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CHEMICAL CHARACTERIZATION OF VOLATILE EXTRACT OF *ARTEMISIA HERBA-ALBA* AND STUDY OF ITS ANTIOXIDANT, ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES AND ITS INHIBITIONORY EFFECT ON CORROSION OF ALUMINUM IN HYDROGEN CHLORIDE SOLUTION

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ABSTRACT

The chemical composition of the essential oil of *Artemisia herba-alba* obtained by hydrodistillation was determined by chromatographic analysis (GC and GC-MS). Seventy-nine components were identified, which accounted for 93.3% of the total essential oil. The major components were chrysanthenone (24.1 %), camphor (16,2%), α-thujone (12.8 %) and 1,8-cineole (9.3%). The investigated volatile extract showed potency antimicrobial activity, against all the studied bacterial strains (MIC values not exceeding 2 µg/mL). Also, this oil showed substantial antifungal activity MIC less than 0.5µg/mL for *Fusarium oxysporum* and *Penicillium expansum*. Compared to BHA and BHT which were acted as positive controls, the essential oil was the less active to inhibit the free radical DPPH insofar it failed to reach a scavenging capability of 50%. The oil was also tested for its corrosion inhibitor of pure aluminum in HCl (1 mol./L) using electrochemical techniques (potentiodynamic polarization, open circuit potential, and electrochemical impedance spectroscopy) and gravimetric measurements. The results reveal that the inhibition effectiveness was dose-dependent of the oil concentration (maximum corrosion inhibition = 94.3% for an optimal concentration of 0.8 g / L). These findings suggest that this essential oil may be a new green inhibitor agent against corrosion in hydrogen chloride solution media and could be used on food systems as an effective inhibitor of foodborne pathogens as well as for pharmaceutical applications.

Keywords: *Artemisia herba-alba*, Essential Oils, Antimicrobial activity, Antioxidant activity, Corrosion, Inhibitor, Metallic Materials, hydrochloric acid

INTRODUCTION

Aluminum exhibits excellent corrosion resistance owing to the formation of a thin protective film of oxide, naturally formed on its surface (**Halambek *et al*., 2013**). It is extensively used in industry, but, the major impediments for its use on wider scale are its sensitiveness to be rusted in humid air and dissolved in acidic media **(Chauhan *et al*., 2007).** Acid solutions such as hydrogen chloride and sulfuric acids are extensively used in pickling processes of metals which are companioned by significant dissolution of the metals (**Hussin et al., 2016; Khadraoui *et al*.,2013)**. The harmful effect of these acids leads to the use of inhibitors which are judged to be the best method of protection of metals against corrosion. (**Chauhan *et al*., 2007; Khadraoui *et al*.,2013**). Use of natural products such as volatile extracts from plants as corrosion inhibitors have been broadly reported by several authors (**Valnet,1984**),out with the aim of developing inhibitors that are acceptable to the environment and less expensive. For this purpose, natural products can be considered as the inexhaustible source (**Ating *et al*., 2010**). Lately, some studies have experienced the efficacy of plant materials as corrosion inhibitors for aluminum in diverse media (**Halambek *et al*., 2013**). The inhibition efficacy of plant extracts is ascribed to the presence of complex organic compounds with diversity of adsorption centers (double bonds, heteroatoms, aromatic rings etc.) (**Halambek *et al*., 2013; Khadraoui *et al*., 2013; Ating *et al*., 2010; Hachelef *et al*., 2016**).

A. herba-alba species are commonly used as a flavoring agent for coffee and in folk medicine due to its multiple therapeutic benefits (**Bailey *et al*., 1981**). It is principally used against the treatment of gastric disturbances and as an antidiabetic agent (**Jouad *et al*., 2001; Mohamed*et al*.,2010**). The chemical composition and biological activity of the plant have been reviewed recently, and the essential oil composition has received ample attention **(Mahmoud *et al*., 1988; Salido *et al*., 2004; Dahmani-Hamzaoui *et al*., (2010, 2015, 2018); Segal *et al*., 1987)**. Thus, several researchers have studied several times the volatile components of *A. herba-alba* collected in Spain **(Salido *et al*., 2004)** and Algeria **(Dahmani-Hamzaoui.,*et al* 2010, 2015, 2018**). Sesquiterpene lactones, flavonoids, some phenolic compounds, and waxes have also been found in the plant**(Segal *et al*., 1987; Dahmani-Hamzaoui *et al*., 2012**). The antibacterial, antifungical and antioxidant activity of *A. herba-alba* has been evaluated several times (**Mahmoud *et al*., 1988)**, the compounds responsible for such activity have been previously isolated **(Dahmani-Hamzaoui *et al*., 2012)**. Nowadays, to the best of our knowledge, there is no work relating on inhibiting properties of *A. herba-alba* volatiles on acidic corrosion of aluminum. On the other hand, some studies were published on the use of this plant as anticorrosive agent. (Hechiche *et al*., 2019;Boudalia *et al*., 2019 ; Ouachikh, *et al*., 2009 ; Benabdellah, *et al*., 2006 ; Bouyanzer et al., 2004)

The aim of this work is to study for the first time the inhibitory effect on corrosion of aluminum in acidic media by *A. herba-alba* essential oil as well as its antioxidant and antimicrobial activities. Thus, the diversity of uses of this oil will offer the possibility of choosing one or more preferred compounds for green corrosion inhibitors, the pharmaceutical and food industry.

MATERIAL AND METHODS

Vegetable material

Artemisia herba-alba aerial parts were collected at the blooming stage of the plant in June 2018 from from Boussâada (245Km south-east of Algiers; 35° 13′ 09″ N, 4° 10′ 54″ E). A Voucher specimen of the plant was filed in the laboratory of plant's Biology in the Faculty of Biological sciences, University of Sciences and Technology Houari Boumediene, Algiers.

Essential oil extraction

Plant samples were shade dried (3 – 6 days) and hydrodistilled, for 3 h. The resulting oil was dried over anhydrous sodium sulphate (Na_2SO_4), and stored at -4°C until use.

