ISSN 0974 – 5211

Journal of Natural Products
(Only ON-LINE, ANNUAL, Science Journal published from INDIA)
Volume 10 (2017)
www.JournalofNaturalProducts.com

Research Paper

Composition and Bioactivity of the Aerial Parts of *Artemisia monosperma* Del. Growing in Libya

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(Received 15 Feb..2017; Revised 20 April-18 August 2017; Accepted 28 August 2017; On-lined 03 Dec. 2017)

ABSTARCT

Criteria for identification and quality control of *Artemisia monosperma* Del**.,** grown in Libya were scanty. Indigenous browse species are considered as useful sources of animal feeds. This prompted the exploration of the nutritional value of the plant. Macro- and micro-minerals were determined. Total carbohydrate, lipid and protein content were estimated. Lipoids were subjected to GLC. HPLC analysis of phenolic contents was carried out. Liquid-liquid fractionation of the ethanol extract of defatted marc (DEAP) was afforded eight compounds characterized through spectral data. Microbial strains were used for antimicrobial susceptibility testing. Larvae of *Culex pipiens* and those of the *Toxocara vitulorum* were used for testing the insecticidal and anthelmintic activities, respectively. *In vivo* studies include LD_{50}, antioxidant and anti-inflammatory activities. The LD_{50} of the aerial parts ascertained its safety, in addition, its good nutritive value indicated by a high protein 8.4%, low fat4.2% and appreciable vitamin A and E contents. Squalene32.5% and linoleic acid26.5% were detected as major lipoids. The triterpenoids ester of Lupeol **1** and *β*-amyrin **2** were characterized from petroleum ether extract (PE). Quercetrin **3**, gallic acid **4**, methylgallate **5**, kaempferol **6**, kaempferide**7** and isoquercitrin **8** were isolated from the ethyl acetate and *n*-butanol extractives of DEAP respectively. PE exhibited a better larvicidal effect against *C.pipiens* and *T. vitulorum* than the DEAP. The latter exhibited significant antioxidant, moderate antimicrobial and larvicidal activities. Finally, the plant could be suggested as safe and valuable fodder. However, it could be considered as anti-inflammatory, antifungal and anthelmintic candidate.

Key Words: *Artemisia monosperma;* Quality control; Nutritive value; Bioactivity.

INTRODUCTION

Artemisia monosperma Del**.** is widespread in Egypt and Libya (Boulos, 2002). The plant grows in different Libyan localities as Gasar Gharian, Ghat area (El Barkat and Wadi Iseien), southern Tripolitania and Saharo-Arabian element (Jafri and El-gadi,

1983). Indigenous browse species are considered as useful sources of animal feeds especially in the dry season (Aregawi et al., 2008).

In spite of the multiplicity of publications on *Artemisia* species, studies related to the chemical composition and biological evaluation of those wild growing in Libya (Jafri and El-gadi, 1983) were scanty, and nothing could be traced regarding *A. monosperma* Del. On the other hand, this species was claimed to exert antispasmodic, anthelmintic and antihypertensive properties (Chakravarty, 1976). Leaves are used in folk Jordanian medicine to induce abortion (Hijazi and Salhab, 2010) and were found to possess antioxidant (Al-Soqeer, 2011), insecticidal (Saleh, 1984), antimalarial (Maia and Moore, 2011) and anticancer (Stavri et al., 2005) activities. Secondary metabolities including coumarins (Hammoda et al., 2008), flavonoids (Elgamal et al., 1997), acetylenes (Saleh, 1984), alkaloids, sesquiterpenoids (Zaki et al., 1984 and 2004), and triterpenoids (Elgamal et al., 1997) were reported from plants growing in localities of close climatic conditions.

The lack of data concerning the plant growing in Libya stimulated the performance of this investigation aiming to explore its composition and bioactivity, and estimate the possibility of its use as raw material in the national pharmaceutical industry and establishes their beneficial effects with regard to some health problems in Libya.

MATERIALS AND METHODS

Plant material: Samples of aerial parts of *Artemisia monosperma* Del. were gathered from plants growing in Tobruk desert (Libya). Plant collection was carried out in November 2013. The identity of the plant material was kindly verified by Dr. Mohamed Al-Gebali, botanist specialist. Voucher specimens (Number 1-12-2013-1) are kept at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Plant extracts

Petroleum ether extract (PE): This was prepared from the air-dried aerial parts (3 kg) by repeated cold maceration with petroleum ether (60-80°C).

Defatted ethanol extracts (DEAP) and fractions: The ethanol (70%) extract was obtained through exhaustive cold maceration of the dried defatted mark (left over after extraction with petroleum ether). The solvent was evaporated under reduced pressure (at 40°C). The residual ethanol-free extractives were suspended in water and sequentially extracted with methylene chloride, ethyl acetate and *n*-butanol saturated with water. The solvent, in each case, was removed by vacuum evaporation and dried extractives saved for further chemical and/ or biological examination.

Material for phytochemical study: The following solvent systems were tried for developing the TLC and PC chromatograms: S_1: *n*-hexane: ethyl acetate (9:1) v/v. S_2: *n*-hexane: ethyl acetate (8:2) v/v. S_3: *n*-hexane: ethyl acetate (7:3) v/v (TLC). S_4: methylene chloride: methanol (9:1) v/v. S_5: *n*-hexane: ethyl acetate: formic acid (5:4.5:0.5) v/v. S_6: *n*-hexane: ethyl acetate: methanol: formic acid (11.5: 13.5: 3.5:0.5) v/v.

Reference materials: For GLC analysis of the unsaponifiable matters (USM) and fatty acid methyl esters (FAME): reference samples, purchased from Nu-Check Prop, were provided by the Central Laboratory at the Faculty of Agriculture, Cairo University, Egypt. Gallic acid and Rutin (Sigma Chemical Co.; St. Louis, MO, USA) were used as standards in spectrophotometric determination of total phenolic and flavonoid contents. For HPLC quantitation of phenolic acids and flavonoids:

reference samples (Sigma Chemical Co.; St. Louis, MO, USA) were supplied by the Food Technology Research Institute, Agricultural Research Center, Egypt.

Material for biological and experimental models

Animals: Adult male albino rats of Sprague Dawley strain (100-120g), were used for determination of LD_{50} and assessment of the anti-inflammatory and antioxidant activities. They were obtained from the National Research Centre, laboratory animal facility, Dokki, Giza. They housed in stainless steel cages (34cm×47cm×18cm) with soft wood shavings as bedcovers, fed with standard pellets diet and given water *ad libitum*. They were allowed to acclimate to standard laboratory conditions (24-28°C temperature, 60-70% relative humidity, and 12h light/dark cycle) for 1 week before the experiments. They were underprivileged of food for at least 18 h previous to experiments but allowable free access to drinking water. The gear usage, management and sacrificing of the animals were performed in accordance with the Ethics committee (No.9-031) in agreement with recommendations for the appropriate care and use of laboratory animals (**NIH Publication No. 80-23; revised 1978**).

