Chemical Characterization and Antioxidant Activity of *Cedrus atlantica Manetti* Tar (Atlas Cedar Tar)

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Cedrus atlantica Manetti Tar was analyzed by gas chromatography coupled to mass spectrometry, it was subjected to analyzes to know their total polyphenolic and condensed tannins contents, also their Ferric-reducing antioxidant power and Total antioxidant capacity. Chemical characterization identified 88 constituents where Himachalene and α-atlantone isomers (14.51 % - 4.07 %), Calacorene (3.52 %) and ar-Turmerone 3.35 %, were the major components, the total polyphenolic content and condensed tannins contents were 57.15 ± 0.15 milligrams equivalent of gallic acid /g tar and 4.41 ± 0.05 milligrams equivalent of catechin /g tar respectively .This extract showed remarkable Ferric-reducing antioxidant power with effective concentration equal to 50 ± 0.075 mg /mL ± 0,00028 and total antioxidant capacity equal to 262.75 mg equivalents of ascorbic acid /g tar ± 14,43. The experimental results indicated that our tar has promotive antioxidant activity.

Introduction

The Cedrus genus gather cedars belonging to the "conifers" family. It contains four species; Lebanon cedar (*Cedrus libani*), Himalayas cedar (*Cedrus deodora*), Cyprus cedar (*Cedrus brevifolia*) and finally Atlas cedar (*Cedrus atlantica*) [1]. The Atlas cedar cover 29,000 hectares of Algerian forests, it is highly wanted for its numerous uses (sawing, coal, tar, resin, tannin, etc.) [2].

The essences of *Cedrus atlantica Manetti* have been always considered as a noble and precious substances [3]

Cedar is generally resistant to insects and pathogens because of various chemical constituents that have been used for many purposes [4].

Cedar wood has been traditionally used for both animal and human diseases treatments in traditional medicine from past to present [5].

Tar is produced by dry distillation (pyrolysis) of wood using different traditional techniques such: canister method, *per descensum* method (combustion furnace technique) [6, 4], Cedrus tar is widely used veterinary as an antiseptic as well as in some human health care applications such: Ulcers, dandruff, eczema [7, 8].

Despite all these traditional uses, the extract was not properly evaluated (few studies have been devoted to these products [4,5,7,9]) especially the *Cedrus atlantica Manetti* tar whose researches focuses mainly on essential oils of wood and oil tar of this specie [10,11, 12, 13, 3, 14,15].

Based on these data, we realized a study of that essence in order to recognize it chemical components and to determine its antioxidant activity using two different methods after quantifying the polyphenolic and tannins contents.

Experimental part

Sample

The Atlas cedar tar (Tar of *Cedrus atlantica Manetti*) samples were traditionally produced by pyrolysis (dry distillation of *Cedrus atlantica Manetti* wood by canister method [4]) in Setif village's, «Algeria », then marketed to different herbalists where they were bought from.

Chromatographic GC-MS analysis

The GC-MS instrumentation consisted of a Hewlett Packard Agilent 6890 gas chromatograph coupled to a quadrupole MS system (model Hewlett Packard Agilent 5973 mass spectrometer). Atlas cedar tar was diluted in methanol and analysed using the following conditions: HP-5MS capillary column (5 % Phenyl 95 % dimethylpolysiloxane, 30 m×0.25 mm i.d., 0.25 μm film thickness); carrier gas, Helium (purity: N 6.0); flow rate, 0.5 mL/min; injection mode, splitless; solvent delay, 3 min ; injection volume, 0.2 μL; injection temperature, 300°C; oven temperature programmed from 50°C to 250°C at 5°C/ minute (min) heating rate and held at 250°C for 15 min; ionization mode, electronic impact (EI) at 70 eV. Identification of the components was with their relative's retention indices on HP5MS column. Then determined with reference to homologous series of C7 - C30 (Product SULPECO ref 49451-U) and by a comparison of their mass spectral fragmentation with the data bank library (NIST/Nist11/EPA/NIH MS) and with the literature [16].