Chemicals

Butylatedhydroxyanisole (BHA), butylated hydroxytoluene (BHT) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), were purchased from Sigma–Aldrich Chemie (Steinheim, Germany), n-alkane standard solution (C_7–C_{28}) was obtained from Fluka Chemika (Buchs, Switzerland). Methanol (analytical grade), Na_2SO_4, Tween 80, Sabouraud Dextrose Agar (SDA) and Mueller-Hinton (MH) were obtained from Merck (Darmstadt, Germany). The authentic standards for chromatographic analysis were purchased from Sigma-Aldrich (St. Louis, USA), Fluka (Buchs, Switzerland) and Merck (Darmstadt, Germany). The aluminum samples of dimensions (1.5×1.5×0.5 cm) were cut from an aluminum foil with the following chemical composition (in wt. %): 0.15 Fe; 0.06 Si; 0.002 Zn; 0.004 Cu and the balance Al. The aggressive solution (1 mol/L HCl) was made from pure-grade HCl provided by Prolabo Chemical Co and deionized water

Chemical analysis

For gas chromatography (GC) analysis a Hewlett-Packard 6890N gas chromatograph equipped with a flame ionization detector (FID) was used. The analysis was made by the mean of two columns under the following operating conditions: HP5MS column (30 m x 0.25 mm LD., film thickness 0.25 µm; constant gas flow 0.3 mL/min) vector gas, N_2; injector and detector temperatures, 250 °C and 320 °C, respectively; injected volume 0.2µL pure oil; split-less mode; and HP wax column (60 m x 0.32 mm LD., film thickness 0.25 µm; constant flow 0.9 mL/min); the oven temperature program was 60 °C for 8 min, rising to 250 °C at 2 °C/min, then held for 30 min at 250 °C. Retention indices were calculated relative to the C_7-C_{28} n-alkanes injected under the same conditions as the oil. Relative amounts of components are based on FID peak areas without using response factor correction.
Gas chromatography coupled to mass spectrometry (GC/MS) analysis was performed using an Agilent 6890N chromatograph. The fused-silica capillary column HP5MS and HP wax (The same as those used in the analysis by GC-FID) were coupled to an Agilent 5973A mass spectrometer. Carrier gas He, injector and detector temperatures, 250 °C flow rate 0.5ml/min; split 1:20; injection volume 0.1µL; oven temperature program for each column is described above for GC; an ionization mode with electronic impact at 70eV was used over the mass range 35-400 units.

Components identification

Compound identification was made by comparison of mass spectral fragmentation patterns with those stored in the MS database (NIST 2007 and Wiley 7N), and with mass spectra literature data, and verified by comparison of linear retention indices of the identified compounds with published index data on apolar and polar columns (**Adams, 2001; Masada, 1979)**. Relative percentage quantities of the constituents of the oils were determined from FID chromatograms using the apolar column (HP-5MS).
The results were expressed as the means ± standard deviation (n=3) by use of Microsoft Excel statistical analysis program.

Antimicrobial activity

The antimicrobial activity was made by the use of the agar dilution method [**NCCLS/CLSI, 2004**]. Cultures of the following microorganisms were employed: four Gram-positive bacteria (*Bacillus coagulans*, *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*), three Gram-negative bacteria (*Agrobacterium tumefaciens*, *Escherichia coli* and *Pseudomonas aeruginosa*) and five fungi (*Mucor ramannianus*, *Aspergillus ochraceus*, *Fusarium oxysporum* f. sp. *albedinis*, *Penicillium expansum* and *Fusarium oxysporum* f. sp. Lini) and two yeasts (*Candida albicans* and *Saccharomyces cerevisiae*). A final concentration of 1% (v/v) Tween-80 was added to the agar after autoclaving in order to enhance oil solubility. Briefly, a series of two fold dilutions of the oil (0.5 to 75 µg/mL) was prepared with Tween-80 (1% (v/v)). Each Petri dish of 5 mm in diameter, contains 3 mL of nutrient agar medium and a known concentration of essential oil was dried prior to inoculation with 2 µL of spots containing approximately 3 x 106 cells (for bacteria and yeasts) or spores (for fungi) of each micro-organism. The experiment was repeated twice to confirm the inhibitory effect. Minimum inhibitory concentrations (MIC) were established after 24 h incubations at 37 ° C for bacteria and yeast and 48 h for filamentous fungi. The MIC value was determined to be the lowest concentration of oil that could inhibit the visible growth of each micro-organism on the agar plate.

Scavenging of DPPH• radical activity

The antioxidant activity of the essential oil was determined based on the ability of the tested samples to scavenge the free radical DPPH• (**Brand-Williams *et al.*, 1995)** by off-line spectrophotometric measurements**.** Solutions (2.4 mL) of DPPH• in methanol (10^{-5}M) with an absorbance of 0.800 ± 0.030AU at 517nm were blended with methanolic solutions (1.2 mL) of samples at different concentrations (300-1000 µg/mL). Triplicate samples were shaken and allowed to stand for 15 min in the dark at room temperature, and the decrease of absorbance at 517nm was measured using a Perkin-Elmer Instruments, Nor-walk, CT, USA). The antioxidant activity of the tested samples, expressed as DPPH scavenging percentage was calculated by the following formula:

Radical scavenging % = $\mathbf{[(A_B - A_S)/ A_B)]x\ 100}$

where A_B is the absorbance of the blank sample (t =0), and A_S is the observance of the tested sample after 15 min.
Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against oil concentration. BHT and BHA were used as positive controls.

Electrochemical measurements

Electrochemical tests were conducted on aluminum (99.90%). The inhibitor solution was prepared by disbanding 30% (V/V) of the essential oil in ethanol (96%). The concentrations of the essential oil, ranged from 0.2 to 0.8 g/L, were obtained by dissolving a desired amount of the inhibitor in HCl (1 mol/L) solution. The electrochemical experiments were performed in a conventional three-electrode electrochemical cell, with Ag/AgCl/KCl saturated electrode as reference, platinum plate of 1 cm^2 surface size as counter electrode and aluminum sample as a working electrode. Tests were made using an Autolab PGSTAT 30 with FRA2 module (Eco Chemie, Netherlands). Autolab Software version 4.9 monitors the experimental sequences. Thereafter, the GPES 4.9 software (General Purpose Electrochemical System) was used for open circuit potential (OCP) and polarization curve measurements and FRA 4.9 software (Frequency Response Analysis) for impedance data measurements.