Microorganisms: A series of bacterial and fungal strains, obtained from the Regional Center for Mycology and Biotechnology (RCMB, Al-Azhar University, Egypt), was used for antimicrobial susceptibility testing. It comprises the following microorganisms: *Aspergillus fumigatus* (RCMB 02564), *Candida albicans* (RCMB 05035), *Geotricum candidum* (RCMB 05096), and *Syncephalastrum racemosum* (RCMB 05922) as representatives of fungi; *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024) *Streptococcus pyogenes* (RCMB 010015) as representatives of Gram-positive bacteria; and *Neisseria gonorrhoeae* (RCMB 010076), *Proteus vulgaris* (RCMB 010085) *Klebsiella pneumonia* (RCMB 010093) *Shigella flexneri* (RCMB 01005420) and *Escherichia coli* (RCMB 010056) as those of the Gram negative group.

Insect and worm larvae: Larvae of the mosquito *Culex pipiens* and those of the nematode *Toxocara vitulorum* were used for testing the insecticidal and anthelmintic activities, respectively. *Culex pipiens* larvae were obtained from a laboratory strain maintained at the Entomology Department, Faculty of Science, Ain-Shams University (Cairo, Egypt). Meanwhile those of *Toxocara vitulorum* were produced from embryonated eggs of worms, released from dissected intestines of slaughtered calves at El-bassatin and Embaba abattoirs (Cairo, Egypt), and handled at the Department of Parasitology, Faculty of Veterinary Medicine, Cairo University (Giza, Egypt).

Laboratory diet and culture media: Experimental animals were kept under hygienic conditions and on standard laboratory diet composed of: vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein (10.5%) and starch (54.3%). The tested organisms were subcultured on nutrient agar medium (Oxoid Laboratories, UK) for bacteria and Sabouraud dextrose agar (Oxoid Laboratories, UK) for fungi**.**

Drugs and biochemical kits: Carrageenan (Sigma, USA) was used for induction of inflammation. Alloxan (Sigma, USA) solution (10mg/0.1ml, i.p.) was used for induction of diabetes. Ampicillin, gentamicin and amphotericin B (Sigma, USA) were used as reference standard antimicrobials for Gram-positive and Gram-negative bacterial and fungal strains, respectively. Indomethacin (Egyptian International Pharmaceutical Industries Co, EIPICO, under license of Merck and Co. INC-Rahawy N.J. USA) was used as standard anti-inflammatory drug. Vitamin E (Pharco Pharmaceutical Co.) was used as reference anti-oxidant. Biodiagnostic kits: Glucose kits (Bio-Merieux Co, France) and Glutathione kits (Wak-Chemie Medical Company,

Germany) were used during evaluation of the antioxidant activity for assessment of blood glucose and glutathione levels, respectively.

Determination of Proximate and Macronutrient Composition

Proximate analysis included the determination of certain analytical standards, as well as that of macronutrient (i.e. total or crude carbohydrate, lipid and protein) contents according to published procedures (Dubois et al., 1956; A.O.A.C., 2000).

Determination of analytical standards: The moisture and total ash contents were determined by adopting the method of the Association of Official Analytical Chemists (A.O.A.C., 2000).

Determination of macronutrients composition: The total carbohydrate content was estimated by the phenol-sulfuric acid colorimetric method, as described by (Dubois et al., 1956). Total lipids were extracted and determined using a rapid Soxhlet extraction system (Gerhardt Soxtherm System) by adopting the A.O.A.C. (2000) procedure. The total protein content was estimated as nitrogen by the A.O.A.C. (2000) method.

Determination of Micronutrient composition

Determination of mineral content: The mineral contents were determined in the digested solution using Inductively Coupled Plasma (6000) Emission Spectrometry. The amounts of the different elements calculated as (mg per 100g dry ash).

Determination of vitamin content

Vitamin A: The content of β-carotene (pro-vitamin A) was estimated according to (Nagata and Yamashita 1992). The dried powdered plant sample (1g) was vigorously shaken with a mixture of acetone-hexane (4:6, 10ml), and filtered. To determine the *β*-carotene content, the absorbance of the filtrate was measured at four different wavelengths 453, 505, 645 and 663nm and its amount deduced from the equation:

$$\beta\text{-carotene (mg/100 mL)} = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452 A_{453}$$

Finally, the corresponding vitamin A content was calculated as follows:

$$\text{Vitamin A (IU/100g)} = (\beta\text{-carotene concentration})/0.6$$

Vitamin C: Extraction of vitamin C from the powdered sample (1g) was carried out. The absorption of the blue color produced was measured at 760 nm, and the vitamin C content in the investigated sample calculated from a pre-established standard curve (Hussain et al., 2010).

Vitamin E: The spectrophotometric estimation of vitamin E was performed by adopting the method of (Naser et al., 2009), based on Emmorie-Engel reaction. The concentration of vitamin E (mg/100g dry wt) was calculated as follows:

$$\text{Vitamin E (mg / 100 g)} = AT - AC / AS$$

Where, AT, AC and AS represent the absorbance of test, carotene and standard samples, respectively.

Taking in consideration that the IU of Vitamin E is the biological equivalent of either 2/3mg dl-*α*-tocopherol or 1mg of dl-*α*-tocopherol acetate; therefore, the concentration of Vitamin E in IU/100g estimated as dl-*α*-tocopherol can be deduced from the following equation:

$$\text{Vitamin E (mg / 100 g)} = \text{Vitamin E(IU/100g)}/0.67$$

Spectrophotometric determination of phenolic and flavonoid contents

The total phenolic and flavonoid contents were determined in the aerial parts of the plant under investigation according to published spectrophotometric procedures (European Pharmacopeia, 2002; Ivanova et al., 2010).

Determination of total phenolic content: Spectrophotometric determination of total phenolic content was carried out by the Folin-Ciocalteu colorimetric method, as described in the European Pharmacopeia (Pharmacopoeia Europaea, Ph. Eur**.,** 2002), and modified by (Ivanova et al., 2010). The defatted ethanol extract of the aerial parts (9.1g/100gdrywt.) of *A. monosperma*, was used. Triplicate experiments were carried out for each sample. The phenolic content of the samples, expressed as Gallic acid

equivalent (mg GAE)/g dry weight of plant extract, was deduced from the pre-established calibration curve.

Determination of total flavonoid content: Colorimetric method was adopted, based on measuring the intensity of the color developed when flavonoids are complexed with aluminum chloride method (Chang et al., 2002). The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight. Experiments were carried out in triplicates.

