

Artemisinin from minor to major ingredient in *Artemisia annua* cultivated in Egypt

El-Moataz Bellah El-Naggar^{1,*}, Mohamed Azazi², Emil Švajdlenka³, Milan Žemlička³

¹ Faculty of Pharmacy, Department of Pharmacognosy, Damanhur University, Egypt.

² Environmental Studies and Research Institute, Department of Natural Resources, Minufya University- Sadat Branch, Egypt.

³ Faculty of Pharmacy, Department of Natural Drugs, Veterinary and Pharmaceutical University, Brno, Czech Republic.

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ABSTRACT

Artemisia annua is currently the only source for the commercial pharmaceutical production of artemisinin as neither its chemical synthesis nor its in vitro production is economically feasible. However, the very low amount of artemisinin in *Artemisia annua* L., (0.01-0.6%) of dry weight has made artemisinin-based drugs remains expensive and is hardly available on a global scale. Limitations in the supply of this promising and most potent antimalarial compound makes the cultivation of this plant is still the only valid source to satisfy the high demands and the vast need of artemisinin at a reduced acceptable market price for most patients. In this paper we attempted to increase the cultivating area of this important plant by cultivating *A.annua* seeds for the first time in the western desert of Egypt to improve its agricultural yields in order to ensure a steady global supply of artemisinin and to study the environmental impact of the cultivation area on the amount of artemisinin production. The high artemisinin concentration reported in this study (4.85-4.90%) of dry weight has identified the Egyptian desert for the first time as a new promising cultivating area for pharmaceutical production of artemisinin. A very simple method was developed using liquid chromatography tandem mass spectrometry (LC-MS/MS) for the quantification of artemisinin in both plant material and pure samples

INTRODUCTION

The medicinal plant *Artemisia annua*, also known as Sweet Wormwood, Sweet Annie, Sweet Sagewort, or Annual Wormwood (Chinese, pinyin, qinghao) is among the top 10 pharmaceutical crops which are receiving intensive worldwide scientific attention as it is currently the only source for the commercial pharmaceutical production of the sesquiterpene lactone artemisinin (Dharam *et al.*, 1996). At the present time, neither total nor semi-chemical synthesis (Delabays *et al.*, 2001) and in vitro production of artemisinin using recent biochemical and molecular genetic metabolic engineering (Arsenault *et al.*, 2008; Teoh *et al.*, 2009) both in planta and (or) in microorganisms (Martin *et al.*, 2003; Ro *et al.*, 2006) are still economically feasible to improve production of artemisinin (Covello *et al.*, 2007; Covello, 2008).

However, the very low amount of artemisinin in *Artemisia annua* L., (0.01-0.6%) of dry weight has made artemisinin-based drugs remains expensive and is hardly available on a global scale. Limitations in the supply of this promising and most potent antimalarial drug weapons against multidrug resistant malaria (*Plasmodium falciparum*) and cerebral malaria compound as part of the ideal strategy for malaria in Africa by WHO, which meets the dual challenge posed by drug-resistant parasites and rapid progression of malarial illness (Klayman, 1985; Roth and Acton, 1987; Loevinsohn, 1994; Robert *et al.*, 2002; Yikang, 2002) makes the cultivation of this plant is still the only valid source to satisfy the high demands and the vast need of artemisinin at a reduced acceptable market price for most patients (Jha, 2011). Thus, *A. annua* has to be cultivated at a large scale to satisfy the vast need for medicine, as 40% of the world's population is threatened by malaria (Dhingra *et al.*, 2000). Therefore, there is a need for a more widespread cultivation of *A. annua* as a medicinal source in additional regions to satisfy this high demands. The aim of the present paper was attempted to increase the cultivating area of this valuable treasure Chinese nation, important plant.

* Corresponding Author

Dr. El Moataz Bellah Aly El Naggar, Lecturer of Pharmacognosy, Department of Pharmacognosy, Faculty of Pharmacy, Damanhour University, Egypt. Tel: 002 01225777579

In this study, *A.annua* seeds were cultivate for the first time in the western desert of Egypt trying to improve its agricultural yield, and to further understand the impact of such environment on its artemisinin production to find out if the cultivation area selected is suitable for the growing of high-yield plants that can be used by pharmaceutical companies to improve the commercial pharmaceutical production of artemisinin at a reduced market acceptable price for most patients. A very simple sensitive method was developed coupling high-performance liquid chromatography (HPLC) with diode-array detector (DAD) and ion trap electrospray ionization mass spectrometry (MSn) for separation, identification and quantification of artemisinin both in plant material and pure samples (standards). The MS, MSn and DAD data together with HPLC retention time (T_R) of artemisinin standard allowed a sensitive and simple method. The main advantages of our method are not only the excellent specificity but also the extremely short and efficient sample preparation. The data reported should help to allow more reliable quantification laboratory analysis of artemisinin in both pure samples and plant materials.