Open circuit potential (OCP)

Open circuit potential were continuously controlled with respect to the saturated Ag-AgCl electrode, for 1 h of immersion in aerated solution of HCl 1M, at 303°K in absence and attendance of *A. herba alba* oil with stirring at 100 rpm.

Potentiodynamic polarization

The Tafel plots based on potentiodynamic polarization were recorded by performing a potential sweep of ± 300 mV around OCPat a scan rate of 1 $mV.s^{-1}$, from negative to positive potential values. The extrapolation of the cathodic and anodic Tafel lines, intersect at E_{corr} and allows the determination of the corrosion current density, (I_{corr}). The inhibition efficiency was calculated by the polarization method using the following equation:

$$\eta' \% = \left(\frac{I_{corr} - I'_{corr}}{I_{corr}}\right) \times 100$$

Where I_{corr} and I'_{corr} correspond to the densities of the corrosion current in the absence and presence of *Artemisia herba-alba* essential oil.

The electrochemical impedance spectroscopy (EIS)

Measurements of electrochemical impedance spectroscopy (EIS) were carried out at OCP after 1 h of immersion in the test solution, with sinusoidal voltage amplitude of 10 mV and scan frequency (*f*) ranging from 100 kHz to 0.01 Hz. The impedance spectra were fitted with the equivalent electrical circuit using the Equivcrt program in FRA software. The inhibition effectiveness was calculated using the equation given by:

$$\eta \% = \left(\frac{R_{ct} - R'_{ct}}{R_{ct}}\right) \times 100$$

Where R'_{ct} and R_{ct} represent the charges transfer resistances with and without inhibitor, respectively.

Gravimetric measurements

Gravimetric measurements were obtained with rectangular aluminum samples, finely mechanically polished using silicon carbide (Si-C) paper up to 1200 grade under water jet, then ultrasonically degreased in absolute ethanol and ultimately air-dried with acetone. The polished samples were weighted before and after 3h of immersion in 100 mL HCl (1mol/L) without and along the addition of inhibitor at different concentrations. The tests were carried out three times with good reproducibility. The weight loss of aluminum sample was used to evaluate the corrosion proportion in milligrams per square centimeter per hour.

$$v = \left(\frac{\Delta m}{s \times t}\right)$$

Where Δm is the weight loss, t is the immersion duration and s the sample surface

$$\eta'' \% = \left(\frac{v_{corr} - v'_{corr}}{v_{corr}}\right) \times 100$$

Where v_{corr} and v'_{corr} are the corrosion rates without and with solution, respectively.

RESULTS AND DISCUSSION

Chemical composition

The yields of essential oil extracted from *A. herba-alba*, calculated from air-dried vegetal material (w/w) are to 0.93 ± 0.06%. Chromatographic analysis by GC and GC-MS of *A. herba-alba* essential oil isolated by hydrodistillation led to the identification of 79 compounds (**Fig1, Table I**), which represent 93.3% of the total oil.

The dominant constituents of the oil were chrysanthenone (24.1%), camphor (16.2%), α-thujone (12.8%), 1,8-cineole (9.3%) and β-thujone (4.8%) (**Fig 2**). Camphene, sabinene and α-pinene have so far only been reported as traces. In this work they accounted (2.4%, 1.5% and 1.0%), respectively.

Camphor is the most prominent component in the essential oil of Boussaada (49.3%) and Djelfa (32%) of Algeria **(Dahmani-Hamzaoui *et al*., 2010; Lakehal *et al*., 2017)**. Chrysanthenone is very important in the essential oil of Bordj Bou Arrérridj (43.8%), M'sila (34.3%) **(Dahmani-Hamzaoui *et al*., 2010)**, southern Spain (36.40%) **(Salido *et al*., 2004)** and Tunisia (17.37%) (**Mighri *et al.*, 2010)**. This compound could come from the hydrolysis and then the oxidation of cis-chrysanthenyl acetate (**Giordani *et al*.,2008)**.

1,8-Cineole (5.61%) is the main compound in southern Spain essential oil (41.0%) **(Salido *et al*., 2004)**, Ichemoul in Algeria (**Bezza *et al*., 2010)** and southern Tunisia (20.00%) (**Akrout, 2004)**.

The α-thujone (12.82%) is the major component in Tunisia (44.0%) and Jordan (16.0%) essential oils (**Akrout, 2004;Hudaib *et al*., 2003)**. On the other hand, it is present in variable compositions in the regions of Algeria (1.50 to 35.1%) (**Khadraoui *et al*., 2013;Dahmani, 2010)**. This compound has been reported to have antibacterial, emmenagogue, insecticide and larvicide properties (**Zouari *et al*., 2010).**

The oil was dominated by ketones (61.6%), followed by alcohols (15.0%). Mono and sesquiterpenic hydrocarbons represented only 8.9% and 4.8% of total oil, respectively.

The oil herein studied is characterized by the chrysanthenone/camphor/α-thujone chemotype, while different Algerian chemotypes, such as camphor (region of Boussâada and Djelfa), camphor/1,8-cineole (Medjedel region), camphor/α-thujone/1,8-cineole/chrysanthenone (Djelfa region), camphor/α-thujone and camphor/trans-pinocarveol/chrysanthenone/α-thujone and 1,8-cineole (regions of Batna and M'sila), α-thujone/β-thujone/1,8-cineole, α-thujone/chrysanthenone (Draâ Ech Chih), chrysanthenone/camphor/α-thujone (Bordj Bou Arreridj) and davanone (region of Bordj Ghedir and Djelfa) have already been reported **(Dahmani-Hamzaoui *et al*., (2010, 2018); Dahmani, 2010)**. Previously, **Salido *et al*., (2004)** reported that most species of Spanish *A. herba-alba* oils are characterized by 1,8-cineole and derivatives of bornane skeletons (camphor, borneol, camphene, etc.)

In general, this variation of the chemical composition of *A. herba-alba* can be attributed to the techniques used for extraction and exogenous factors: the sunshine and the nature and the composition of the soil.