Total condensed tannins (CTC)

The analysis of condensed tannins was carried out for Atlas cedar tar sample according to the method of [17]. 3 mL of the vanillin solution (4% in methanol) and 1.5 mL of concentrated HCl are added to 25 μL of diluted Atlas cedar tar. The absorption is recorded at 500 nm after 15 min of reaction, a calibration curve is carried out in parallel under the same operating conditions using catechin. The results are expressed in equivalent milligrams of catechin per gram of the weight of the sample (mg CE/g S). All the required measures were determined in three replications.

Total polyphenolic content (TPC)

The total polyphenol content of Atlas cedar tar was determined according to [18] using the folin – ciocalteu reagent. 200 µL of diluted tar was added to a mixture (1 mL of folin ciocalteu diluted 10 times and 0.8 mL of sodium carbonate at 7.5 %). The tubes are shaken and stored for 30 min. The absorbance is measured at 765 nm using the UV / Vis spectrophotometer. Gallic acid was used as a standard and total polyphenol content was expressed as milligrams equivalent of gallic acid per gram of the weight of the sample (mg GAE / g S). the test was done in triplicate.

In vitro antioxidant activities

Antioxidant activity of *Cedrus atlantica .M* tar was determined according to two different methods, which were Ferric-Reducing antioxidant Power (FRAP) and total antioxidant capacity (TAC).

1.Total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity (TAC) of our tar was evaluated according to the phosphomolybdenum method described by [19] withe littles modifications .300 µL of Atlas cedar tar sample at various concentrations were mixed with 3 mL phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate V/V/V). All tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. The mixtures were then allowed to reach room temperature. The absorbance of the solutions is measured at 695 nm against the blank which contains 3 mL of the reagent solution and 0.3 mL of methanol and it is incubated under the same conditions as the sample. As a positive control, ascorbic acid was chosen to be employed. The total antioxidant activity was presented as "mg of ascorbic acid equivalent per g of sample" mg AAE / g S). Standard graph of ascorbic acid was employed in order to calculate the equivalents of ascorbic acid (AA). The experiment was conducted in triplicate.

2.Ferric-reducing antioxidant power (FRAP)

The FRAP assay was performed followed by the method previously by [20]. Briefly,1 mL of Atlas cedar tar sample diluted was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (III) ($K_3Fe(CN)_6$) solution (1 %). The mixtures are incubated at 50 ° C for 20 min. After that 2.5 mL (10 %) trichloroacetic acid (TCA) was added and the mixture was centrifuged at 3000 rev / min for 10 min. At the end, the upper layer 2.5 mL of each concentration was mixed with 2.5 mL of distilled water and 0.5 mL of 0,1 % iron (III) chloride anhydrous ($FeCl_3$) then the absorbance was recorded at 700 nm. Ascorbic acid was used as a positive control. A higher absorbance indicates a higher reducing power. the test is carried out in tripli-

cate. Result is expressed as Effective Concentration of tar at which the absorbance was 0.5 (EC_{50} in (mg/mL) obtained from linear regression analysis [21].

Statistical analysis

The results of measurements of total condensed tannins, total polyphenolic components, and antioxidants activities are expressed as mean values ± standard deviation (SD).

Results and discussion

Chemical composition of *Cedrus atlantica Manetti* tar

76 % of the Atlas cedar tar is identified with 88 components; himachalene and α -atlantone isomers, α-Calacorene, occidentalol, (z) nuciferol and ar-Turmerone, are the majority components accounting about 59 % of the tar (**Table 1, Figure 1**).

Table 1: Constituents of *Cedrus atlantica Manetti* tar

Components	tR (min)	KI	%
heptane	3.23	700	0.05
2,5-dimethylfurane	3.33	708	0.03
methylcyclohexene	3.89	751	0.04
toluene	4.09	767	0.10
butanoic acid	4.30	783	0.04
3-methylbut-2-enal	4.43	793	0.01
mesityl oxide	4.54	801	0.10
furfural	5.18	833	0.34
4-hydroxy-4-methylpentan-2-one	5.32	840	0.04
furfuryl alcohol	5.58	853	0.05
(z)-salvene	5.71	859	0.02
p-xylene	5.94	871	0.05
3-methylbut-2-enoic acid	6.35	891	0.02
o-xylene	6.48	898	0.01
2-methyl-2-cyclopenten-1-one	6.76	909	0.06
acetylfuran	6.86	913	0.04
(2)-hexenyl formate	7.24	928	0.04
5-methyIfurfural	8.20	965	0.41
methyl 2-furoate	8.50	977	0.01
phenol	8.65	983	0.22
1,3,5-Trimethylbenzene	9.02	997	0.04