MATERIAL AND METHODS

Chemicals and reagents

Pure reference standard of artemisinin, 98% Sigma–Aldrich (Bornem, Belgium) was kindly offered from Prof. Milan Zemlicka and Dr Email svajdlenka, Department of Natural Drugs, faculty of Pharmacy, Veterinary and Pharmaceutical University, Brno, Czech Republic. All solvents and reagents were of the highest purity needed for each application. HPLC–MS grade Methanol, water and formic acid (99.8%) were purchased from Sigma Aldrich Fluka (6 of October city, Cairo)

Plant material

The seeds of *Artemisia annua* L.were kindly provided from Royal Herbal Company Egypt (Horizon Herbs, LLC). The plants were grown under controlled conditions at (ESRI) Environmental studies and research Institute Minufiya University Farm at Sadat desert. The plant was harvested just before flowering. This period is recommended to obtain a maximum extraction yield of artemisinin in the plant. Voucher specimens (A.A-2) are deposited at the Herbarium of the Department of Natural Resources, Environmental Studies and Research Institute – Minufiya University.

Artemisia cultivation method

Artemisia annua seeds were cultivated according to the cultivation method reported in the WHO's monograph on good agricultural and collection practices (GACP) for *Artemisia annua* L. cultivation (Castilho *et al.*, 2008). Cultivation of this plant requires a minimum of 6 months. The growth cycle of *A. annua* from sowing to withering consists of six stages, namely the seedling, branching, flower-budding, flowering, fruiting and withering phases. The length of each growth period of *A. annua*

varies with seed source, cultivation techniques, site and conditions. Following emergence, the biomass of *A. annua* increases with the increase in the length of growth period and reaches its maximum before flower budding. Biomass decreases from the time of flowering to plant withering

Planting

Artemisia annua is a robust plant once established, but the seeds are tiny (12–14,000 seeds/gram) and the young seedlings are very delicate so planting in the field is not easy. The process is very labour-intensive and losses are frequently high. The seeds were sprinkled uniformly onto the surface of soil and covered very lightly with compost (1-2 mm) and germinated in a greenhouse at the beginning of October (2011). The surface was kept moist and not let to dry out.

Transplanting

When the plantlets have about 10 true leaves and are about 10-14 cm high, approximately 8 weeks after sowing. They were pricked out, taking care not to damage the roots and then transplanted to the field. This process is extremely delicate, if the taproot is bent or if the young plant is stressed in any way (especially by water shortage) it responds by flowering prematurely leading to greatly reduced yield. Until the transplanted seedlings start to actively grow away the soil should be kept moist but after this period *Artemisia* plantations can with stand dry conditions. In these experiments seed drilling and transplanting took place in December 2011. The plants were harvested just before flowering (July 2012). This period is recommended to obtain a maximum extraction yield of artemisinin in the plant. The leaves and small stems of the plant were dried in the shade, at ambient temperature not exceeding 40°C for 72 hours. They were then crushed into powder with a mixer.

Preparation of Extracts and sample solution

An aliquot of 25 gram of grinded air dried aerial parts of *Artemisia annua* was exhaustively extracted with 750 ml of methanol in a Soxhlet apparatus at 60 °C then filtered through 0.45 µm membrane prior to use. The collected methanolic extracts evaporated via rotavapour at 40-50 °C under reduced pressure yielded 5.06 gm of the crude methanolic extract. Artemisinin extracts 100 mg was accurately transferred into a 10 ml volumetric flask and dissolve in methanol. Final volume was made up with methanol to give 10mg/ml solution of *A.annua*. Aliquots of 10µl was injected into the LC-DAD/MS analysis system

HPLC conditions

Analyses were performed using a Dionex Ultimate 300 (Bremen, Germany) composed of a quaternary pump with an on line degasser, a thermostatted column compartment, a photodiode array detector (DAD), an auto sampler, and Chromelon software. The HPLC separation was performed on Zobrax SB-C18 column (150 mm×4.6 mm, 1.8 µm, Agilent Company, USA). Mobile phase consisted of two solvents, (A) methanol and (B) 0.2 %

formic acid. Separation of compounds was carried out with gradient elution profile: 0 min, A: B 10:90; 36 min, A: B 70:30; 50 min, A: B 100:0; 60 min. Chromatography was performed at 30 °C with a flow-rate of 0.8 ml/min. UV traces were measured at 254, 290 and 280 nm and UV spectra (DAD) were recorded between 190 and 900 nm.

