetracosanol | 13.84 | 16.31 | 4.62 | |
| 5 | $C_{25}H_{52}O$ | Pentacosanol | 14.05 | – | 23.37 | 23.50 |
| 6 | $C_{26}H_{54}O$ | Hexacosanol | 14.06 | 11.85 | – | – |
| 7 | $C_{27}H_{56}O$ | Heptacosanol | 15.10 | – | – | 16.14 |
| 8 | $C_{28}H_{58}O$ | Octacosanol | 19.12 | 13.30 | – | – |
| 9 | $C_{29}H_{60}O$ | Nonacosanol | 19.92 | 12.47 | – | 26.21 |
| 10 | $C_{30}H_{60}$ | Triacoontene | 20.31 | – | 24.73 | – |
| 11 | $C_{31}H_{60}O$ | Hentriacontanol | 24.08 | – | 16.49 | – |
| 12 | $C_{32}H_{66}O$ | Dotriacontanol | 26.37 | – | 2.45 | – |
| 13 | $C_{34}H_{78}$ | Tetratricontane | 32.00 | – | 15.95 | – |

**Table 3**
GLC data of unsaponifiable fraction.

| Peak No. | Compounds | $R_t$ (min) | Plants relative (%) | | |
|---|---|---|---|---|---|
| | | | *B. andreuzzianz* | *T. zanonii* | *V. tenuisecta* |
| 1 | $C_3$ | 4.91 | – | 0.37 | – |
| 2 | $C_5$ | 5.84 | – | 0.93 | – |
| 3 | $C_8$ | 6.32 | – | 0.87 | – |
| 4 | $C_9$ | 7.58 | – | 3.18 | – |
| 5 | $C_{10}$ | 8.60 | – | 7.50 | 0.06 |
| 6 | $C_{11}$ | 8.79 | 0.08 | – | 0.24 |
| 7 | $C_{12}$ | 9.01 | 0.22 | 4.55 | 0.50 |
| 8 | $C_{13}$ | 9.90 | 1.14 | – | 1.06 |
| 9 | $C_{14}$ | 10.80 | 0.96 | 1.93 | 1.57 |
| 10 | $C_{15}$ | 11.12 | – | – | 0.82 |
| 11 | $C_{16}$ | 13.00 | – | 0.83 | 4.77 |
| 12 | $C_{17}$ | 13.43 | – | 1.89 | 3.68 |
| 13 | $C_{18}$ | 14.20 | – | 2.04 | 3.40 |
| 14 | $C_{19}$ | 14.53 | – | – | 3.52 |
| 15 | $C_{20}$ | 17.20 | – | 46.98 | 2.99 |
| 16 | $C_{22}$ | 17.38 | 6.23 | 4.08 | 9.68 |
| 17 | $C_{23}$ | 17.46 | 8.45 | – | 2.07 |
| 18 | $C_{24}$ | 17.54 | – | – | 2.39 |
| 19 | $C_{25}$ | 20.40 | 53.91 | 1.70 | 1.96 |
| 20 | $C_{26}$ | 21.84 | 1.66 | 6.29 | 2.97 |
| 21 | $C_{27}$ | 23.02 | 1.81 | – | 7.32 |
| 22 | $C_{28}$ | 24.50 | 1.33 | 3.85 | 2.03 |
| 23 | $C_{29}$ | 25.44 | – | 1.14 | 5.74 |
| 24 | $C_{30}$ | 27.47 | – | 2.36 | – |
| 25 | $C_{32}$ | 29.03 | – | 1.99 | – |
| 26 | Cholesterol | 31.75 | 15.47 | 4.48 | 2.12 |
| 27 | Stigmasterol | 34.01 | 0.83 | 0.36 | 16.59 |
| 28 | β – Sitosterol | 34.93 | 5.62 | 1.36 | 13.77 |
| 29 | Campasterol | 35.40 | – | 0.86 | – |
| 30 | β – Amyrine | 39.41 | 2.29 | 0.41 | 10.74 |