Polyphenols content: HPLC analysis was performed according to published procedures (Mattila et al., 2000). The simultaneous separation and quantization of flavonoids, catechins and phenolic acids were performed on an analytical absorbance values recorded.

HPLC analysis of polyphenol contents: HPLC system consisting of GBC-LC high performance chromatograph equipped with a UV detector set at two different wavelengths 280 and 330nm. Analysis was achieved on a Hypersil BDS C18 column (250mm×4.6mm, 5µm particle size). The external standard method was applied, calibration curves for standards being made by diluting stock solutions in methanol to yield 2-20µl. Polyphenols were identified in the sample (at 280 and 330nm), their retention times and relative area percentages (expressed as ppm) .

Investigation of the lipoidal content

Gas chromatographic profiling of the lipoids: The unsaponifiable and saponifiable lipoids were prepared from the petroleum ether extract (PE) of the aerial parts of the plant and subjected to gas liquid chromatography (GLC). The unsaponifiable matter (USM) was prepared from the petroleum ether extract (PE, 1.0g) according to Vogel (1975). The solvent-free residue (0.52g), representing the USM, was saved for further GLC analysis. The aqueous alkaline solution, left after separation of the USM, was acidified with dilute hydrochloric acid (5N) to liberate the free fatty acids (FA). These were extracted with diethyl ether (4×50ml). The extract was then dehydrated over anhydrous sodium sulphate and the solvent evaporated to dryness yielding a 0.43g residue representing the free FA (Vogel, 1975).

Preparation of the fatty acid methyl esters (FAME): The FA mixture as well as the standard fatty acids was, separately, dissolved in small amounts of anhydrous methanol (Finar, 1973). After 10 min, the solvent was evaporated at room temperature under a stream of nitrogen and the dried residue saved for GLC analysis.

GLC analysis of the unsaponifiable matter (USM): The USM was subjected to GLC on Hewlett-Packard HP-5890 N system equipped with an FID detector,280°C; air flow rate: 350ml/min and H2 flow rate 50ml/min. Analysis was performed on a ThermoTR-5-MS coated with 5% phenyl polysilphenylene siloxane column (30mx0.25mmx0.25µmfilm thickness); injector temperature 270°C, using N_2 as carrier gas and adopting a temperature programming as initial temperature, 70°C,kept isothermal for 2 min, increased to 280°C by the rate of 5°C/min, then kept isothermal. Flow rate 30ml/min. Aliquots, 2µl each, of 2% chloroformic solutions of the USM and reference samples were co-chromatographed. Identification of the component hydrocarbons, phytosterols and triterpenoids was based on comparison of the retention times observed for the different peaks in the GLC chromatogram of the sample to those of the available authentic samples. The relative amount of each component was calculated *via* peak area measurement using a computing integrator.

GLC analysis of the fatty acid methyl esters (FAME): The FAME sample was analyzed using GLC Trace GC Ultra system equipped with a FID detector. Analysis was performed using a Thermo TR-FAME column (70%Cyanopropyl Polysilphenylene Siloxane) (30mx 0.25mmx 0.25µm film thickness); injector

temperature 200°C, using N_2 as carrier gas and adopting a temperature programming as initial temperature, 140°C, increased to 200°C by the rate of 5°C/min, then kept isothermal for 3min. Flow rate 30ml/min. with N_2 as carrier gas. Aliquots, 2µl each, of 2% chloroformic solutions of the analyzed FAME and reference fatty acid methyl esters were analyzed under the same conditions. Identification of the component fatty acids (FA) was carried out by comparing the retention times of their methyl esters to those of the available references. The amounts of individual FA were computed as mentioned under the USM.

Fractionation and isolation of the components of the petroleum ether extract: A weighed amount (10g) of the petroleum ether extract of the aerial parts was subjected to fractionation by vacuum liquid chromatography (VLC) on a silica gel H 60 column. Elution was performed starting with *n*-hexane, and the polarity gradually increased. Fractions (200ml, each) were collected, concentrated and monitored. Fractions showing similar chromatographic pattern were pooled together.

*Fraction **I***: (4g, eluted with 30-40% $CHCl_3$ in *n*-hexane) were rechromatographed, after pooling, on a silica gel column (50×2.5cm) and isocratic elution performed with *n*-hexane**:** EtOAc mixture (8:2 v/v); sub-fractions (10ml, each) were collected and monitored by TLC as previously mentioned. Sub-fractions were combined, then evaporated under reduced pressure to yield 1.5g residue. The latter was further purified by rechromatography on another silica gel column (50×1.5cm) using *n*-hexane**:** EtOAc (7:3 v/v) as eluent, to yield pure compound **1** (45mg, R_f = 0.79, in S_1) as white needle crystals.

*Fraction **II***: (3.4g, eluted with 50-90% EtOAc in $CHCl_3$) were similarly rechromatographed on a silica gel column (50×2.5cm) using *n*-hexane: EtOAc (1:1 v/v) as eluent, fractions (10ml, each) were collected. Best resolution was obtained on developing the chromatograms with S_1 (*n*-hexane: ethyl acetate 7:3v/v).), yielding white needle crystals of compound **2** (30mg, R_f = 0.61, in S_1).

Fractionation and isolation of the components of the ethyl acetate fraction of the defatted ethanol extract (DEAP): A weighed amount (12g) of the ethyl acetate fraction was adsorbed on silica gel, dried, powdered and subjected to vacuum liquid chromatography (VLC) (10×5.5cm, 200g). Elution was carried out starting with chloroform with gradual elution. Fractions (200ml, each) were collected.

*Fractions **A***: eluted with 30-55% EtOAc in $CHCl_3$ yielded on evaporation 3 g of solvent-free residue. This was rechromatographed on a Sephadex LH-20 column (50×2.5cm) using methanol as an eluent. Sub fractions were pooled together to give 1.9g residue. This residue was purified by reapplication on another Sephadex LH-20 column (50×1.5cm); elution was performed with methanol: H_2O (80:20 v/v), yielding yellowish needle crystals of compound **3**(25 mg, R_f = 0.76 in S_5).

*Fractions **B***: eluted with 70-85% EtOAc in $CHCl_3$**,** yielded 4.5g of solvent-free residue upon evaporation. Further column chromatographic fractionation on a Sephadex LH-20 column (50×2.5cm) was carried out using methanol as an eluent. Sub fractions (10 ml), were pooled together and evaporated under reduced pressure to give a 2.3g residue. The latter was applied to silica gel column (50×1.5cm) using chloroform: methanol (90:10 v/v) as an eluent. Rechromatography on a Sephadex column using 50% methanol in water as eluent resulted in isolation of two pure compounds **4** (35mg, R_f = 0.73 in S_5) and **5** (40mg, R_f = 0.69 in S_5).