Mass spectrometric conditions

The HPLC–MS system consisted of electrospray ionization (ESI) interfaced Bruker Daltonik Esquire-LC Amazon SL Ion Trap Mass spectrometer (Bremen, Germany) and Dionex Ultimate 300 (Germany) composed of a quaternary pump with an on line degasser, a thermostatted column compartment, a photodiode array detector (DAD), an auto sampler, and Hystar software. The ionization parameters were as follows: positive ion mode; capillary voltage 4000 V, end plate voltage –500 V; nebulizing gas of nitrogen at 50 p.s.i.; drying gas of 10 l/min nitrogen at 350 °C. Mass analyzer scanned from 15 to 1000 u. The MS/MS spectra were recorded in auto-MS/MS mode. The fragmentation amplitude was set to 1.0 V. MS3 data were acquired in positive mode.

Calibration curve

Calibration curve was obtained from a methanolic dilution series of the reference standard artemisinin. Accurately transfer 50 mg of artemisinin working standard into a 10 ml volumetric flask and dissolve in methanol. Concentrations ranging between 0.001 and 3.0 mg/ml of artemisinin were prepared from the standard stock solution by serial dilution with methanol for the calibration data.

The calibration curves were obtained by the external standard method on six levels of concentration of standard (0.001, 0.0025, 0.005, 0.010, 0.020 and 0.030 mg/ml), with three injections per level. Linear regression was used to establish the calibration curve. Results were calculated using the peak areas.

RESULTS AND DISCUSSION

The cultivation of *A. annua* and the consequence quantification of its artemisinin content and purity in both pure samples and plant materials to date have been characterized by a considerable inconsistency in values. This is likely to be due to the adoption of varied analytical procedures and the use of inappropriate to the specific applications analytical techniques (Lapkin *et al.*, 2009). The WHO's monograph on *A. annua* cultivation (WHO Press 2006) and the artemisinin monograph by the International Pharmacopeia (The International Pharmacopeia Geneva 2003) contains descriptions of several analytical methods for artemisinin quantification and provides a detailed description of the techniques required for the cultivation and collection of *Artemisia annua* L. This developed WHO monograph model on good agricultural practices for *Artemisia annua* L. have led to facilitate cultivation of this particular medicinal plant with reliable quality and to ensure a sustainable supply to meet market demands providing practical and specific technical guidance on the

cultivation and collection of *Artemisia annua* L. of good quality and a high yield (WHO Press 2006).

HPLC-DAD and HPLC-MS analysis of the extracts

The most common method for analysis of artemisinin in the WHO monograph and in International Pharmacopeia are based on HPLC method using UV detection at 214 nm and dihydroartemisinin (Castilho *et al.*, 2008) as an internal standard. This method is validated only for the pure artemisinin and is not suitable for quantification of artemisinin in extracts. Many authors discard the WHO HPLC method on the basis of very low UV absorbance of artemisinin (Zhao, 1987; Sahai and Vishwakarma, 1998; Christen and Veuthey, 2001; Qian *et al.*, 2005; Jansen and Soomro, 2007; Liu *et al.*, 2007). It was hence suggested to use pre- or post-column hydrolysis of artemisinin into more UV-active compounds to allow the use of the most wide-spread HPLC-UV instruments (artemisinin is UV-transparent therefore derivatisation is required) are time-consuming and not suited for routine analysis (Elsobhy *et al.*, 1987; Zhao, 1987; Qian *et al.*, 2005). There are also reports on using other detectors, such as electrochemical reduction (Acton *et al.*, 1985; Vandenberghe *et al.*, 1995) and evaporative light scattering (Avery *et al.*, 1999; Liu *et al.*, 2007).

However, the issues surrounding accurate determination of artemisinin quantity will not be resolved by simply replacing the detector on an LC system. More important is the fact that most of these methods lack specificity (TLC, UV-detection, FID, ECD, ELSD). As an *A. annua* plant extract may contain hundreds of components, some structural analogues of artemisinin, good specificity of the detector is essential (Lapkin *et al.*, 2009). Liquid chromatography coupled with mass spectrometry (LC/MS) is a powerful tool for the rapid identification of chemical constituents in plant extracts. The high sensitivity of MS as an LC detector is a very efficient method for detection, particularly in cases of constituents without chromophore moieties (Ranasinghe *et al.*, 1993; De Rijke *et al.*, 2003).

The developed analytical system HPLC/DAD/MSn in our study made it possible to separate ($R_t = 35.2$) and quantify artemisinin in both plant materials and pure samples. Identification of artemisinin was based on retention time, injection of different dilutions of artemisinin standard and confirmed by comparing the mass spectral fragmentation pattern of both pure standard and artemisinin peak in the methanolic extract of *Artemisia annua* with each others and to those reported in literature.