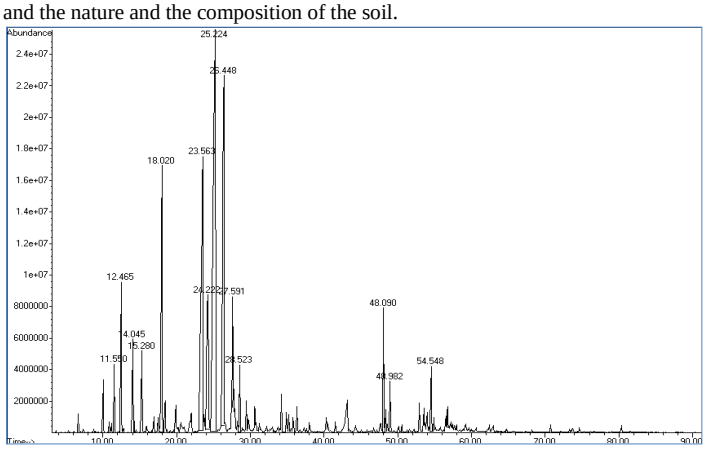

Figure 1 Chromatogram of *A. herba Alba* essential oil

Table 1 Composition (%) of *A. herba-alba* oil isolated by hydrodistillation

N°	Compound	RI_1	RI_2	%	Identification
1	Santalinatriene [a,i,j,l]	908	1028	0.7 ± 0.03	MS,RI, Std
2	Tricyclene [a,g,h,i,j]	921	1007	0.2± 0.01	MS,RI
3	α-Thujene[a,d,e,g]	926	1027	0.1± 0.01	MS,RI
4	α-Pinene [a,b,c,f,g,h,i,j,k]	933	1022	1.0± 0.05	MS,RI, Std
5	Camphene [c,d,e,f,g,h,i,j]	947	1061	2.4± 0.11	MS,RI, Std
6	Thuja-2,4(10)-diene [g,j]	948	1120	tr	MS,RI
7	1-Octen-3-ol[h,i]	971	1431	tr	MS,RI, Std
8	Sabinene[a,b,d,h,i,j,k]	971	1116	1.5± 0.08	MS,RI, Std
9	β-Pinene[a,b,c,d,e,f]	973	1100	0.3± 0.01	MS,RI, Std
10	1,3,5-Trimethylbenzene[a,h]	986	1257	tr	MS,RI
11	Myrcene [a,b,g,h,k]	992	1160	1.5± 0.08	MS,RI, Std
12	α-Phellandrene [d,f,g,h]	1001	1163	0.1± 0.01	MS,RI, Std
13	α-Terpinene [a,c,d,h,i,j,k]	1015	1179	0.4± 0.01	MS,RI, Std
14	1,2,3-Trimethylbenzene[a,h]	1015	1287	tr	MS,RI
15	β-Phellandrene [a,g]	1023	1207	tr	MS,RI, Std
16	p-Cymene [d,e,f,g,h,i,j,k]	1024	1256	0.3± 0.01	MS,RI, Std
17	1,8-Cineole[a,b,c,d,e,f,g,h,i,j,k]	1031	1204	5.6± 0.13	MS,RI, Std
18	Santalina alcohol[a,e,f,g,i]	1037	-	0.5± 0.02	MS,RI
19	β-Oimene[d,f,h,g]	1041	1214	tr	MS,RI, Std
20	γ-Terpinene[a,c,d,e,f,g,h,i,j,k]	1058	1238	0.5± 0.02	MS,RI, Std
21	cis-Sabinene hydrate[e,i,j]	1068	1469	0.4± 0.01	MS,RI
22	Artemisia alcohol[a,e,f,g,i]	1085	1481	0.8± 0.03	MS,RI
23	Terpinolene[a,c,d,f,g,h]	1087	1277	tr	MS,RI
24	trans-sabinene hydrate[a,c,g]	1095	1450	tr	MS,RI
25	Filifolone[a,h]	1103	1423	0.1± 0.01	MS,RI
26	α-Thujone[a,b,e,f,g,h,i,j,k]	1106	1409	12.8± 0.16	MS,RI, Std
27	β-Thujone[a,b,e,f,g,h,i,j,k]	1118	1422	4.8± 0.10	MS,RI
28	Chrysanthenone[a,c,d,e,f,g,h,i,j,k]	1130	1505	24.1± 0.45	MS,RI
29	trans-Pinocarveol[a,b,d,e,f,g,h]	1134	1622	0.6± 0.03	MS,RI, Std
30	Camphor[a,b,c,d,e,f,g,h,i,j,k]	1148	1514	16.2± 0.24	MS,RI, Std
31	trans-Verbenol	1149	1648	0.2± 0.01	MS,RI
32	Isothujanol[b,h]	1155		0.1± 0.01	MS,RI