propenylbenzene	9.15	1002	0.08
o-Cymene	9.78	1025	0.02
p-Cymene	9.87	1028	0.15
3-methylcyclopentane-1,2-dione	9.97	1031	0.29
3-methyl-cyclohex-2-en-1-one	10.28	1042	0.08
o-cresol	10.70	1057	0.25
3,5-octadien-2-one	10.95	1066	0.02
p-cresol	11.31	1079	0.48
o-guaiacol	11.75	1095	1.06
2,6-dimethylphenol	12.22	1111	0.13
maltol	12.40	1118	0.10
ethyl-2-hydroxy-2-cyclopenten-1-one	12.58	1124	0.05
4-acetyl-1-methylcyclohexene	12.93	1136	0.48
p-ethylphenol	13.08	1142	0.04
2,4-dimethylphenol	13.39	1152	0.37
2-phenyl-1,3-butadiene	13.64	1161	0.03
3,4-dimethylphenol	13.98	1173	0.26
2-methoxy-p-cresol	14.26	1183	0.17
p-methylacetophenone	14.48	1191	0.22
5-methylguaiacol	14.72	1199	1.83
catechol	14.89	1205	0.45
2,3-dimethylbenzofuran	15.11	1213	0.07
4,7-dimethylbenzofuran	15.34	1222	0.08
2,4,6-trimethylstyrene	15.49	1227	0.04
2-ethyl-6-methylphenol	15.62	1232	0.13
5-ethyl-2-methylphenol	15.91	1242	0.12
dihydrochavicol	16.45	1262	0.12
3-methylcatechol	16.63	1268	0.38
p-ethylguaiacol	17.10	1286	1.32
4-methylcatechol	17.47	1299	0.54
p-vinylguaiacol	18.02	1320	0.29
silphiperfol-4,7(14)-diene	19.02	1358	0.10

eugenol	19.18	1364	0.54
dihydro eugenol	19.45	1374	0.58
ß-cubebene	19.80	1388	0.74
4-ethylcatechol	19.90	1392	0.53
isovanillin	20.34	1409	0.34
longifolene	20.60	1419	0.88
2,3-dimethylnaphthalene	20.97	1434	1.03
6,9-guaiadiene	21.20	1443	0.61
dihydrocurcumene	21.51	1455	1.55
(E)-isoeugenol	21.62	1460	0.77
α-himachalene	21.76	1465	**4.53**
γ-muurolene	21.96	1473	0.10
ar-curcumene	22.12	1480	0.45
trans-β-guaiene	22.25	1485	0.40
γ-himachalene	22.46	1493	**4.07**
himachala-1,4-diene <11α-H>	22.54	1496	0.85
ß-himachalene	23.11	1520	**14.51**
cuparene	23.19	1524	0.85
himachalene<γ dihydro-ar->	23.29	1528	1.36
cis-calamenene	23.48	1536	0.68
α-calacorene	23.68	1544	**3.53**
occidentalol	23.87	1552	**2.51**
ß-calacorene	23.98	1557	0.04
ar-dihydoTurmerone	25.08	1604	0.92
longiborneol	25.34	1615	1.12
α-cadinol	26.20	1653	0.33
ar-turmerone	26.75	1678	**3.35**
(E)-γ -atlantone	27.36	1705	1.35
(Z)-α -atlantone	27.57	1715	**4.96**
(z)-nuciferol	27.83	1727	**2.61**
(E)-2-hexyl-Cinnamaldehyde	28.18	1744	1.82
(E)-α -atlantone	29.18	1791	**5.15**

trans-isovalencenol	29.33	1798	0.24
ß-vetivone	30.05	1833	0.79
9,10-dimethyl-1,2,3,4,5,6,7,8-octahydroanthracene	30.43	1851	0.78
Others			23.7
Grand total			100

Legend: tR: Retention Time; **KI**: Kovàts Index.