Typical HPLC-positive mode ESI-MS base peak chromatogram (BPC) of the crude methanolic extract of *Artemisia annua* showing artemisinin peak eluted at 35.2 min as the main major active ingredient is represented in Figures 1 (A).

Typical HPLC-DAD chromatogram of the crude methanolic extract of *Artemisia annua* monitored at 214, 217, 290 and 280 nm and UV spectra (DAD) recorded between 195 and 500 nm showing very low absorbance of artemisinin peak in the ultraviolet (UV) regions compared with the LC-positive mode ESI-MSn base peak chromatogram (BPC) profile is represented in Figures 1 (B).

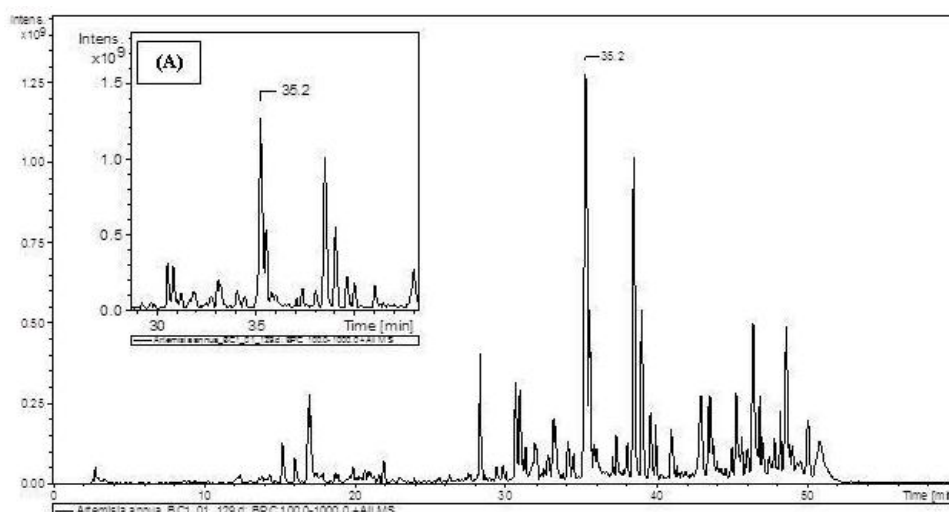


Fig. 1(A): Typical HPLC-positive mode ESI-MSn base peak chromatogram (BPC) profile of the methanolic extract of *Artemisia annua* showing artemisinin peak eluted at 35.2 min as the major active ingredient.

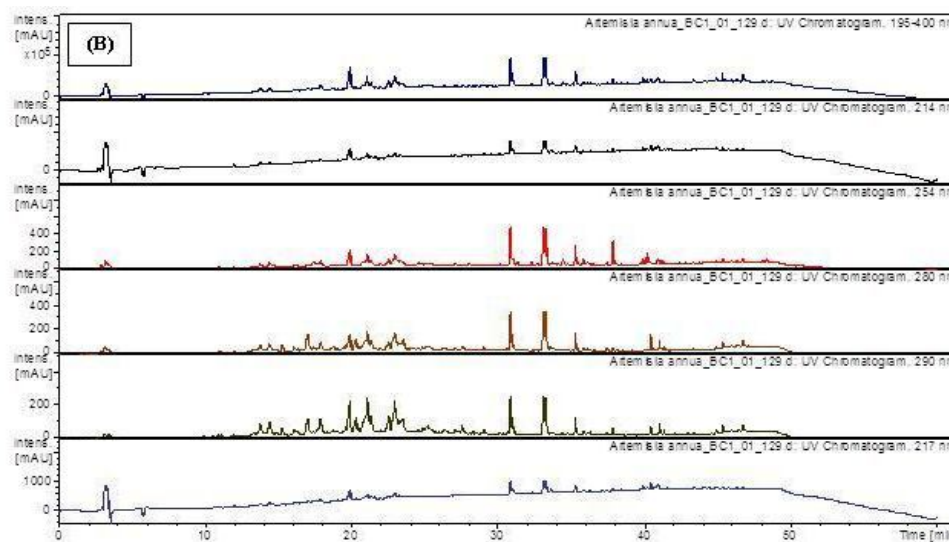


Fig. 1(B): Typical HPLC-DAD chromatogram of the crude methanolic extract of *Artemisia annua* monitored at 214, 217, 290 and 280 nm and UV spectra (DAD) recorded between 195 and 500 nm showing very low absorbance of artemisinin peak in the ultraviolet (UV) regions compared with the LC-positive mode ESI-MSn base peak chromatogram (BPC) profile.