33	Sabina ketone[g,,i,j]	1156	-	0.1± 0.01	MS,RI
34	Pinocarvone[a,e,g,h,i,j,k]	1165	1570	0.5± 0.02	MS,RI, Std
35	Borneol[a,b,c,d,e,f,g,h,i,k]	1169	1675	0.8± 0.02	MS,RI, Std
36	Terpinen 4-ol[a,c,d,e,f,g,h,i,j,k]	1175	1589	0.6± 0.02	MS,RI, Std
37	Santalinyl acetate[a,e]	1176	-	0.1± 0.01	MS,RI
38	Thuj-3-en-10-al[g]	1185	1566	0.1± 0.01	MS,RI
39	p-Cymen-8-ol[a,c,d,e,g,i]	1187	1830	0.6± 0.02	MS,RI
40	α-Terpineol [a,b,c,d,e,g,h,i,k]	1191	1672	0.3± 0.01	MS,RI, Std
41	Myrtenol[a,c,e,f,g,h]	1199	1774	0.5± 0.02	MS,RI, Std
42	trans-Piperitol[a,b,c,d,e,f,g,h]	1208	1740	0.7± 0.03	MS,RI
43	Verbenone[a,b,f,g,h,i,j,k]	1209	1683	0.1± 0.02	MS,RI
44	trans-Carveol[a,f,g,h]	1218	1820	0.1± 0.01	MS,RI
45	cis-Carveol[a,g,h,j]	1229	1848	0.1± 0.01	MS,RI
46	Cumin aldehyde[a,c,d,g,h]	1243	1770	0.1± 0.01	MS,RI, Std
47	trans-Ocimenone	1244	1813	tr	MS,RI
48	Carvone[a,f,g,h,i,j]	1244	1721	0.1± 0.01	MS,RI, Std
49	Carvotanacetone[a,g]	1255	1713	0.2± 0.01	MS,RI
50	Piperitone[a,b,c,f,g,h]	1255	1710	0.1± 0.01	MS,RI, Std
51	cis-Chrysanthenyl acetate[a,d,e,g,h,i,j,k]	1262	1605	0.9± 0.04	MS,RI
52	Isobornyl acétate	1286	1572	0.3± 0.01	MS,RI
53	Bornyl acetate[a,b,c,d,e,g,h,i,j]	1294		0.5± 0.02	MS,RI, Std
54	Carvacrol[d,f,i]	1299	2150	0.1± 0.01	MS,RI, Std
55	Cis-Pinocarvyl acétate	1313		0.1± 0.01	MS,RI
56	1,6-Dimethylhepta-1,3,5-triene	1321		0.2± 0.01	MS,RI
57	Dihydro Carveol Acetate	1356		0.4± 0.01	MS,RI
58	Eugenol[d,g,h,k]	1359	2155	0.2± 0.01	MS,RI
59	α-Copaene[a,d,e,f,g,h,i]	1375	1476	0.2± 0.01	MS,RI
60	cis-Jasmone[g,i]	1398	1932	1.5± 0.07	MS,RI
61	Methyl eugenol[a,g,h]	1399	2000	0.2± 0.01	MS,RI
62	β-Caryophyllene[a,c,d,f,h,k]	1422	1594	0.2± 0.01	MS,RI, Std
63	Aromadendrene[g]	1442	1615	0.2± 0.01	MS,RI
64	(E)-Ethyl cinnamate[f,g]	1471		0.2± 0.01	MS,RI, Std
65	β-Chamigrene[g]	1477		tr	MS,RI
66	γ-Murolene[a,d,g,h,k]	1480	1691	2.3± 0.09	MS,RI
67	Bicyclogermacrene[g]	1500	1718	0.2± 0.01	MS,RI
68	α- Muurolene[a,f,g,h]	1503	1706	tr	MS,RI, Std
69	Cubebol[g]	1516	1926	0.1± 0.01	MS,RI
70	δ-Cadinene[a,c,d,f,h,k]	1523	1742	0.1± 0.01	MS,RI
71	Cadina-1,4-diene	1533	2056	0.9± 0.06	MS,RI
72	Ledol[a,j]	1563	2020	tr	MS,RI
73	trans-Nerolidol[d,f,g,i]	1564	2031	tr	MS,RI
74	Palustrol	1566		0.5± 0.02	MS,RI
75	Spathulenol[b,d,f,g,h,i,k]	1578	2115	0.2± 0.01	MS,RI, Std
76	Caryophyllene oxide[d,f,g,h,i,k]	1583	1975	0.4± 0.01	MS,RI, Std
77	β-Copaen 4-α ol[g]	1588	2140	0.6± 0.01	MS,RI
78	Viridiflorol[f,g]	1593	2075	1.2± 0.08	MS,RI
79	Cedrol	1600		0.2± 0.01	MS,RI
Monoterpene hydrocarbons			8.9		
Sesquiterpene hydrocarbons			4.7		
Alcohols			15.0		
Ketones			61.6		
Ether oxides			0.5		
Esters			2.4		
Others			0.2		
Identified components (%)			93.3		

Components listed in order of their elution from the HP 5MS column; tr = trace (amount< 0.05%); $RI_1$and RI_2: Linear retention indices relative to HP5-MS and HP-Wax capillary columns, respectively; Values are the averages of three measurements ± SD; RI, comparison of retentions indices with those of published data; MS, comparison of the mass spectra of peaks with MS libraries and published data; Std, comparison of RI and mass spectra with that of with authentic compound (standard).
a : (**Benjilali., 1980, 1981**); b : (**Boutekdjiret, 1992**); c : (**Feuerstein, 1988**); d : (**Salido., 2001, 2004**); e : (**Feuerstein., 1986; Segal., 1987**); f : (**Dob, 2006**), g : (**Dahmani-Hamzaoui.,2010, 2015, 2018**); h: (**Vernin., 1994, 1995, 2001**); i: (**Haouari, 2009**); j: (**Paolini, 2010**); k: (**Bezza, 2010**).

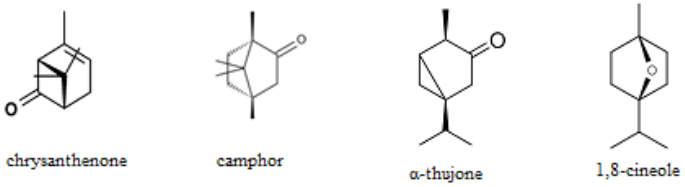

Figure 2 Major compounds of *A. herba-alba* essential oil (Bousâada region)

Antimicrobial activity

The MICs of essential oil from *A. herba-alba* against fourteen species of microorganisms by the agar dilution method are summarized in **Table 2**. It shows a good inhibitory potency against all tested microorganisms (MIC not exceeding 2 µg/mL) except for *Aspergillus ochraceus* (MIC= 5 µg/mL). The data obtained from the agar dilution method using *A. herba-alba* essential oil indicated that *Bacillus coagulans*, *Staphylococcus aureus*, *Agrobacterium tumefaciens*, *Fusarium oxysporum*, *Penicillium expansum* and *Pseudomonas aeruginosa* were the most sensitive microorganisms tested (MIC< 0.5 µg/mL).

Globally, the studied oil exhibited a wide antimicrobial spectrum and exerted strong microbial growth-inhibiting properties against all microorganisms. The essential oil antimicrobial activity could be ascribed to its major constituents as chrysanthenone, α-thujone and camphor (**Mahmoud *et al*., 1988)**. However, according to some authors, components present at lower concentrations might be engaged in some type of synergy with the other compounds (**Oussalah *et al*., 2007; Vagionas *et al*., 2007)**. The biological activity of any essential oil is intimately linked to its chemical composition, the functional groups of the major components and the possible synergistic effects of these constituents, it is likely that minority compounds act in synergy; in this way, the value of the essential oil is due to all of these components (**Andreani *et al*., 2016**; **Salhi *et al*., 2017)**.