Fractionation and isolation of the components of the n-butanol fraction of the defatted ethanol extract (DEAP): A weighed amount (13g) of the *n*-butanol fraction was subjected to vacuum liquid chromatography (VLC) on a silica gel column (10×5.5cm, 200g). Elution was carried out starting with chloroform and the polarity

gradually increased. Fractions (200ml, each) were collected, and combined together, based on chromatographic profile, to yield three main fractions.

Fractions ***a*****:** eluted with 30-45% EtOAc in $CHCl_3$, yielded on evaporation a residue (3g), which was rechromatographed on a Sephadex LH-20 column (50×2.5cm) using methanol as eluent, sub fractions, 10ml each, were collected. Sub fractions were pooled together, based on TLC monitoring. They were evaporated under reduced pressure to yield a residue 1.2g residue which showed a major spot on TLC (R_f = 0.83 in S_6) together with minor spots. Purification by application to on another Sephadex LH-20 column (50×1.5cm), using methanol: H_2O (80:20) for elution, afford compound **6** as yellow powder (45 mg, R_f = 0.83 in S_6).

Fractions ***b***: eluted by 65-100% EtOAc in $CHCl_3$, yielded on evaporation a residue **(**4g), which was applied on a Sephadex LH-20 column (50×2.5cm), elution being carried with methanol (sub fractions, 10 ml each being collected). Sub fractions were combined; upon solvent evaporation under vacuum, a residue of 1.7g was obtained. This residue was subsequently purified on another Sephadex LH-20 column (50×1.5cm), using methanol: H_2O (90:10) as eluent, to afford pure compound **7** as yellow powder (30mg, Rf = 0.68 in S_6), after vacuum evaporation of the solvent.

Fractions ***c***: eluted with 40-90% MeOH in EtOAc, yielded on evaporation a 2g residue. The latter was applied on a Sephadex LH-20 column (50 ×2.5cm), subjected to refractionation using methanol for elution; monitoring being proceeded through TLC. Sub fractions (each 10ml) were combined and evaporated under reduced pressure to give a 0.8g residue; which upon purification by subsequent chromatography on another Sephadex LH-20 column (50×1.5cm) and elution with methanol: H_2O (90:10) yielded compound **8** as a brownish yellow powder (60mg, R_f = 0.49 in S_6).

Characterization and identification of the isolated compounds: Compounds **1** and **2** isolated from the petroleum ether extract of the aerial parts of *A. monosperma* Del., three compounds **3-5** from the ethyl acetate extract. While, three compounds, **6-8** were isolated from the *n*-butanol fraction of the deffated ethanol extract of the aerial parts. Eight isolated compounds were identified through their physicochemical, chromatographic and spectral data.

In-vivo Assessed Bioactivities

Determination of Median Lethal dose (LD_{50}): The acute toxicity resulting from oral administration of the total ethanol (90%) extract of the aerial parts of *A. monosperma* (prepared by repeated maceration of 100g air-dried powder) was investigated. In this respect, its median lethal dose (LD_{50}) was determined according to (Karber, 1931).

Evaluation of the anti-inflammatory activity:Acute anti-inflammatory activity of the (DEAP) was assessed *in-vivo* by means of the paw swelling, or foot edema method. Indomethacin used as standard anti-inflammatory drug. This model is commonly used to assess the efficiency of non steroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin production (Whiteley and Dalrymple, 2001). The method described by (Winter et al., 1962) was adopted. Experiments were carried out in triplicates. The percentage edema was recorded and percentage edema inhibition calculated as follows:

$$\% \text{ Edema inhibition} = (D_0 - D_t)/ D_0 \times 100,$$

Where D_0 = Mean paw diameter of Control group at a given time,
D_t = Mean paw diameter of treated (extract or standard) group at the same time.

Evaluation of the antioxidant activity: The (DEAP) was subjected to evaluation of their antioxidant activity**.** The effect of the tested sample was compared to that of Vitamin E, as reference antioxidant drug. Blood glutathione was determined in Alloxan-diabetic rats by adopting the spectrophotometric method of (Beutler et al.,

1963). *Diabetes mellitus* was induced by a single intra-peritoneal injection of Alloxan (150mg/kgb.wt.) followed by an overnight fast. Hyperglycemia was assessed, after 72 h of diabetes induction, by measuring the blood glucose levels according to (Trinder, 1969). Experiments were carried out in triplicates. The blood GSH level was recorded, and percentage change from control computed as follows:

$$\% \text{ Change} = (G-G_0) \times 100/G_0$$

Where G_0 is the GHS level in diabetic animals prior sample administration and G that measured after.

In-vitro Assessed Bioactivities

Antimicrobial Activity: The activity of the defatted ethanol extract of the aerial parts was subjected to evaluation of their antimicrobial potential against selected microorganisms. The activities were compared to those of ampicillin and gentamycin as standard antibacterial agents, and amphotericin B as reference antifungal drug. The antimicrobial activity of extracts under investigation was screened by the agar well-diffusion method, as described by Holder and Boyce (1994). Results are recorded, as mean diameter of zone of inhibition in mm ± Standard deviation (Agwa et al., 2000). Potencies of the sample solutions relative to those of the appropriate antibiotics are also calculated.

Determination of minimal inhibitory concentrations (MIC): Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation (Andrews, 2001). MIC of the most active sample was estimated against each of the aforementioned microorganisms. The broth dilution method, as described by Doughari (2006), was adopted; and experiments carried out in triplicates. The procedure was repeated on the same organisms using the standard antibiotics (ampicillin and gentamicin for bacteria and amphotericin B for fungi). Meanwhile, a tube containing broth media only was seeded with the organisms to serve as control. Tubes containing the bacterial cultures were incubated at 37°C, for 24h; while those of fungal cultures were kept at room temperature (25-30°C), for 3-7 days. After incubation, the tubes were examined for microbial growth by observing for turbidity.

Insecticidal Effect

The dried petroleum ether and ethanol (70%) extracts, prepared by successive maceration of the aerial parts of the plant, were used. Stock solutions (1mg %) was prepared, from 100 mg samples, in distilled water by the aid of Tween 80 (0.3%). The efficacy of each extract was tested using three upgraded concentrations (1ppm, 3ppm and 5ppm), prepared by dilution with the vehicle.

Insect rearing: *Cx. Pipiens* larvae were obtained from Research Training Center and were identified according to Harbach (1988). Adult mosquitoes were reared in breeding cages under laboratory conditions of temperature (26±2^{o}C), relative humidity (70–80% RH), and 12 h photoperiod. Female mosquitoes were fed on blood of pigeon for egg deposition. Rearing was conducted according to the method described by Gad et al. (1988).