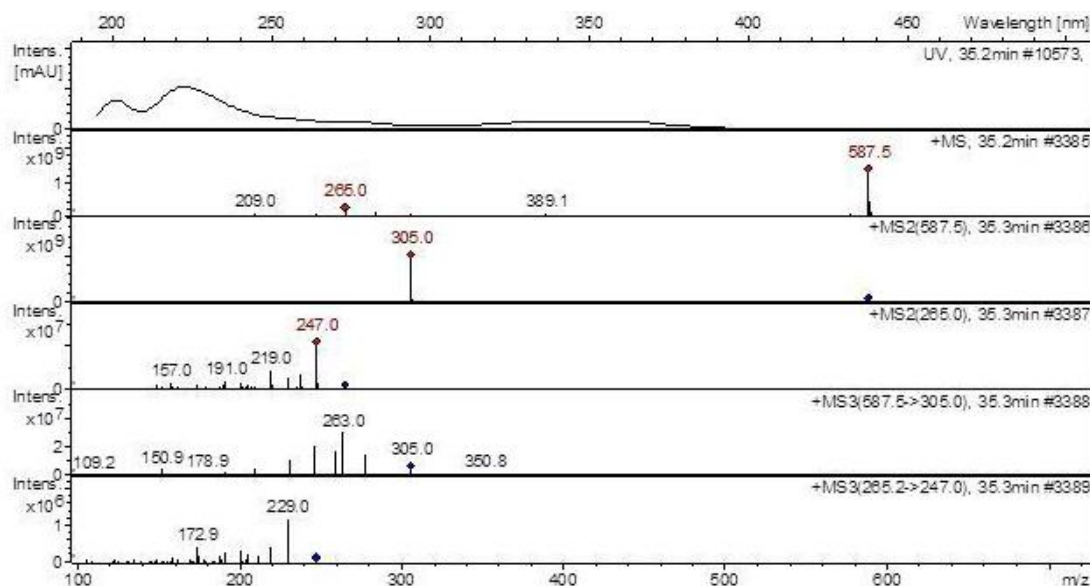


Fig. 2: The HPLC/DAD/MS3 mass spectra and fragmentation pattern of artemisinin peak present in the methanolic extract of *A. annua* as well as that of artemisinin standard.

The mass spectral data of artemisinin and artemisinin standard obtained in the positive ion mode using an electrospray ionization source and eluted at retention time 35.2 represented in Figure 2 shows the characteristic quasi-molecular ions as well as the characteristic molecular fragments of artemisinin $[M+H]^+$ (m/z 283.1) ion that corresponds to the molecular formula of $C_{15}H_{23}O_5$. The characteristic quasi-molecular peaks are represented by $[2M+Na]^+ = 587$ m/z that corresponds to dimer peaks cationized by Na^+ and $[M+Na]^+ = 305$ m/z that corresponds to monomer peak of artemisinin cationized by Na^+ while the characteristic fragments of artemisinin are represented by 265.14, 237.15 and 209.15 that corresponds to loss of H_2O , loss of H_2O and CO , and loss of H_2O and two CO , respectively. HPLC/DAD/MS3 mass spectra and fragmentation pattern of artemisinin peak present in the methanolic extract of *A. annua* as well as that of artemisinin standard showing both the characteristic quasi-molecular peaks and fragments of artemisinin is represented in Figure 2.

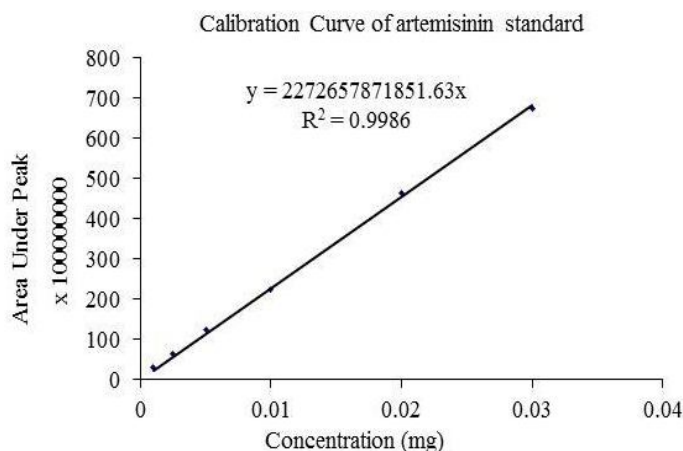


Fig. 3: Calibration Curve of Artemisinin obtained by six levels of concentrations of standard, with three injections per level.

