RESEARCH ARTICLE Agriculture, active compounds investigation of cola herb (*Artemisia abrotanum* L.) recently introduced in Iraq

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ABSTRACT

Background: This study was designed to study the adaptation of cola herb (Artemisia abrotanum L.) for semi-arid condition of middle region of Iraq, pharmacognostical evaluation including macroscopical and microscopical characters. Furthermore, this study conducted to phytochemical investigation of this plant. Aims and Objectives: This type of Artemisia was introduced recently to Iraq for ornamental purposes without any study about adaptation this plant for environmental conditions of semi-arid of Iraq and no studies about active compounds and pharmacognostical characters of this plant, therefore, this study conducted to agriculture and investigation of active compounds of this plant. Materials and Methods: The methods of this study were included plantation of herb cola at medicinal plants garden of College of Pharmacy of Al-Mustansiriya University in Baghdad. Active compounds of the leaves of this plant were investigation using different reagents and the essential oil was extracted by clevenger with analysis by gas liquid chromatography. Results: The results were referred to good adaptation of this plant for semi-arid conditions of middle region of Iraq because this plant has different morphological characteristics prevent water losing from leaves surface. Furthermore, the results were referred to this plant have different active compounds such as saponin, tannin, coumarin, and flavonoids. The volatile oil study was referred to 2-4% of volatile of plant leaves, and the most important compounds of this volatile oil were soloinene, limonene, myrecen, camphene, thusene, α -pinene, and others. **Conclusion:** The results were gave the best indication for the possibility of this plant to grow and more distributed under Iraq conditions and need more chemical and clinical studies of active compounds with open field experiments or trails to improve quality and quantity of volatile oil.

KEY WORDS: Cola Herb; Artemisia abrotanum; Gas Liquid Chromatography; Volatile Oils

INTRODUCTION

Different extracts of medicinal plants have evoked interest as sources of natural products, their potential uses as alternative remedies for treatment of many infection diseases.^[1] *Artemisia* is a genus of small herbs and shrubs found in northern temperature regions; it belongs to the important family

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compositae (Asteraceae).^[2] *Artemisia abrotanum* its semievergreen subshrub in southern europe and hardy perennial in the eastern half of the United states and it reach from 3 to 5 feet in height.^[3,4] *A. abrotanum* was traditionally considered as an antiseptic, astringent, emmenagogue, expectorant, febrifuge, stomachic, stimulant, tonic, anti-inflammatory, rermifuge, spasmolytic and used for treating upper respiratory tract disease. It's used against cancer, cough, fever, and tumors.^[5] This type of *Artemisia* was introduced recently to Iraq for ornamental purposes without any study about adaptation this plant for environmental conditions of semi-arid of Iraq and no studies about active compounds and pharmacognostical characters of this plant, therefore, this study conducted to agriculture and investigation of active compounds of this plant.

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MATERIALS AND METHODS

Plantation of Plant

The field experiment was conducted to evaluation the *A. abrotanum* plant adaptation of growing under semiarid conditions of Iraq during 2015-2016 growing season. The field experiment was conducted at the medicinal plants garden of College of Pharmacy of Al-Mustansiriyah University at Baghdad location. After preparation of soil to plantation, 2 months old seedlings with an average height of 10 cm were used. During growing season NPK fertilizer (18, 10, 16) was added 1 month after transplanting at 250 kg/ha. The plot area was $2 \times 3 m^2$, leaving 50 cm between plants and 50 cm between rows (Figure 1).

Collection of Plant Samples

Samples of plant collected at pre-flowering stage and 50 cm above soil level, samples were dried at room temperature 25°C in a laboratory room. The plant identification by national herbarium of the agriculture ministry.

Pharmacognostical Evaluation

Macroscopic examination

Fresh specimens of *A. abrotanum* plant were used to study the morphological character of the plant such as the shape of leaves, stems, and margins.

Microscopic examination

Powdered microscopy

Shade dried leaves were finely powdered and examined under microscope. Small quantity of the powder was placed on the slide which then was mounted two drops of chloral hydrate and covered with a cover slip and examined under microscope. Different cell components were observed and photography was done using digital camera.

Leaf microscopy

The lower and upper epidermal layer of fresh leaf (in fragments) were mounted in chloral hydrate and observed under a microscope. Determination of the components of leaves (stomata, trichomes, and stomatal index) were carried out under a microscope. The stomatal index was carried out using the following equation.^[6]

Stomatal index = Number of Stomata Number of Stomata+Number of epidermalcells ×100

Phytochemical Screening

The preliminary phytochemical study of ethanolic areal parts extract of plant was carried out by standard methods of phytochemical screening such as mayers, dragendroff's, borntrager's test, for alkaloids, and glycosides. The foam test, lead acetate test, ferric chloride test, alkaline test and Salkowski test were used for saponin, tannins, flavonoids, and terpenoids examination extracted using a clevenger - type apparatus.^[7] The distillation was carried out with 50 g of plant material and 300 ml distilled water. Series of distillation, each lasting exactly 1 h respectively.^[8-12]

Essential Oil Extraction

Essential oil was, from 1 h to 5 h. the essential oil yield percentage was measured, and the oil was collected, dried over by anhydrous sulfate (Na_2So_4) and stored at $-5^{\circ}C$ in 2 ml vials for further analysis.