Bioassay: Extracts were assayed against 3rd instar larvae of *Cx. pipiens*. Bioassays were conducted in the same laboratory conditions mentioned above. Three replicates, each of 25 larvae, were assayed in 100ml volume, against each concentration. A Control group exposed to the vehicle was run simultaneously for each plant extract. Mortality readings were recorded 24 h post-treatment, as described by WHO (2005). Rearing and bioassays were performed at the Entomology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

Statistical analysis: Mortality readings were corrected according to Abbott's formula (1925), and lethal concentrations (LC_{50}, and LC_{95}) were calculated. Statistical analysis was carried out using the Probit Analysis Program (Finney, 1971).

Anthelmintic Effects

The 2nd stage larvae of *Toxocara vitulorum* (*Neoascaris vitulorum***,** the species hosted by cattle) were selected as experimental models for *in vitro* testing of the anthelmintic activity of *A. monosperma*. Stock solutions (1mg %) of the petroleum ether and ethanol (70%) extracts of the aerial parts were prepared from 100mg samples as previously described (Insecticidal activity). The susceptibility of *Toxocara vitulorum* to the tested samples was estimated by adopting the larval paralysis and motility assay as described by (WHO, 1996). Four upgraded concentrations (2.5, 5.0, 10 and 20ppm) of each plant extract were used. Adult females of *Toxocara vitulorum*, collected from slaughtered buffaloes at Cairo abattoir, were washed repeatedly with saline solution (0.85%), and the interior part of the gravid uteri removed by dissection. Mature eggs (containing 2nd stage larvae, L2) were extracted from the distal parts of the uteri, passed through fine sieve, washed and sedimented several times using 1% normal saline. Larvae were stored, as egg stocks, in enough solution at 4°C until use.

Bioassay: The eggs (about 25 per group) were spread as thin sheets in clean petri dishes for exposure to the plant extracts. The tested samples, adjusted to the required concentrations with distilled water, were added as a thin film on the eggs. The exposure times were 3, 6, 12 and 24 hours for each of the 4 tested concentrations. A control group consisting of eggs covered with distilled water was handled simultaneously for each concentration. Experiments were carried out in triplicates. The eggs were repeatedly washed (3-5 times) with distilled water and precipitated. Both exposed and control eggs, were kept for embryonation in an incubator at 28°C, for 7 days. Three days post incubation the eggs were examined microscopically. Bioassays were conducted at the Parasitology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. The number of eggs containing larvae was recorded, and percentage larval mortality calculated according to (Kovendan et al, 2012) as follows:

Mortality %= Number of dead larvae /Number of exposed larvae X 100

RESULTS

Reports concerned with the evaluation *A. monosperma* as either food or fodder were lacking. This prompted the exploration of the proximate and micronutrient composition of its aerial parts.

The tested sample could be considered as moderately nutritious with crude protein content (8.4%). Moreover, its crude fat content being distinctly low (4.29%) renders it more easily metabolized. In addition, these data provide reliable criteria for identification and quality control of *A. monosperma* Del., either for interspecies differentiation or as ingredient of herbal formulations (Table 1).

Table-1: Proximate composition of the aerial parts of *A. monosperma* Del.

Parameter	Percentage (g/ 100g dry wt)
Moisture	4.5
Total ash	12.7
Total carbohydrate	9.13
Total lipid	4.29
Total protein	8.4

Table-2: Mineral content of the aerial parts of *A. monosperma* Del.

Element	Content(mg/100g dry wt)	RDA/AI*(mg)
Phosphorus*	1260	100-1250
Potassium*	1310	4500-5100
Sodium*	1110	1000-1500
Calcium*	3070	200-1300
Magnesium*	356	30-420
Copper**	4.3	0.9
Manganese**	12	0.003-2.6
Zinc**	6.1	2-13

RDA= Recommended dietary allowances **(**The George Mateljan Foundation, 2009)
AI = Adequate Intake; *, macro-minerals (required in amounts > 100mg/day); **, micro-minerals (required in amounts < 100mg/day).

The data recorded in Table 2 revealed that the analyzed plant sample contains appreciable amounts of Ca and P; which exceed the required daily allowances (RDA) of these macro-minerals. Similarly, the detected amounts of the micro-minerals Cu and Mn were distinctly high (4.3 and 12 mg/100g) and over their respective RDA.

Vitamins are essential dietary components; which are necessary for optimal body growth and health. From data presented (Table 3), it could be concluded that, the aerial parts of. *A. monosperma* Del. contains appreciably high amounts of the two fat-soluble antioxidant vitamins, A and E.

Table-3: Vitamin contents of the aerial parts of *A. monosperma.* Del.

Vitamin	Content	RDA
Vitamin A	5457.83 IU/ 100g*	700-1300µg/d
Vitamin C	30mg/100g	65-115mg/d
Vitamin E	309.96 IU/ 100g**	4-19mg/d

RDA= Recommended dietary allowances (Lutz and Przytulski, 1994; Parfitt, 1999);
*Equivalent to 3274.2µg β-carotene/100g;**Equivalent to 207.67mg α-tocopherol /100g.

Spectrophotometric determination of phenolic and flavonoids content showed that the average absorbance of the ethanol extracts of the aerial parts (0.5, 0.1mg/ml) was 0.437, 0.265 corresponding to 65.68µg/ml gallic acid and to 61.86µg Rutin/ml, respectively. The total phenolic content, expressed as mg GAE/g extract was thus 131.3 for the aerial parts. Apparently, the flavonoid content of the aerial parts (expressed as mg RE/g extract) was nearly alike to its phenolics content.

HPLC analysis of the phenolic compounds in the methanol extract (5g/100ml) of the aerial parts of *A. monosperma* Del. enabled the identification and quantification of 29 phenolic compounds among which: 13 phenolic acids, 14 flavonoids, a phenylethanoid (3-OH-tyrosol) and a stilbenoid (resveratrol). The majority of phenolic acids were identified at 280 nm, while flavonoids were mostly detected at 330 nm (Table 4, 5). The major identified flavonoids were hesperidin, rutin, 7-OH flavone and hesperetin. On the other hand, the main detected phenolic acids were salycilic acid, vanillic acid and *p*-coumaric acid.

Table-4: Phenolics identified by HPLC, at λ=280nm, in the methanol extract of aerial part of *A. monosperma* Del.