The most intensive quasimolecular peaks in the spectra of artemisinin molecules are the monomer and dimer peaks cationized by Na^+ represented by $[M+Na]^+$ (m/z 305) and $[2M+Na]^+$ (m/z 587), respectively. The high intensity of the protonation and cationization of artemisinin is obviously conditioned on the peculiarities of artemisinin structure in particular, the presence of endoperoxide bridge and carbonyl group in the drug structure provides the centers to connect protons and more heavy cations. The analysis of the spectra of artemisinin also pointed to the fact that artemisinin tends to form ions of dimers and trimers under the ESI experimental conditions. Apparently the chemical structure of the artemisinin-type agents and in particular presence of the polar functional groups (CO , OH , endoperoxide bridge) in the structures can provide the dimmer and trimer noncovalent complexes formation by van der Waals interactions, hydrogen bonds and other types of noncovalent interactions (Cheng *et al.*, 2002). Especially that cationization as a way of ion formation is characteristic for the electrospray method of ionization (Laskin and Lifshitz, 2006) and solvent drops spray

with solvent evaporation steps included in ESI technique also promotes the formation of ions of dimers and trimers of analyzed molecules. The peaks of cationized monomeric and dimeric molecules of the artemisinin-type drugs were also registered in previous ESI mass spectrometry study (Pashynska *et al.*, 2004) of these agents. The peaks characteristic for artemisinin observed in the present work are in a good agreement with the peaks registered for the drugs in other previous ESI investigation of these antimalarial agents (Pashynska *et al.*, 2004) and can be used for the further analysis of artemisinin in both its pure form as well as in plant extracts.

Calibration curve

The linearity of the method was determined at six concentration levels ranging from 0.001 to 0.03 mg/ml. The Six different concentrations of standard prepared were (0.001, 0.0025, 0.005, 0.010, 0.020 and 0.030 mg/ml). The calibration curve was obtained by the external standard method on six levels of concentration of standard, with three injections per level. The calibration curve was constructed by plotting peak area against concentration of drugs. Linear regression was used to establish the calibration curve. Results were calculated using the peak areas. ($R^2 = 0.9986$). The results show an excellent correlation between peak area and concentration of Artemisinin within the concentration range indicated above. The calibration curve is shown in Figure 5. Typical HPLC-positive mode ESI-MSn base peak chromatogram (BPC) profile of the methanolic extract of *Artemisia annua* showing artemisinin peak as the major active ingredient, Typical HPLC-positive mode ESI-MSn extracted ion chromatogram (EIC) of artemisinin peak present methanolic extract of *Artemisia annua* and the compared with two Typical HPLC-positive mode ESI-MSn profile of two concentrations from the six levels of artemisinin standard that were used to obtain the calibration curve for artemisinin quantification are shown in Figure (A, B, C and D) respectively. *Artemisia annua* L. is grown worldwide; however, this does not mean that all the plants necessarily contain artemisinin, which is the effective ingredient for antimalarial treatment. In some places, although *Artemisia annua* L. may contain artemisinin, the content may be very low and without industrial value. Based on the experiences in various countries, the highest range of artemisinin content obtained from *A. annua* harvested from different production areas in different countries is very wide and the highest content of artemisinin reached is up to 1– 2% (Guoyue *et al.*, 1998) expressed as dry weight of leaves of *A. annua*. In contrast the higher unexpected content of artemisinin that ranges between 4.85-4.90% of dry plant weight in *A. annua* cultivated in this study, have reported the Sadat desert to be one of the most promising area for the cultivation of *Artemisia annua* with high artemisinin content. The high unexpected content of artemisinin obtained from current study has extensively gained our attention to study the impact of various environmental, climatic and geographical factors of Sadat desert on artemisinin production and to compare them with those reported in the WHO monograph of *Artemisia annua*.