The extracted oil has been mixed with n-hexane, injected into gas-liquid chromatography using an auto-sample and the different compounds have been separated on an HP-INOWOAX ($60 \times 0.25 \times 0.25$ mm) capillary column. Helium was used as carrier gas (flow rate 1.5 m/min⁻¹). The temperature program was 35-230°C (2.5° C/min.) in course of time (92 mm), injector temperature was 205°C and flame ionization detector used, area percentage were obtained using a PC programmer (maestro chromatograph data system), for identification of single compound internal and external standard substances have been used, the external standard was obtained from Oma company for chemical compounds.

RESULTS

The results of field experiment of plant were referred to the adaptation of *A. abrotanum* to the environmental condition of Iraq (semi-arid condition). The adaptation of this plant may be belong to have this plant fine glandular hairs and wooly hairs which covered the leaves, that useful in preventing the water losing by transpiration and evaporation from leaves surface. Another reason of its adaptation may be belong to possibility of this plant to grow in clay-sandy soil of Iraq. Maybe belong to short, and crowded leaves of this plant, that lead to a reduction of water lose by both transpiration or evaporation from leaves surfaces.^[13]

Pharmacognostical Evaluation

The results of macroscopical examination of the plant were showed that it was semi-erect, branched and green, up to 45 cm in height. Stems were angular rarely slender, green. The results were showed some typical xeromorphic features in the stem and leaf structure, which are more strongly. Microscopical examination of the leaf showed the presence of anomocytic stomata in which the guard cells are surrounded by epidermal cell that are not distinguishable from other epidermal cells as shown in Figure 2. Multicellular unbranched trichomes were represented. Furthermore, the microscopic examination was showed about 16.6% as stomatal index of upper surface while 25% for lower surface of leaves shown in Table 1.



Figure 1: (a and b) Artemisia abrotanum L



Figure 2: Micrograph of leaf of Artemisia abrotanum L. showing the stomata and trichomes. (a) Multicellular unbranch trichomes, (b) anomocytic stomata

Phytochemical Screening

The results of phytochemical screening were presented in Table 2 and these results were referred to the cola herb was contained different active compounds such as saponin, tannin, coumarin, and flavonoids.

DISCUSSION

The results of this study were referred to the percentage of volatile oil of this plant was reached to 2.4%. The values of specific gravity, oil density, and refractive index were reach to 0.98. 0.66 mg/ml and 1.5 mg degree, respectively. In addition, the results were obtained in Table 3 referred to 13 compounds of volatile oil of cola herb leaves. The most main compounds of volatile oil were soloinene (21.458%), limonene (14.368%), myrecen (13.634%), camphene (12.680%), thusene (4.163%), α -pinene (4.106%), α -terpinone (2.841%), trycyclene (1.541%), terpinolene (0.843%), β-pinene (0.515%), β-phellanderne (0.056%), and β -ocimene (0.433%), respectively. These results were gave best indication for the possibility of this plant to grow under semi-arid conditions at middle region of Iraq and need more studies about active compounds and medical activity of these compounds. In addition, the results of quality and quantity of volatile oil of this plant encourage to increasing the different field experiment to obtain more quality and quantity of volatile oil.

CONCLUSION

The good adaptation of this plant for a semi-arid condition such as higher temperature with dry climate with very important active compounds of this plant lead to increasing the research or isolation of active compounds of this plant.

Table 1: Stomatal index					
Number of stomata	Number of epidermal cell	Stomatal index (%)			
Upper surface					
2	10	16.6			
Lower surface					
4	12	2			

Table 2: Results of phytochemical screening of cola herb		
Test	Result	
Saponin	+	
Tannin	+	
Coumarin	+	
Flavonoid	+	

Table 3: Components of volatile oil of A. abrotanum L.which analysis by GLC					
Diagnosis	RT	Area	Conservation (%)		
Trycyclene	5.696	504243	1.541		
Thusene	7.833	1361801	4.163		
β-pinene	8.435	1343253	4.106		
Camphone	8.975	4147914	12.68		
α-pinene	10.048	178420	0.515		
Soloinene	12.208	7010503	21.458		
Myrecen	14.877	4459878	13.634		
a_terpinone	15.002	929273	2.841		
Limonene	15.725	4700010	14.368		
$\beta_phelladreene$	16.578	18349	0.056		
β_ocimene	16.848	141527	0.433		
Terpinolene	17.847	275681	0.843		
β_thyjone	18.537	23317	0.071		

GLC: Gas liquid chromatography, RT: Radiotherapy,

A. abrotanum: Artemisia abrotanum

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