**Table 4**
GLC analysis of fatty acid methyl ester.

| Peak No. | Compounds | | $R_t$ (min) | Plants relative (%) | | |
|---|---|---|---|---|---|---|
| | | | | *B. andreuzzianz* | *T. zanonii* | *V. tenuisecta* |
| 1 | Lauric acid | ($C_{12:0}$) | 4.27 | – | 1.36 | – |
| 2 | Myrstic acid | ($C_{14:0}$) | 10.05 | – | 1.22 | 1.72 |
| 3 | Palmitic acid | ($C_{16:0}$) | 11.61 | 27.28 | 13.95 | 26.52 |
| 4 | Stearic acid | ($C_{18:0}$) | 15.92 | – | 15.05 | – |
| 5 | Oleic acid | ($C_{18:1}$) | 16.56 | 2.48 | 13.69 | – |
| 6 | Linolic acid | ($C_{18:2}$) | 17.44 | 57.05 | 35.25 | 19.50 |
| 7 | Linolenic acid | ($C_{18:3}$) | 18.88 | – | 11.21 | 44.66 |
| 8 | Arachidic acid | ($C_{20:0}$) | 20.40 | 2.56 | 1.58 | – |
| 9 | Gadolic acid | ($C_{20:1}$) | 22.97 | 3.57 | – | 4.04 |
| 10 | Behenic acid | ($C_{22:0}$) | 24.91 | 5.44 | – | 2.42 |
| 11 | Erucic acid | ($C_{22:1}$) | 26.59 | 1.62 | 1.21 | 1.04 |
| 12 | Lignoceric acid | ($C_{24:0}$) | 28.36 | – | 3.58 | – |
| 13 | Tetracosenoic acid | ($C_{24:1}$) | 32.25 | – | 1.90 | – |

**Table 5**
Effect of different extracts of *V. tenuisecta* on some microorganism.

| Microbes | | Bacteri | | | Fungi |
|---|---|---|---|---|---|
| *Ext.* | *Concentration* | *S. aureus* | *B. subtillis* | *M. phlei* | *C. albicans* |
| Petroleum ether | a | – | – | 11.330±1.155 | – |
| | b | – | – | 13.330±1.155 | – |
| | c | – | – | 14.670±0.577 | – |
| Fatty alcohol | a | – | – | – | – |
| | b | – | – | – | – |
| | c | – | – | – | – |
| Unsap | a | 9.000±1.000 | – | – | – |
| | b | 9.670±0.577 | – | – | – |
| | c | 11.000±1.000 | – | – | – |
| Fatty acid | a | – | – | 7.330±0.577 | – |
| | b | – | – | 9.000±1.000 | – |
| | c | – | – | 11.000±1.000 | – |
| Methanol | a | – | – | 11.330±0.577 | – |
| | b | – | 7.330±0.577 | 12.670±0.577 | – |
| | c | – | 8.000±0.000 | 13.330±0.577 | – |
| Chloroform | a | – | – | – | – |
| | b | – | 8.000±0.000 | – | – |
| | c | – | 11.000±1.000 | – | – |
| Butanol | a | 8.670±1.528 | – | 13.000±4.359 | – |
| | b | 9.330±1.155 | – | 15.000±4.359 | – |
| | c | 11.330±1.155 | – | 16.330±4.933 | 10.670±0.557 |
| Acetone | a | – | – | 10.000±1.00 | – |
| | b | – | 7.670±0.577 | 11.670±1.155 | – |
| | c | – | 8.670±0.577 | 14.670±2.517 | – |

a=0.05 g/mL, b=0.10 g/mL, c=0.5 g/mL.

*albicans* and antioxidant and antioxidant activity of different extracts of *T. zanonii* were shown in Table 5 & 6.