The most abundant component was ß-himachalene (14.51 %) mentioned in other studies carried out on Lebanon cedar tar by both methods; traditional and artificial (Jenkner Retort method carried out on different parts of the tree) [7,4].

The abundance was considered in the Atlas cedar wood essential oils of different origins [12]. However, it was absent in the wood essential oil of the same plant [13]. This sesquiterpene and its derivatives are known for their insecticidal and antifungal activities [22].

The second major component was E)-α -atlantone (5.15 %) present with 3.74 % in Lebanon cedar tar of roots produced by Jenkner Retort [7], totally absent in the tar produced by the same technique from the branches and in the traditional Lebanon cedar tar [4]. while, too high percentages were observed in Atlas cedar wood essential oils 19.30 % [11,10].

The (Z)-α -atlantone (4.96 %) was present with 7.40 % in the traditional Lebanon cedar tar [4], but not detected in the study [7] of Lebanon cedar tar produced traditionally and by the Jenkner Retort method (for the different parts of the tree). Contrarily, considerable contents were mentioned in the Atlas cedar wood oil produced by hydrodistillation 4.02 % [13], 5.16 % [3,14]. These α-atlantone isomers are known for their insecticidal and antifungal [23, 24].

The fourth component was α-himachalene (4.53 %). Other studies carried out on Lebanon cedar tar produced traditionally or by Jenkner Retort (on roots and branches) all found high percentages of this component [7,4], as well as an increase percentage were noted in the Atlas cedar wood essential oil produced by hydrodistillation [14,3], and steam distillation [14], but this component was absent in the analysis of [13].

The last component of the himachalene family was γ-himachalene where 4.07 % was found. It was identified only in Atlas cedar wood essential oil [3] and Lebanon traditional tar [4]. Concerning α-calacorene (3.53 %), occidentalol (2.51 %) and (z)-nuciferol (2.61 %)**;** a total absence of these constituents was noted in all samples of Lebanon cedar tar produced traditionally or by