No.	Identified phenolics	Rt	Conc. (ppm)
1	Gallic acid	7.100	167.9
2	Pyrogallol	7.179	130.975
3	3-OH-Tyrosol (phenyl ethanoid cpd)	8.407	86.145
4	Protocatechuic acid	8.541	57.575
5	Catechin	8.696	7.957
6	Chlorogenic acid	9.412	322.31
7	Catechol	9.584	92.78
8	Epicatechin	9.928	16.30
9	*P*-Hydoxy benzoic acid	10.140	285.95
10	Caffeic acid	10.455	30.854
11	Ferulic acid	12.293	30.158
12	Isoferulic acid	12.624	44.572
13	Resveratrol (stilbenoid cpd)	13.256	183.634
14	Ellagic acid	13.643	44.031
15	Vanillic acid	13.719	3574.197
16	3,4,5-Methoxy-cinnamic	14.631	21.400
17	Salicylic acid	14.987	4761.383
18	*p*-Coumaric acid	15.400	893.21
No. of identified phenolic acids			**12**
No. of identified flavonoids			**4**

Rt: retention time ; Conc.: concentration, average of three determinations.

Table- 5: Phenolics identified by HPLC, at λ=330 nm, in the methanol extract.

No	Identified phenolics	Rt	Conc. (ppm)
1	Naringin	12.802	62.839
2	Rutin	12.928	1289.554
3	Hesperidin	13.045	3195.857
4	Rosmarinic acid	13.341	23.561
5	Quercetrin	14.050	287.717
6	Quercetin	15.594	6.853
7	Naringenin	15.878	26.626
8	Kaempherol	16.062	73.558
9	Hesperetin	16.191	429.023
10	Apigenin	17.337	159.810
11	7-OH flavones	18.200	530.530
No. of identified flavonoids			**10**
No. of identified phenolic acids			**1**

Rt: retention time; Conc.: concentration, average of three determinations

Table- 6: Components identified by GLC in the USM of the aerial parts of *A. monosperma* Del.

Identified Components	RRt	Relative Percentage
n-Hexadecane **(C_{16})**	0.39	4.181
n-Heptadecane **(C_{17})**	0.46	3.615
n-Nonadecane **(C_{19})**	0.60	2.695
n-Eicosane **(C_{20})**	0.66	5.757
n-Heneicosane **(C_{21})**	0.72	4.978
n-Docosane **(C_{22})**	0.78	5.788
n-Tricosane **(C_{23})**	0.83	2.458
n-Tetracosane **(C_{24})**	0.88	2.494
n-Pentacosane **(C_{25})**	0.93	8.607
Squalane **(C_{30})**	1.00	32.525
n-Octacosane **(C_{28})**	1.06	0.733
β-Sitosterol **(C_{29})**	1.73	1.796
Stigmasterol **(C_{29})**	1.82	0.973
% Total identified components		**76.6**
% Identified hydrocarbons		**73.83**
% Identified phytosterols		**2.77**

RRt: retention time relative to squalane; Rt of squalane =17.35

From the data concerning the USM and FAME of the aerial parts of *A. monosperma* Del. and as recorded in Tables 6 and 7, the following could be concluded: The USM and FA fractions represented 52 and 43% of the parent PE extract corresponding to 0.23 and 0.19g/100g dry wt. of the plant material, respectively. The GLC profile of the USM revealed the presence of at least 13 components representing 76.6% of the total composition. The percentage of identified hydrocarbons prevailed reaching 73.83% while that of phytosterols was minor Squalane predominated the hydrocarbon fraction and *β*-Sitosterol that of phytosterols.

GLC analysis of FAME allowed the identification of 11 components representing 83.53% of the total fatty acids. The percentage of detected unsaturated fatty acids was slightly higher than that of the saturated ones (47.56 *vs* 35.97%). The major identified FA in the mixture was the polyunsaturated ω-6, Linoleic acid followed by the saturated Palmitic acid.

Column chromatographic fractionation of the petroleum ether, ethyl acetate and the n-butanol soluble fraction of the ethanol extract of the aerial parts of *A. monosperma* (DEAP) allowed the isolation of compounds (**1**-**8**) which, were identified through their physicochemical, chromatographic and spectral data. Eight Compounds were characterized as lupeol acetate (Jamal et al., 2008) *β*-amyrin acetate (Feleke and Brehane, 2005), quercetin (Charisiadis et al., 2014), gallic acid (Mena et al., 2012), methyl gallate **(**Méndez and Mato, 1997), kaempferol (Gohari et al., 2011), kaempferide, quercetin-3-*α*-*O*-glucopyranoside. As far as the available literature is concerned, this is the first report on isolation of lupeol acetate, methyl gallate from genus *Artemisia*.

Table- 7: Components identified by GLC in the FAME of the *Artemisia monosperma* Del.

Fatty acid corresponding to identified FAME	RRt of FAME	Relative percentage
Myristic acid (C_{14})	0.60	2.02
Palmitic acid (C_{16})	0.78	16.90
Palmitoleic acid ($C_{16:1}$)	0.80	0.90
Stearic acid (C_{18})	0.93	4.02
Petroselinic acid ($C_{18:1}$)	0.95	11.11
Linoleic acid ($C_{18:2}$)	1	26.50
Linolenic acid ($C_{18:3}$)	1.03	8.72
Arachidic acid (C_{20})	1.07	4.55
Erucic acid ($C_{22:1}$)	1.14	0.33
Behenic acid (C_{22})	1.21	5.48
Lignoceric acid (C_{24})	1.33	3
% Total identified fatty acids		**83.53**
% Saturated fatty acids		**35.97**
% Unsaturated fatty acids		**47.56**

RRt: Retention time relative to linoleic acid; Rt of linoleic acid =30.53

Despite the previously reported data on *β*-amyrin acetate isolation from other *Artemisia* species (Lao et al., 1984) yet, this is the first on isolation of *β*-amyrin acetate from *A. monosperma* Del. Quercetin, gallic acid, kaempferol and isoquercetrin were previously identified in genus *Artemisa* (Maggio et al., 2013).

The acute toxicity resulting from oral administration of the total ethanol extract of the aerial parts of *A.monosperma* Del. was investigated. Results obtained revealed that the tested extract could be considered as safe in the range of the orally administered doses, since its determined LD50 was up to 6.1g/kg b.wt.

Results, of anti-inflammatory effect evaluation as represented in Table 8, revealed that the defatted ethanol extract exhibited a significantly high potency ranging from (36.22-73.47%) relative to the reference; and its efficiency appeared the highest after 1h of treatment. This remarkable effect could be correlated to its relatively high terpenoid content with reported anti-inflammatory activity (de Cássia da Silveira e Sá et al, 2013).

Results of the antioxidant activity evaluation presented in Table 9 revealed that the DEAP (defatted ethanol extract) restored the reduced blood GHS level in diabetic animals almost as efficiently as Vitamin E (relative potencies 97.03%). Meanwhile, the remarkable efficiency of the defatted ethanol extract is probably due to the synergistic action of its phenolic components (Rice-Evans et al., 1997).

Table- 8: Acute anti-inflammatory activity of the defatted ethanol extract (DEAP) of the aerial parts of *A. monosperma* Del., as compared to Indomethacin.