Table 2 MICs (µg/mL) of *Artemisia herba-alba* essential oil

Microorganism	Minimal inhibitory concentration (µg/mL)
Bacteria	
Bacillus subtilis ATCC6663	1
Bacillus coagulans CIP6625	<0.5
Micrococcus luteus ATCC9314	2
Staphylococcus aureus CIP7625	<0.5
Agrobacterium tumefaciens N°2410	<0.5
Escherichia coli CIP54.8	0.5
Pseudomonas aeruginosa CIPA22	<0.5
Fungi	
Mucor ramannianus NRRL1829	2
Aspergillus ochraceus	5
Fusariumox ysporum f.sp. *albedinis* CURZA	<0.5
Penicillium expansum 8932	<0.5
Fusarium oxysporum f. sp. *lini* CINRA	1
Yeast	
Candida albicans CLM	2-5
Saccharomyces cerevisiae ATCC4226	0.5

ATCC: American Type Culture Collection; CURZA: collection de l'Unité de Recherche sur les Zones Arides (Alger) ; CIP: collection de l'Institut Pasteur de Paris, France; CINRA: collection of the National Institute of Agronomic Research of Dijon, France; NRRL: Northern Regional Center, Peoria (U.S.A) ; CLM: collection of the Microbiology Laboratory of ENS Kouba, Algiers

Scavenging of radical DPPH•

The action of antioxidants on the scavenging of DPPH radicals comes from their ability to donate hydrogen. DPPH is a stable free radical which can accept hydrogen radical or an electron to become a stable diamagnetic molecule. During the interaction of an antioxidant with DPPH, there will be an electron or atom transfer to DPPH what leads to the neutralization of its free radical character and convert it to 1-1 diphenyl-2-picryl hydrazine which will result in a decrease of the degree of coloration which indicates the activity of scavenging the antioxidant. In this study the essential oil had significant scavenging effect on the DPPH radical which was dose dependent of the concentration (Table 3).

Table 3 DPPH• radical scavenging activity (%) and IC_{50} (µg/mL) of *A. herba alba* essential oil

Sample	Concentration (µg/mL)					IC_{50}
	300	400	500	700	1000	
	Radical scavenging [a]± 0.05					
HE	37.20	38.84	41.46	44.15	46.34	NA
BHA						41.38
BHT						24.91

[a] Values are means of three independent replicates with RSD is less than 1%; NA : Value not available in the gamut of the experimented concentrations..

Thus, there was an increase of the percent of radical scavenging effect of essential oil on DPPH with the increase of the concentrations of the oil from 300-1000 µg/mL. The inhibition rate of the DPPH radical ranged from 37.20% (for 300 µg / mL) to 46.34% (for 1000 µg / mL).
However, the oil could not reach the IC_{50} in the range of concentrations studied (300-1000 µg/L). For all concentrations, the essential oil showed lower percentage of inhibition of DPPH than the standards BHT and BHA). The relative low DPPH radical scavenging activity of this oil compared to those of BHT and BHA could be attributed to the absence of phenolic components such as thymol, carvacrol, eugenol, which play an important role in the antioxidant activity of an oil **(Hazzit *et al.*, 2009; Hadjadj *et al.*, 2020)**.

Electrochemical measurements

Open circuit potential (OCP)

Fig 3 displays the temporal evolution of OCP with and without the presence of the essential oil. There is no obvious influence of the essential oil on the open-circuit potential even if a very slight evolution can be observed to more negative values. However, on these chronopotentiograms, fluctuations in potential are observed which originate from an activating action of the material, probably because of the presence of Cl^-.

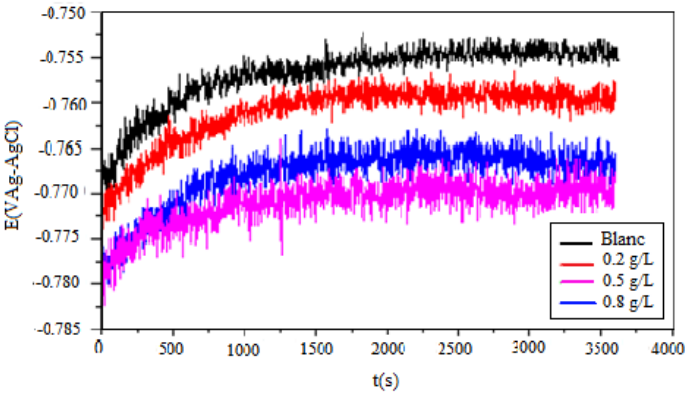

Figure 3OCP-time curves of Al in HCl (1 mol./L) solution with and without essential oil

Potentiodynamic curves

The curves of Potentiodynamic polarization for aluminum in HCl solution (1 mol./L) at 303 ° K, without and with various concentrations of *A. herba-alba* oil are shown in Fig 4. As can be seen, in the absence of oil from *A. herba-alba*, there was no passive film formation during the anodic polarization because there is no plateau on the anode curves. Indeed, according to the Pourbaix diagram, aluminum is passive for pH ranging from 4 to 8.5. Beyond these limits, aluminum undergoes corrosion in aqueous solutions because its oxides are soluble, especially in hydrochloric medium where the formation of Al^{3+} ions in the former and AlO_2^- ions in the latter occurs (**Branzoi *et al.*, 2003)**. There is no difference in the anodic portion of the polarization curve with the addition of *A. herba-alba* oil. The shape of the potential-intensity curves seems to indicate that the Tafel relationship is followed, showing that both anodic and cathodic reactions are activation-controlled.