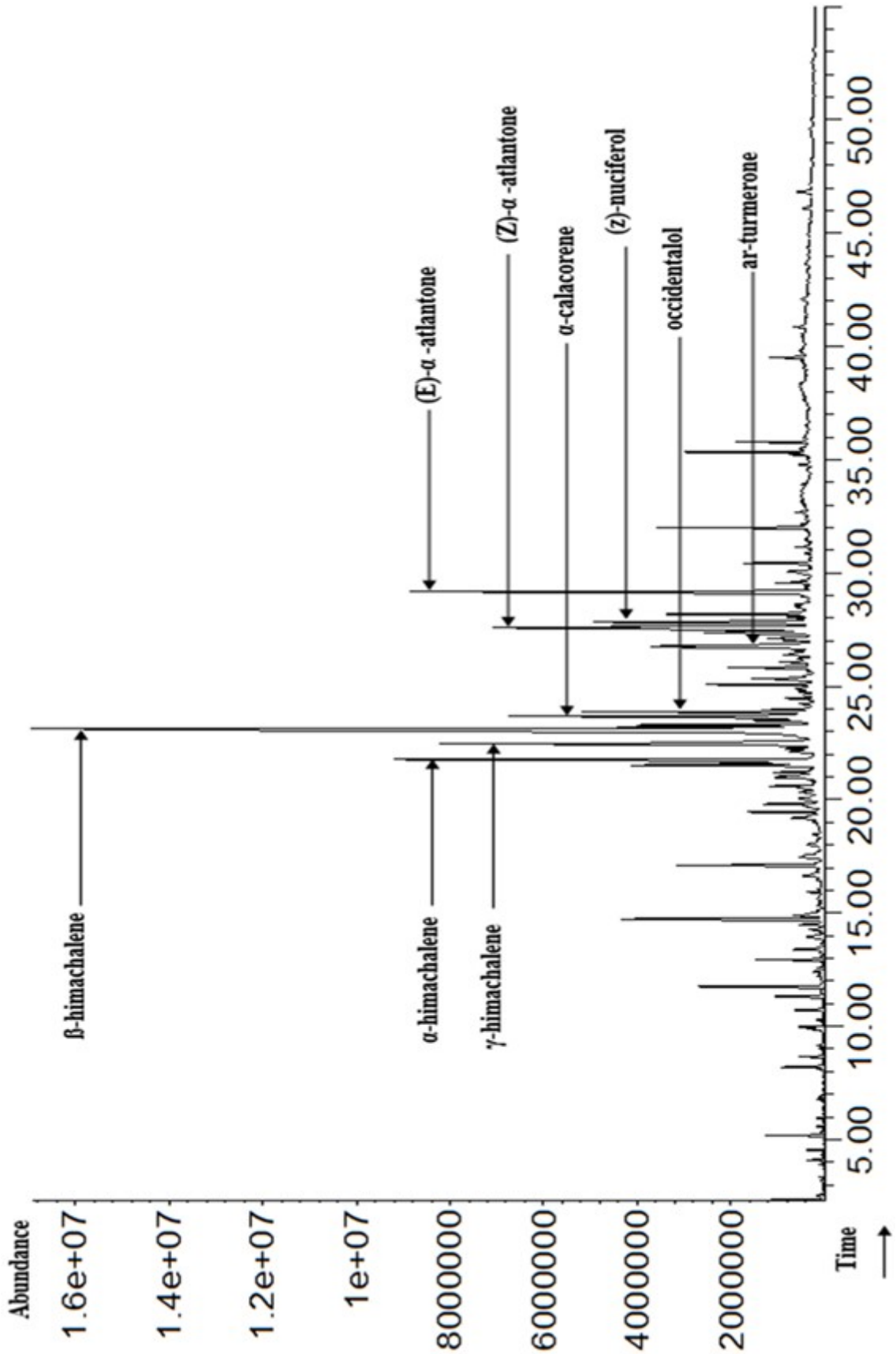

Figure 1. GC/MS chromatogram of *Cedrus atlantica Manetti* Tar

Jenkner Retort [7,4] and even Atlas cedar wood essential oils produced by hydrodistillation or steam distillation in the studies cited above.

Ar-turmerone which was among the major components in our sample with a percentage of 3.35 % was identified only in Lebanon cedar tar produced by Jenkner Retort from the roots and branches with minimal contents [7].

Total polyphenolic and condensed tannins contents

Atlas Cedar tar have a high polyphenolic content (57.15 ± 0.15 mg GAE/ g tar) compared to that found [5] on Lebanon cedar tar (0.85 ± 0.06 mg GAE/g). This divergence may be due to the variation of the species used and even the extraction technique [4]. The amount of condensed tannins in terms of equivalent catechin was calculated as 4.41 ± 0.05 mg CE /g tar, the calibration curves of gallic acid and catechin are presented below (**Figure 2, 3**).