**Table 6**
Antioxidant activity of different extracts of *T. zanonii*.

| Sample | Absorbance | I% |
|---|---|---|
| Blank (MeOH) | 0.363 | – |
| Total alcoholic extract | 0.045 | 87.6 |
| Chloroform extract | 0.172 | 52.4 |
| Ethyl acetate extract | 0.023 | 93.6 |
| Butanol extract | 0.028 | 92.1 |
| Petroleum ether extract | 0.363 | 0.0 |
| Unsaponifiabl fraction | 0.361 | 2.0 |
| Blank ($H_2O$) | 0.353 | – |
| Aqueous extract | 0.080 | 77.5 |

## 4. Discussion

### 4.1. Pale yellow oil obtained from the three plants

The obtained pale yellow oil of *B. andreuzziana* (0.20% v/w) was analyzed by GC/MS. The results revealed that it consists of a mixture of twenty compounds (five of them constitute about 95.0%) belonging to many classes as saturated hydrocarbons, unsaturated hydrocarbons, alcohols, aldehydes, ketones, ester, oxides and aromatics. The sesquiterpene content was found the highest value (95.25%) of the oil, among which caryophllene (63.10%), cis γ – bisabolene (26.30%) and α – Selinene (5.03%) are the most abundant compounds. The oxygenated sesquiterpen constitute about 2.45% in which caryphyllene oxide is the major one (2%). While the monoterpenes represents about 2.4% in which thujene, (bicyclic monoterpen 0.7%) is the main one. These data were in accordance with that reported by Couladis *et al* in 2002[33] from *B. pseudodictamnus* and Bader *et al* in 2003 from *B. undulate*[34].

The GC/MS data showed the hydrodistillation extraction of volatile oil of *T. zanonii* is a mixture of 80 compounds only 74 compounds were identified representing 92.98% of the total (nine of them constitute about 85%). The identified compounds represent several chemical classes, viz.: saturated hydrocarbons 0.56%, unsaturated hydrocarbons 41.79%, alcohols 31.68%, aldahydes 0.09%, ketones 2.39%, esters 15.16%, oxides 0.64% and aromatics 0.67%, with the highest abundance of β –Pinene, linalyl acetate, linalool, germacrene–D in addition to γ –elemene (14.13%, 11.10%, 11.00%, 8.81% and 7.79% respectively). These results were coincided with that reported by Cavaleiro *et al* [35], where they identified more than seventy components from the oil of *T. lusitanium* and *T. algarbiensis* in which β –Pinene and germacrene–D are the major compounds. While the GC/MS analysis of the volatile oil extracted by solvent extraction (n–hexane∶ether 50∶50) showed a mixture of 16 compounds representing 86.90% of the total oil. The identified compounds represent several chemical classes, viz.: saturated hydrocarbons 16.08%, unsaturated hydrocarbons 60.94%, alcohols 0.91%, ketones 1.24%, esters 7.93% and about 13.10% unknown compounds with the germacrene–D, β –Pinene and linalyl acetate as the main components, (20.04%, 18.19% and 7.93% respectively).

The GC/MS analysis of *V. tenuisecta* volatile oil showed it is a mixture of thirteen compounds 98.64% (six of them constitute about 91.0%. The identified compounds represent several chemical classes, vis.: alcohols (60%), bicyclic monoterpenes (16.55%), monocyclic monoterpenes (11.95%), aromatics (6.69%), aldehydes (3.25%), acyclic monoterpenes (0.59%). Also, the results showed that 1–octen–3–ol (52.87%), bicyclo[3.2.0] heptan–2–one–6–hydroxy–5–methyl–6–vinyl (13.89%) and limonene (9.33%) are the main compounds. These data were in agreement with that reported by Mohammad *et al*[36] where they investigated the volatile oil of *V. officinalis* in 2003 and identified 1–octen–3–ol as one of the main compound, also Chalchat and Garry[37] were identified limonene in the volatile oil of the same plant in 1999.

### 4.2. Acetone insoluble fraction (fatty alcohols mixture)

The GC/MS analysis of fatty alcohols mixtures obtained from *B. andreuzziana* and *V. tenuisecta* revealed the presence of a mixture of 5 fatty alcohols. For *B. andreuzziana*, octadecanol being the main constituent (46.07%) while for *V. tenuisecta*, heptadecanol (46.07%) is the main one, but for *T. zanonii* it showed the presence of tricosanol (5.10%), tetracosanol (4.62%), pentacosanol (23.37%), nonacosanol (26.21%), triacontene (24.73%), tetratricontene (15.95%).