If we consider the cathode branches of the tafel plots, the aluminum corrosion potential moved 2–18 mV cathodically in comparison to the control (blank) and also a modification in cathodic Tafel slopes were noted. It is well admitted that an inhibitor is classified as anodic or cathodic type if the shift in potential corrosion is upper than 85 mV with respect to potential of corrosion of the control. (**Yan*et al.*, 2008)**. This indicates that *A. herba-alba* oil acts like mixed-type inhibitor with predominating cathodic efficiency. The cathodic Tafel plot (**Fig 4**) provided parallel lines denoting that the addition of *A. herba-alba* oil to the $HCl(1\ mol.L^{-1})$ solution did not change the mechanism of hydrogen evolution and the reduction of H+ ions at the aluminum surface occur principally through a charge transfer mechanism. The extract molecules were firstly adsorbed on the metal surface and blocked the reaction sites of the aluminum surface. Thus, the surface area disposable for H^+ ions has been reduced, justifying the reduction of the cathodic current while the reaction mechanism itself remains unchanged (**Solmaz *et al.*, 2008)**. A larger coverage of the surface by the extract was got in solutions with the higher concentrations.

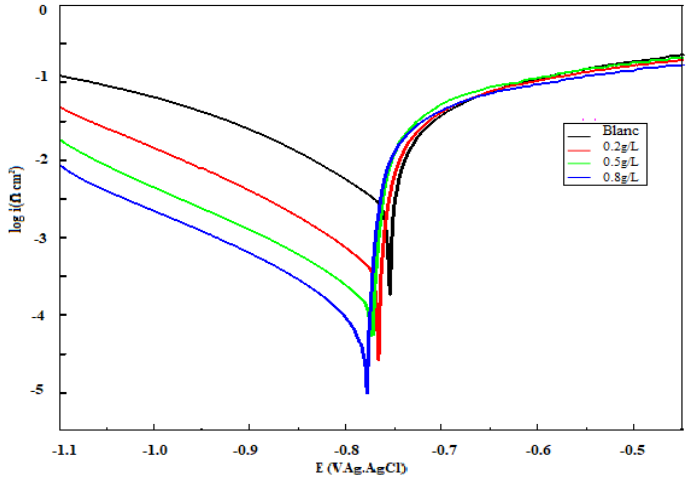

Figure 4 Polarization curves for aluminum in HCl (1mol./L) solution with and without *A.herba-alba oil, v=1mV/s, T= 303°K*

Cathodic Tafel slope bc, increase slightly upon addition of A. herba Alba oil, which signify that cathodic sites are blocked by the adsorption of the molecules resulting in an inhibition of the cathodic reduction reactions. Cathodic Tafel lines were extrapolated to the corrosion potential so as to establish polarization settings got for aluminum corrosion in $HCl\ (1\ mol.L^{-1})$ solution without and in the presence of diverse concentrations of *A. herba-alba oil*. Alike fitting method had been

previously used for aluminum in hydrogen chloride solution (**Şafak *et al*.,2012)**. Corrosion potentials (E_{corr}), corrosion current densities (Icorr), cathodic Tafel slope values (bc) deducted from the polarization curves, matching inhibition efficacies (η'(%)) and surface coverage degrees (θ) are presented in **Table 4**.
The displacement of corrosion potentials in the cathodic meaning and diminution of corrosion currents with the augmentation of inhibitor concentration is a clue that the molecules of the cathode site are adsorbed on the surface of the aluminum **(Bereket *et al*., 2004)**. By addition of *A. herba-alba* oil to the HCl solution, an upturn in corrosion conduct is evident. This could be chiefly ascribed to the physical adsorption process of active molecules, which besides form a protective layer on aluminum surface.

Table 4 Potentiodynamic polarization settings recorded for aluminium in HCl solution 1M with and without *A. herba-alba* oil at 303°K.

C_{inh} (g/L)	E_{corr} (mV/AgAgCl)	$-b_c$ (mV/dec)	I_{corr} (mA.cm^{-2})	θ	η'(%)
0	-755	155.0	3.20	-	-
0.2	-770	183.9	0.80	0.75	75.0
0.5	-774	190.0	0.25	0.92	92.1
0.8	-780	198.0	0.16	0.95	95.0

Electrochemical impedance spectroscopy (EIS)

The EIS spectra of an aluminum electrode, in a molar solution of hydrochloric acid in the absence and presence of different *A. herba alba* oil concentrations, plotted at OCP, after 1 hour of stabilizing in solution ventilated at 303°K, are shown in **Fig 5**. The Nyquist plots display ulike form composed of depressed capacitive loop in the high frequency (HF) region followed by an inductive loop in the low frequency (LF) region. The HF capacitive loop is assigned to the charge transfer process of the electric double layer at the electrode/electrolyte interface. As can be seen, lower diameters of capacitive and inductive loops are observed in blank solution, which suggest higher charge transfer and higher deterioration of the protective film of corrosion products. The LF inductive loop translated an adsorption and pitting corrosion phenomenon owing to the presence of chloride ions when the electrode undergo active dissolution followed by the destruction of the passive film (**Metikoš-Huković et al., 1994)**. Moreover, with addition of *A. herba-alba* oil in acidic solution, remarkable increase in impedance magnitude is noticed. This indicates that with the rise of the inhibitor concentration, charge transfer process and pit propagation mechanism are impeded.

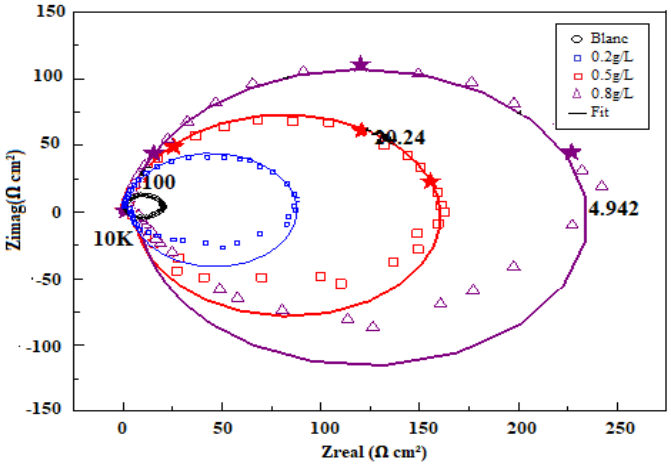

Figure 5 Nyquist plot for Aluminum in HCl (1mol./L) solution with and without *A. herba-alba* oil after 1h at OCP T= 303 K

The equivalent circuit model (ECM) shown in **Fig 6**, was proposed in order to permit a precise analysis of the diagrams of impedance. The electrical elements corresponding to the dynamic processes can be defined as follows:
Rs is the resistance of the solution, CPE represent the constant phase element and RP is the polarization resistance. Inductive elements (R_{ind} and L) are quite common for aluminum impedance behavior in acidic solution (**Metikoš-Huković et al., 1994)**. The sum of RP and R_{ind} represents the charge transfer resistance R_{ct}. CPE was used in lieu of an ideal capacitor, since capacitive loops are not perfect circles, and this can be correlated to the frequency dispersion as a result of the in homogeneity and ruggedness of electrode surface **(Khaled *et al*., 2009)**.