Group	Zero	1h		2h		3h		4h	
	PD (mm)	P D (mm) Mean ± S.E	% EI	P D (mm) Mean ± S.E	% EI	PD (mm) Mean ± S.E	% EI	P D (mm) Mean ± S.E	% EI
Control	3.56±0.08	4.41*±0.1	-	4.71*±0.13	-	4.81*±0.12	-	4.85*±0.07	-
DEAP (100mg/k	3.48±0.02	4.34*±0.09	24.71	4.21*±0.08	20.97	4.03*±0.09	15.80	3.84*±0.09	10.34
Indomethacin (20mg/kg	3.30±0.08	4.16±0.09*	33.63	3.99±0.06*	35.07	3.92±0.01*	30.00	3.84±0.01*	28.54

DEAP: defatted ethanol extract of aerial parts; S.E. = standard error;
*Statistically significant from the control normal inflamed group at the corresponding time: $P< 0.05$.
Statistical analysis was carried out using repeated measures one way ANOVA followed by Least significant test for multiple comparison.
PD:Paw Diameter (mm) FI:Edema Inhibition

The antimicrobial activity of the sample was evaluated against a set of selected microorganisms, including 4 fungal and 8 bacterial strains. The results as represented in Table 10, 11 revealed that: the tested samples exhibited moderate to remarkable growth inhibitory potential against most of the tested strains as compared to the appropriate standard antibiotics. Yet, the tested extract was inactive against the filamentous fungus *Syncephalastrum racemosum*, the Gram-positive bacterium *Streptococcus pyogenes* and the Gram-negative *Proteus vulgaris*. On the other hand, (DEAP) revealed pronounced growth inhibitory activity against all susceptible microorganisms. The DEAP extract appeared distinctly effective on *Klebsiella pneumonia* (potencies relative to gentamicin, 80.22 %; MICs 0.98µg/ml).

Table-9: Effect of DEAP of the aerial parts of *A. monosperma* on GHS blood level in Alloxan-induced diabetic rats, as compared to Vitamin E.

Group (n=6)	Dose / Kg b.wt.)	Blood GHS (mg %) Mean±SE	% Change from control	Relative potency %
Control	-	36.2±1.4	-	-
Diabetic	-	21.4±0.5	-	-
Diabetic + Vit E	7.5mg	35.8±1.3	67.89	100
Diabetic + DEAP	100mg	35.5±1.4	65.88	97.03

GHS: reduced glutathione; DEAP: defatted ethanol extract of aerial parts

The toxicity of upgraded concentrations of the extracts of the aerial parts of *A. monosperma* to *Cx. pipiens* larvae was estimated. The lethal concentrations (LC_{50} and LC_{95}, in ppm), recorded 24h post-exposure, were taken as a measure for toxicity. The results compiled in Table 12 revealed that the petroleum ether extract is more efficient than the defatted ethanol (70%) extract, as depicted by its lower LC_{50} and LC_{95}. This effect could be related to the terpenoid nature of its components (especially, the volatiles); whereas, the defatted ethanol extract is more enriched in phenolics. However, this effect could be considered as moderate when compared to the previously recorded LC values of the reference insecticide, Temephos (0.5ppm, after 24-48h exposure; (Taher et al., 2012).

Table- 10: Antimicrobial activity of the defatted ethanol extract (DEAP) of the aerial parts, of *A. monosperma* Del., expressed as diameters of zones of inhibiton in mm.

Tested microorganisms	DEAP	Standards
Fungi		**Amphotericin B**
Aspergillus fumigatus (RCMB 02564)	20.3± 0.58	23.7+0.10
Candida albicans (RCMB 05035)	16.2± 0.44	21.9+0.12
Geotricum candidum (RCMB 05096)	22.6±0.37	26.4+0.20
Syncephalastrum racemosum (RCMB 05922)	NA	25.4+0.16
Gram-positive bacteria		**Ampicillin**
Staphylococcus aureus (RCMB 010027)	14.1±0.14	28.9+0.14
Staphylococcus epidermidis (RCMB 010024)	19.1±0.18	25.4+0.18
Streptococcus pyogenes (RCMB 010015)	NA	26.4+0.34
Gram-negative bacteria		**Gentamicin**
Neisseria gonorrhoeae (RCMB 010076)	16.2± 0.16	19.9+0.18
Proteus vulgaris (RCMB 010085)	NA	23.4+0.3
Klebsiella pneumoniae (RCMB 010093)	21.1±0.13	26.3+0.15
Shigella flexneri (RCMB 01005420)	15.7± 0.15	24.8+0.24
Escherichia coli (RCMB 010056)	17.3±0.12	25.3+0.18

DEAP: defatted ethanol extract of the aerial parts; NA: Not active.

Table-11: Minimum inhibitory concentrations (µg/ml) of the *A. monosperma* Del. DEAP sample.

Tested microorganisms	DEAP	Standards
Fungi		Amphotericin B
Aspergillus funuatus(RCMB 02564)	1.95	0.12
Candida albicans (RCMB 05035)	31.25	0.24
Geotricum candidum(RCMB 05096)	0.24	0.03
Gram-positive bacteria		Ampicillin
Staphylococcus aureus(RCMB 010027)	62.5	0.007
Staphylococcus epidermidis (RCMB 010024)	3.9	0.06
Gram-negative bacteria		Gentamicin
Neisseria gonorrhoeae(RCMB 010076)	31.25	3.9
Klebsiella pneumonia (RCMB 010093)	0.98	0.03
Shigella flexneri (RCMB 01005420)	31.25	0.06
Escherichia coli (RCMB 010056)	15.63	0.06

DEAP: defatted ethanol extract of the aerial parts.

Table-12: Comparative susceptibility test of 3ed instar larval of *Culex pipiens* to the extracts of the aerial parts of *A. monosperma* (expressed as lethal concentrations), with synergistic ratio.

Extract	LC values in ppm (95% CL)	
	LC50 (95% CL)	LC95 (95% CL)
Petroleum ether	1.58 (2.9 - 0.68)	86.3 (7737.3 - 6.6)
DEAP	3.04 (5.6 - 1.79)	136.7 (13564.7 - 8.3)

LC_{50}: median lethal concentration; LC_{95}: 95% lethal concentration; 95% CL: Ninety-five percent confidence limit; DEAP: defatted ethanol (70%) extract of aerial parts

The susceptibility of *Toxocara vitulorum* 2nd instar larvae to upgraded concentrations of the extracts of the aerial parts of *A. monosperma* was tested at different time intervals. The number of dead larvae per 100 eggs was taken as a measure for toxicity (percentage mortality).