### 4.3. Unsaponifiable fraction

The unsaponifiable fraction of the studied species was investigated by GLC. The results proved that it mainly contain a mixture of a series of n–alkanes, a sterols fraction and triterpene fraction. for *B. andreuzziana* the unsaponifiable fraction contain a series of hydrocarbons in which n–C–25 is the main one , sterols with cholesterol as the highest percentage and triterpene β –amyrin (2.29%), these data were in accordance with Ahmed *et al*[38] who reported that *B. limbita* contain β – sitsterol and oleanelic acid, while of *T. zanonii* the hydrocarbon fraction contain n–C3 ton–C32, the sterol fraction contain cholesterol, stigmasterol, β –sitosterol and campasterol in addition to β –amyrine (0.41%). Finally for *V. tenuisecta*, n–C16 (9.68%), sterols fraction of stigmasterol (16.59%) and β –amyrine (10.74%) are the main compounds respectively. These data were coincided with that reported by Makboul in 1986[39] and Liu C and Liu Y in 2002[40].

### 4.4. Fatty acids methyl esters

The study of the total fatty acids of *B. andreuzziana*

revealed the presence of saturated fatty acids (35.28%) and unsaturated fatty acids (64.72%) in which palmatic and linoleic acids are the main compounds respectively, while *T. zanonii* revealed the presence of lauric (1.36%), myristic (1.22%), palmitic (13.95%), stearic (15.05%), oleic (13.69%), linoleic (35.25%), linolenic (11.21%), arachidic (1.58%), erucic (1.58%), lignoceric (3.58%), tetracosenoic (1.90%). The saturated and unsaturated fatty acids represents 36.74% and 63.27% respectively. Also stearic and lineoleic acids are the major acids. But for *V. tenuisecta*, the results proved the presence of saturated and unsaturated fatty acids in percentages of 30.66% and 69.34% in which linolenic acid and palmitic acid are the major acids respectively. Also $C_{18:2}$ and $C_{18:3}$ constituted about 64.16% of fatty acids. These data were in accordance with that reported by Jose *et al*[41] in 1999 where they studied the composition of fatty acids in *V. officinalis* and they found that the major fatty acids are $C_{18}$, $C_{18:2}$ and $C_{18:3}$.

### *4.5. Antimicrobial and antioxidant activity activity analysis*

The results of antimicrobial activity of *V. tenuisecta* extracts of different concentrations against some selected microorganisms showed that: The petroleum ether extract, fatty alcohols and unsaponifiable fraction had no antimicrobial activity against all the tested microorganisms. The fatty acids fraction had high activity against *M. phlei*, moderate activity against *B. subtillis*, but had no effect against the *S. aureus* and *C. albicans*. The methanol extract showed high inhibition against *M. phlei*, moderate activity against *B. subtillis*, but had no effects on *S. aureus*. Moreover had high activity against *C. albicans* at concentration (c, b). But at the concentration (a) had no effect. The chloroform extract exhibited moderate activity against Staphylococcus aureus at the high concentration (c) only. While Bacillus subtillis was affected at concentration (b, c) as a moderate activity. Whereas did not effect on both *M. phlei* and *C. albicans*.

The butanol extract showed a moderate inhibition against *M. phlei*, *C. albicans* and *S. aureus*, but the conc. (a) did not affected with S. aureus. The acetone extract had moderate activity against *M. phlei* only in a concentration dependent mode and did not affect on the other microbes.

These results were coincided with that reported by El-Aziz *et al*[33]. where they proved the antibacterial activity of butanol and ethyl acetate extracts of *V. bipinnatifida*. Also, Gamboa and Castro in 2004[28] reported about the antibacterial activity of some compounds isolated from *V. littoralis*.

The studies of antioxidant activity of different extracts against DPPH showed that the ethyl acetate, butanol fractions and aqueous extract have the highest antioxidant activity. This activity may be mainly due to the presence of flavonoids (aglycones or glycosides) in these fractions. These observations were in accordance with that reported by Sonja *et al*[43].

## Conflict of interest statement

We declare that we have no conflict of interest.

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