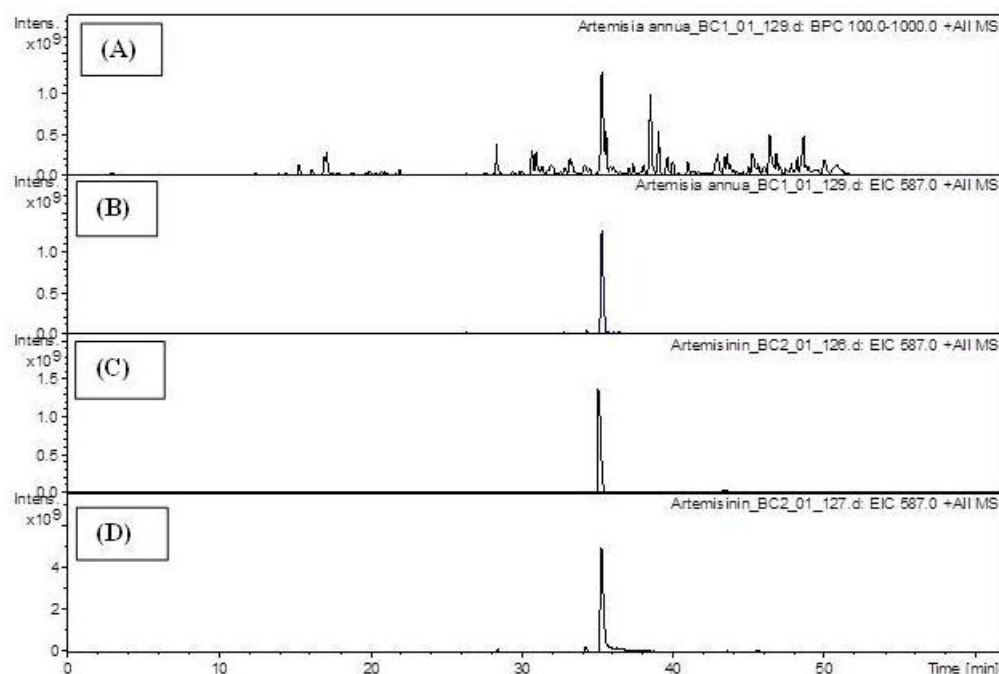


Fig. 4 (A): Typical HPLC-positive mode ESI-MSn base peak chromatogram (BPC) profile of the methanolic extract of *Artemisia annua* showing artemisinin peak as the major active ingredient.

Fig. 4 (B): Typical HPLC-positive mode ESI-MSn extracted ion chromatogram (EIC) of artemisinin peak present methanolic extract of *Artemisia annua*.

Fig. 4 (C and D): Two Typical HPLC-positive mode ESI-MSn profile of two concentrations from the six levels of artemisinin standard that were used to obtain the calibration curve.

WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants

WHO encourages and supports developing countries in promoting their local medicinal products, by developing a WHO monograph model on good agricultural practices and guide lines on numerous environmental and geographical factors that can lead to facilitate the cultivation of this particular medicinal plant with good quality and a high yield of artemisinin. Based on the experiences in various countries *A. annua* grows well in warm climates. The most suitable conditions for the growth of *A. annua* are found in tropical humid monsoon climatic regions, where the average temperature during the growth phase is 17.6–28.4 °C. Experience has shown that the annual number of hours of sunlight should be about 1000 hours. The temperature for seed germination should be above 7 °C. *A. annua* grows in most soil types provided that the pH of the soil is between 4.5 and 8.5 and it has deep topsoil and good drainage properties (Ferreira *et al.* 1995). For *A. annua*, the maximum absorption of nitrogen occurs during the initial branching and alabastrum stages, and that of phosphate occurs prior to the initial branching and blooming stages, whereas the absorption of potassium increases linearly from the seedling to alabastrum stages. Therefore, potassium should be used as the base fertilizer. Prior to the appearance of the sixth leaf blades, *A. annua* seedlings are susceptible to drought or waterlogging. Once established the plants exhibit strong adaptability and resistance to drought or waterlogging, because of their abundant and dense lateral roots. However, *A. annua* has relatively strict requirements for water supply when the seedlings are young and during the

preliminary phase of growth. As a result, sufficient water supply and/or the necessary drainage should be ensured in this period. Harvesting at the appropriate time is critically important to ensure optimum content of artemisinin in *A. annua*. Studies in countries in which *A. annua* is produced have shown that the best harvesting time is the early stage of flower budding. The yield of *A. annua* leaves and the content of artemisinin are reduced if harvesting is too early or is delayed. Different drying methods can affect the yield of artemisinin. Comparisons between sun-drying, shade-drying and oven-drying at 60 °C have shown that natural sun-drying is the best method (Fenglin *et al.*, 1997).

Interestingly and according to the meteorological data of Sadat City 2003 – 2012 (Table 1) which was obtained from the Egyptian Meteorological Department and the solid analysis data (Table 2) that shows the Physical and chemical properties of soil samples profile of the study area (Ranasinghe *et al.*, 1993; Christen and Veuthey, 2001).

The environmental and geographical factors of our studied area were in a good agreement with the guide lines provided in the WHO monograph as shown in Table (1 and 2) at least during the growth period (from December to July) represented in the soil conditions, climatic conditions, water requirement and different types of fertilizers rich in this area during the cultivation period, especially after the transplantation of the plantlet to the field that starts at the first of December 2011 till harvesting in July 2012 because before this time the seeds were germinated in green house under the controlled conditions described in WHO monograph.