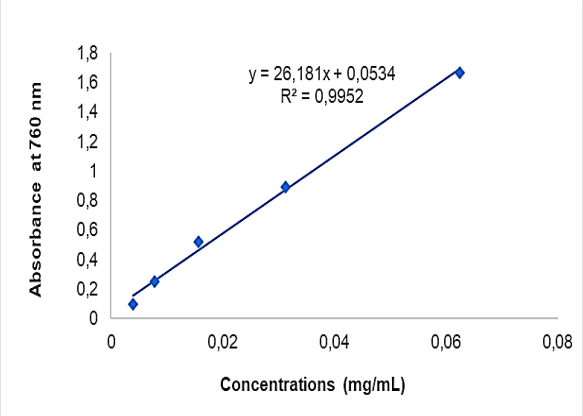

Figure 2. Calibration curve of Gallic acid

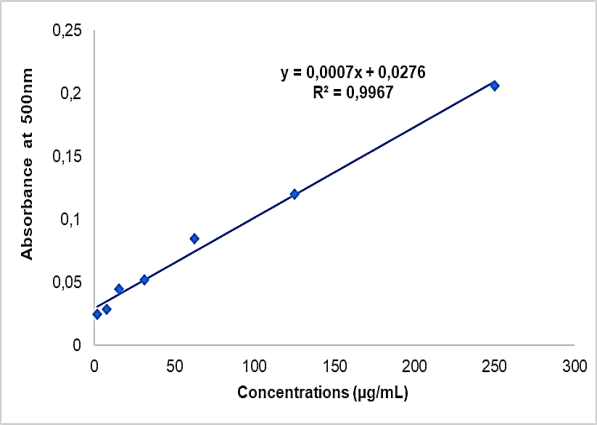

Figure 3. Calibration curve of Catechin

Antioxidant activities of Atlas cedar tar

1. FRAP assay

The Ferric-reducing antioxidant power of a component serves as a significant indicator of its antioxidant activity, it is based on the reduction of the ferric ion (Fe^{3+}) present in the [$K_3Fe(CN)_6$] (yellow color), complex to ferrous (Fe^{2+}) (blue color), a higher absorbance at 710 nm indicates higher reducing power and vice versa. Based on the obtained results (**Figure 4**). Atlas cedar tar had an increase reducing capacity but it was always inferior to ascorbic acid (known to be a strong reducing agent). The EC_{50} value of reducing power for Atlas cedar tar was 0.075 ± 0.00028 mg/mL this low value means a high reducing power of (Fe^{3+}) of Atlas Cedar tar.

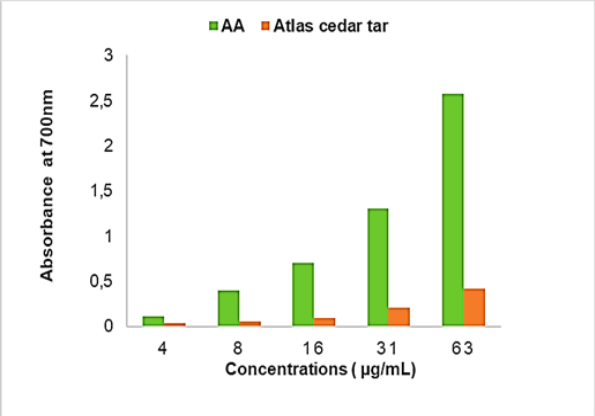

Figure 4. Antioxidant capacity of *Cedrus atlantica Manetti* tar, using ferric reducing power method

2. TAC assay

The Atlas cedar tar was also used to determine its antioxidant capacity from the formation of the green phosphomolybdenum complex. This greenish complex result from the reduction of Mo (VI) to Mo (V) by the antioxidant component, detectable in the visible region at 695 nm. The total antioxidant capacity of our tar using the calibration curve below is 262.75± 14.43 mg AAE / g tar.

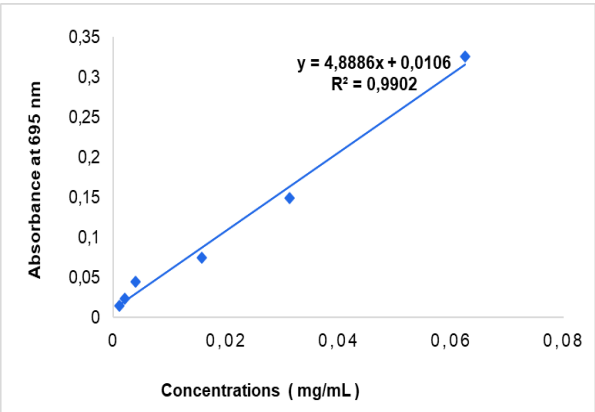

Figure 5. Calibration curve of Ascorbic Acid

Conclusions

In this study, the chromatographic analysis (GC/MS) of Atlas cedar tar obtained by dry distillation (canister method) identified 88 components. Himachalene and α-atlantone isomers were the major components, they were predominant almost in most of *Cedrus atlantica Manetti* wood essential oils and *Cedrus libani A. Rich* tars that we used to compare with. Regarding the antioxidant activities, we did not find available studies to compare. it is the first study of the antioxidant activity of traditional *Cedrus atlantica Manetti* tar using these two methods: FRAP, TAC and even of other traditional tars; the increase in antioxidant activity tested by FRAP and TAC is very probably due to the significant quantity of sesquiterpenoids. (Himachalene isomers) known for their antioxidant activity [25] these results indicated that our tar has promotive antioxidant activity.

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