Table-13: Susceptibility of second stage larvae of *Toxocara vitulorum* to the extracts of the aerial parts of *A. monosperma*, expressed as percentage mortality.

Concentration (ppm)	Percentage Mortality							
	After 3h		After 6h		After 12h		After 24h	
	PE	DEAP	PE	DEAP	PE	DEAP	EP	DEAP
2.5	37	33	50	40	57	44	60	45
5.0	38	35	57	44	62	58	73	66
10.0	45	38	66	50	69	62	75	76
20.0	94	83	100	100	-	-	-	-

PE: pertroleum ether extract; DEAP: defatted ethanol extract of the aerial parts

From data compiled in Table 13, it is evident that the petroleum ether extract was more active at all tested concentrations. The effect appeared to be concentration dependent. 100%t mortality was observed after 6h of exposure for both extracts.

DISCUSSION

The tested extract of the aerial part could be considered as safe in the range of the orally administered doses. In addition, it could be considered as moderately nutritious with a crude protein content (8.4%) exceeding those of both *A. campestris* and *A. herba-alba* (7.7 and 7.9%, respectively), although reaching about half that of red clover (Barseem, *Trifolium pratense*, 15-25%) (INRA, 2007). Moreover, its crude fat content (4.29%) being distinctly lower than that of *A. herba-alba* (4.29 *vs* 9.0 %) (Houmami et al., 2004) renders it more easily metabolized.

The analyzed plant sample contains appreciable amounts of Ca and P which exceed the required daily allowances (RDA) of these macro-minerals. Similarly, the detected amounts of the micro-minerals Cu and Mn were distinctly high and over their respective RDA. Therefore, the aerial parts of *A. monosperma* Del. could be considered a valuable source of these elements. Alike 35 elements had been determined in *A.annua* therefore, when used effectively, can assist in meeting some of the important nutrient and mineral requirements of monogastric and polygastric animals (Alassane et al., 2013).

The aerial parts of *A.monosperma* Del. contain appreciably high amounts of the two fat-soluble antioxidant vitamins, A and E. These data provide reliable criteria for identification and quality control of *A. monosperma* Del., either for interspecies differentiation or as ingredient of herbal formulations.

Several flavonoids, phenolic acids, and coumarins have been isolated and identified in a number of Artemisia species (Valant-Vetschera et al., 2003; Deng et al., 2008). Among phenolic acids commonly occurring in the genus are caffeic and vanillic acids were isolated from several *Artemisia* species (Deng et al., 2008). Apparently, the flavonoid content of the aerial parts nearly alike to its phenolics contents in the aerial part of *A. monosperma* Del.

Lipoidal components, especially phytosterols and triterpenoids, of certain *Artemisia* species have been investigated. Still, the data reported regarding those of *A. monosperma* Del. were scarce (El gamal et al., 1997); in addition, nothing could be traced in the available literature concerning those of the plant growing in Libya. GLC profile of USM showed hydrocarbons as major while that of phytosterols was minor. Squalane predominated the hydrocarbon fraction and *β*-Sitosterol that of phytosterols.

GLC analysis of FAME allowed detection of unsaturated fatty acids which was slightly higher than that of the saturated ones (47.56 *vs* 35.97%). The major identified FA in the mixture was the polyunsaturated ω-6, linoleic acid followed by the saturated palmitic acid. The results obtained in this study are comparable to the previously reported data (Elgamal et al., 1997; Ivanescu et al., 2013).

The defatted ethanol extract exhibited a significantly high potency as anti-inflammatory agent relative to the reference indomethacin. This remarkable effect could be correlated to its relatively high terpenoid content with reported anti-inflammatory activity (de Cássia da Silveira e Sá et al, 2013). Where, the remarkable efficiency of the defatted ethanol extract an antioxidant is probably due to the synergistic action of its phenolic components (Rice-Evans et al., 1997).

Artemisia species are among of the most popular components of traditional herbal medicines used for treatment of infections by fungi, bacteria and viruses (Abad et al., 2012). Several reports were traced concerning the evaluation of the antimicrobial potential of various members of the genus, collected from different localities (Ashgari et al., 2012; Fedhila et al., 2015). The plant exerted an antimicrobial, and especially antifungal effect, similar to most other *Artemisia* species (Panek et al., 2014; Fedhila et al., 2015), and including those growing in Libya other than *A. monosperma* (Janaćković et al., 2015). However, the antimicrobial activity of the investigated plant could not be correlated to neither a single constituent nor a specific group of metabolites, but rather to a synergistic effect of different components including volatiles and phenolics.

The majority of mosquito control projects focus on the larval stage i.e. to produce larvicides rather than adulticides, since the latter might just decrease the grown-up populace briefly (Knio et al., 2008). Therefore, a more efficient way to reduce mosquito population is to target the larvae. Indeed, the insect repellent and insecticidal activities of certain *Artemisia* extracts and essential oils have been investigated; some are even recognized as valuable bioinsecticides, particularly those of *A. vulgaris* (European Food Safety Authority, 2014), *A. absinthium* (Dhen et al. 2014), and *A. annua* (Tripathi et al., 2000). Meanwhile, this is the first report concerning the toxic effect of *A. monosperma* growing in Libya against *Culex pipiens* larvae. Concerning the insecticidal effect, the petroleum ether extract is more efficient than the defatted ethanol extract, as depicted by its lower LC_{50} and LC_{95}.

The susceptibility of *T. vitulorum* 2nd instar larvae to upgraded concentrations of the extracts of the aerial parts of *A. monosperma* was tested at different time intervals. Petroleum ether extract was more active at all tested concentrations. The effect appeared to be concentration dependent. 100% mortality was observed after 6h of exposure for both extracts. Among secondary metabolites, terpenoids and tannins are reported to be the most common in anthelmintic plants (Wink, 2012). The growth inhibitory activity exhibited by terpenoids towards parasites was related to their interference with metabolic pathways. Meanwhile, condensed tannins were reported to act mainly through inhibition of egg hatching and larval development (Nugyen et al., 2005). In this respect, the effect of the petroleum ether extract of *A. monosperma* could be referred to a synergistic action of its terpenoid components and that of the ethanol extract to its condensed tannin content. It is worthy to note that several *Artemisia* species have been reported to as promising commercial anthelmintic alternatives, especially against nematodes (Veerakumari, 2015).

CONCLUSION

As far as the available literature is concerned, none of the constituents, isolated herein, was previously reported in the plant under investigation. The previous findings recommend cultivation and broad propagation of this wild growing species for both economical and pharmaceutical purposes. Yet, in order to maximize a targeted bioactivity, appropriate selection of time of collection, plant organ and extraction process should be taken in consideration. The plant could be suggested as safe condiment and valuable fodder. However, it could be considered as natural anti-inflammatory, antifungal and anthelmintic candidate.

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