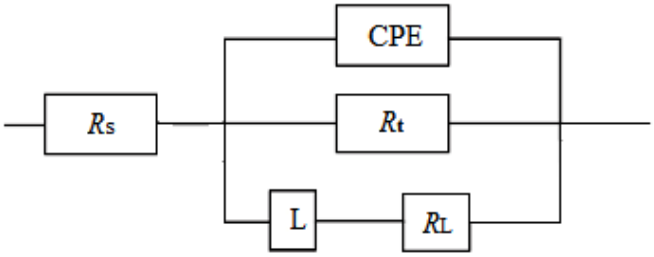

Figure 6 Equivalent circuit used to adjust impedance data

The impedance settings deduced from Nyquist plots obtained for aluminum in hydrochloric acid (1 mol./L) and the impedance parameters and inhibition efficacies ηeis (%) for the addition of essential oil in HCl solution, are presented in **Table 5**

Table 5 Characteristic settings deducted from Nyquist plot

C (g/L)	Rs (Ω cm²)	Rp (Ω cm²)	f max (Hz)	CPE (µF/cm²)	n	R_{ind} (Ω cm²)	L (H cm²)	R_{ct} (Ω cm²)	η(%)
0	1.20	1.94	602.10	62.1	0.9609	2.25	7.68	14.25	-
0.2	1.18	2.86	147.70	53.3	0.9435	3.00	17.68	62.00	77.0
0.5	1.50	5.79	92.49	24.8	0.9366	6.00	36.31	165.00	91.4
0.8	1.25	8.68	57.90	6.3	0.9299	8.00	53.98	247.00	94.3

The results reveal that R_{ct} values rise significantly, whereas CPE decreases with the augmentation of the concentration of *A. herba-alba* oil. This diminution of CPE values compared to that in the solution of HCl not containing essential oil can arise from an increase in the thickness of the electrical double layer and suggests that molecules present in *A. herba alba* oil are obstructing the metal surface by their adsorption at the interface of the metal/solution (**Singh et al., 2011)**.The highest inhibitor efficiency (94.3%) has been recorded in the presence of 0.8 g/ L of *A. herba-alba* oil. This result is close with that obtained from potentiodynamic polarization method. A similar study reported by **Hechiche et al., (2019)** showed an inhibitory efficacy more or less low (92% for a concentration of 3 g/l) in comparison to our results.This may be due to the difference in chemical composition of the essential oil of *A. herba alba*, knowing that the major compound reported in this study is camphor 26.2% and chrysanthenone 12.4% while our study presents chrysanthenone24.1% and camphor 16.2% as the major compounds.

Gravimetric measurement

The effect of *Artemisia herba-alba* oil concentration on the corrosion of Al in 1 mol/L HCl solution was examined by weight loss measurements at 303 K after an immersion of 1 h. **Table 6** exhibits the calculated values of corrosion rates, surface coverage and inhibition effectiveness in the absence and presence of various concentrations of Artemisia oil. As can be seen, corrosion rate decreases with the increase concentration of *A. herba-alba* oil. This decrease of "v" values in comparison with that in HCl solution without essential oil can result mainly from the formation of a protective film originated by the strong adsorption of the inhibitor molecules at the metal/solution interface (**Metikoš-Huković et al., 1994).** The inhibitory effect of *Artemisia herba-alba* essential oil is superior at higher concentration; the maximum of 94.3 % is attained at 0.8 g/L. These results agree well with those recorded from potentiodynamic polarization method.

Table 6 Weight loss measurements after 1h at 303°K

C (g/L)	Δm (g)	v (mg.cm^{-2}.h^{-1})	η'' (%)	Θ
0	0.157	14.0	-	-
0.2	0.040	3.50	`75.0	0.75
0.5	0.017	1.53	89.1	0.89
0.8	0.010	0.81	94.3	0.94

CONCLUSIONS

The analysis of essential oil isolated from *A. herba-alba* plant shows that its composition is dominated by Ketones (61.1%). The major constituents are chrysanthenone (24.1 %), camphor (16.2%) and α-thujone (12.8%). The essential oil showed a good activity against all microorganisms tested (MIC not exceeding 2 µg/mL) except for *Aspergillus ochraceus* where the MIC is 5 µg/mL. *Bacillus coagulans*, *Staphylococcus aureus*, *Agrobacterium tumefaciens*, *Fusarium oxysporum*, *Penicillium expansum* and *Pseudomonas aeruginosa* were the most sensitive microorganisms tested (MIC 0.5 µg/mL). The essential oil was found to be less active by inhibiting free radicals compared to the synthetic antioxidants (BHT and BHA). Maximum value of inhibition efficiency (94.3 %) was reached at 0.8 g/L. SEM studies revealed improvements in Al surface in presence of *A. herba-alba* essential oil which confirms the formation of an adsorbed and protective barrier of *A. herba-alba* essential oil molecules. The polarization studies showed that *A. herba-alba* essential oil acts as mixed-type inhibitor without changing the hydrogen evolution mechanism. From EIS measurement it is clear that the charge tra a perfect correlation between the magnitude of the inductive loop and the propagation of pitting corrosion. Good agreement was obtained between the different methods employed in this study and showed that *A herba-alba* can be a good candidate for aluminium corrosion protection in hydrochloric acid medium.A transfer resistance increase with the inhibitor concentration and charge transfer process and pit propagation mechanism are impeded. SEM analysis allows

According to these findings, this essential oil may be suggested as new green inhibitor agent against corrosion in hydrochloric acid media and the possible use of the essential oil on food systems as an effective inhibitor of foodborne pathogens, and for potential pharmaceutical applications. However, further research is needed in order to determine the toxicity, antibacterial, and antioxidant effects in edible products.

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