Table. 1: Average Meteorological data of Sadat City 2003 – 2012

Climatic factors	Rainfall (mm)	Temperature(°C)	Relative humidity (%)	Evaporation (mm/day)	Wind velocity (km/hr)
Months					
January	10.1	17	56	2.7	10.2
February	7.0	19	53	2.9	11.5
March	5.2	26	43	3.5	12.8
April	1.2	30	48	4.6	12.2
May	0.0	34.6	49	5.5	10.3
June	0.0	35	53	5.7	10.5
July	0.0	36.5	57	4.9	10.2
August	0.0	35.5	58	4.7	8.5
September	0.0	30	58	4.1	8.4
October	1.0	27	58	4.3	8.6
November	2	24	57	3.1	8.7
December	9.1	19	57	2.9	10.3

Table. 2: Physical and chemical properties of soil samples profile of the study area.

Depth /Cm	M%	Physical					PH	CaCo3 %	N ppm	E.C Ms / Cm	Chemical				Cations / ppm			
		Particle size %									Anions / ppm				Cations / ppm			
		G	Sand			SC					Cl ⁻	SO4 ⁴⁻ CO3 ³⁻ HCO3 ⁻			Na ⁺ K ⁺ Ca ⁺ Mg ²⁺			
			C	M	F													
0-25	7.5	9	3.8	38.9	36	12.3	8.6	7.6	23	1.7	2.25	3.93	-	1.2	3.4	1.15	14.2	4.8
25-50	8.0	17	5.4	37.5	29.	10.6	8.4	5.9	22	154	2	1.5	-	1.0	2.5	0.92	10.7	2.3
50-75	9.3	19	7.5	36.1	28.	9.3	8.2	3.0	20	0.97	1.75	1.18	-	0.9	2.4	0.86	10.5	2.1
75-100	12.6	12	5.7	36.2	36	9.8	8.1	2.5	17	0.58	1.5	0.95	-	0.8	1.7	0.67	6.3	1.6
100-125	13.1	10	3.2	32.7	37.2	15.3	7.9	2.2	9	0.36	1.0	0.82	-	0.7	1.8	0.43	3.8	0.4
125-150	13.9	9	2.8	32.5	40.1	15.6	7.2	2.0	6	0.35	0.75	0.12	-	0.5	0.97	0.35	0.6	0.1

M= Moisture content – G= Gravels – C= Coarse sand – M = medium – F= fine sand – SC= finer sediments – T.N = Total nitrogen

Geographical, Ecological, Climatic and Environmental studies on the area of cultivation

Sadat City was established in 1976: to become a new residential based on industrial and agricultural activities, it has been a unique location between Cairo and Alexandria and adjacent Delta – making it a center for attracting domestic and foreign investments. Sadat City is located in the north and west of Cairo city at the Kilo 93 through Cairo-Alexandria Desert Road and an area of 500 Km². Latitude 30, 3750 (3022'30.000"N), and Longitude 30, 5003 (3030'1.080"E), while Altitude (feet) 137.

Meteorological Studies

The data showed in table (1) was obtained from the Egyptian meteorological Department .The monthly mean rainfall varied from 9.1mm in December (transplantation period) to no rainfall in July (harvesting period) and minimum value in October and November (the period of germination in green house under the controlled conditions described in WHO monograph). The monthly mean temperature was 15 °C in December and remains at the range of 17.6-28.4 °C during the growth phase (the optimal temperature during the growth period) reaching 35 °C at harvesting time in July. The relative humidity varied between 57% December and 36.5% July. The evaporation varied from 2.9 mm/day December to 4.9 mm/day July. The wind velocity varied from 10.3 km/hr December to 10.2 km/hr July.

Soil Analysis

The solid analysis data shown in Table (2), indicates that the main bulk of the soil mainly of fine and medium sand ensuring a good drainage property ,while the salinity (E.C) decreased with depth ; this may due to the continuous evaporation and decreased

leaching of salts from the surface layers. The anions were mainly chlorides, partly sulphates and rarely bicarbonate. The soil reaction PH was alkaline. The soil salinity, fine sediments, organic matter and soil moisture content were demonstrated to be related closely with desert vegetation patterns (Abd El-Ghani, 2000).

CONCLUSION

This study reported El Sadat desert and probably all the surrounding areas that shares the same environmental, ecological and geographical factors for the first time as a promising cultivation area for *Artemisia annua* with high artemisinin yield. This new promising cultivation area may be used in a large scale in order to improve the overall supply of artemisinin at a reduced acceptable price for most patients.

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