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Total Flavonoids and Total Phenolic Content, Antioxidant, Antibacterial and Corrosion Activities of Plant Extracts of *Santolina africana* Jord. Et Fourr

Tarak Mekhelfi^{1,2*}, Manel Zaoui-Djelloul Daouadji¹, Safa Benhamed¹, Lahcene Zaiter², Fatma Tabai¹

¹Faculty of Mathematics and Matter Sciences, Department of Chemistry, Kasdi Merbah University, Route de Ghardaia, Ouargla, Algeria

²Research Unit Valorization of Natural Resources, Bioactive Molecules and Physicochemical and Biological Analyzes (VARENBIOMOL), Faculty of Exact Sciences, Department of Chemistry, Constantine University 1, Constantine, Algeria

***Corresponding author:** Mekhelfi T, Faculty of Mathematics & Matter Sciences, Department of Chemistry, Kasdi Merbah University, Route de Ghardaia, Ouargla, Algeria. Tel: +213790570555; E-mail: tarakmekhelfi@gmail.com

ABSTRACT

Santolina africana Jord. Fourr is of the Asteraceae family, an endemic plant that grows in northeastern Algeria, which has great benefits in traditional medicine (used as antiperspirants, antiseptics, antiseptics, bactericidal and anti-diabetic). [1] The studies have demonstrated antioxidant activity and bacteria for volatile oils of this plant [2]. Firstly, Preliminary tests for chemical families have been shown presence of (flavonoids, tannins, coumarins, alkaloids, and sugars). The content of phenols, flavonoids was estimated by spectrophotometric methods. Also the antioxidant activities of the extracts of *S. africana* were investigated by DPPH test and phosphomolybdenum assay. The results of the quantitative estimation of total phenols by Folin-Ciocalteu reactif showed that the *n*-butanol phase had the highest ratio (123,3875 mg GAE /g). The results of the quantitative evaluation of flavonoids by trichlorure d 'aluminum showed that the phase of the petroleum ether contained an excess of flavonoids in the unusual results for the test anti-oxidant of test the DPPH . The *n*-butanol phase showed the best result followed by the acetate phase (IC₅₀=45.90 ± 0.573 & 38.80 ± 1.201 mM respectively). The result of TAC test the *n*-butanol extract gave the highest

inhibitory ($TAC=1.227 \pm 0.02$ (mM)) concentration followed by the petroleum ether, acetate and, chloroform phase. Antibacterial activity was performed on two Gram negative strains (*Escherichia coli* ATCC25921, *Pseudomonas aeruginosa* ATCC29733), and a third Gram-positive strain (*Staphylococcus aureus* ATCC25923). The phase gave the petroleum ether the highest inhibition (CMI =12 mm) against strain *Staphylococcus aureus*. Finally, the results anti-corrosive by Tafel method effects of the crude ethanol extract showed that the latter was effective a large percentage.

Keywords: *Santolina africana* Jord. Fourr, Preliminary tests, Phenol, Flavonoid, DPPH, Total antioxidant activity.

INTRODUCTION

The relationship between man and nature has existed for many centuries, and this correlation increases according to his requirements especially in the field of pharmacy, medicine and beauty. Traditional medicine has been widely known and widespread among our predecessors, especially in the countries of the African continent, including Algeria. *Santolina* species are a complex group of botanical species whose chemo-taxonomic classification is periodically revised. [3] It is present in Algeria with 3 species: *Santolina africana* Jord. et Four. *S. lonadioides* Coss, *S. nobilis* L. J. Gay. [2] species are a rich source of essential oil and polyphenolic compounds where phenolic compounds are responsible on promoting the health properties of natural products. Soaking of the flower heads of this plant is used in traditional medicine against intestinal heaters, as an anti-spasmodic, anti-inflammatory, antiseptic, bactericidal and for different kinds of dermatitis [3], [4]. Inflorescences of this plant are mixed with honey and used for the treatment of cardialgia ulcer and stomach pain [5]. Her essential oils are against the two-spotted spider mite (*Tetranychus urticae*) [6]. Our goal in this study is to estimate the quantification, polyphenols and flavonoids by the Folin-Ciocalciu reagent and aluminum trichloride respectively in addition to promoting this work by the study of biological effectiveness and effectiveness of the anticorrosion of the plant studied.

Harvesting of plant material

The plants were collected in May 2015 in the region of Biskra (South-east of Algeria), It was dried under shade for several weeks and kept away from light and moisture.

EXTRACTION METHOD

227 g of the antenna part were macerated in a mixture of EtOH-H₂O (70:30, v/v) for 24-72 h at times; the ethanol extract is then filtered and dried in 35°C by the rotary evaporator, and then treated with distilled water. Extraction by gradient in polarization of solvents (petroleum ether) < (CHCl₃) < (ACOET) < (n-butanol) .

The weights of the four phases obtained from the extraction steps after concentration are: 1.5318 g (Petroleum ether), 1.268 g (ChCl₃), 1.1967 g (ACoET), 1.5152 g (n-butanol).

Preliminary tests to detect the chemical families in the ethanol extract [7]

Alkaloids: Add Dragerouf Wagner reagent drops

Flavonoids: Add concentrated HCl + Mg chip

Tannins: Add FeCl_3 1%

Sugars: Add Fehling's reagent (A + B)

Saponines: Agitation vigorously

Terpenes: Add CH_3Cl + H_2SO_4

Coumarins: Add NH_4OH 10%

Total phenolic content

The total phenolic content in the extracts of *S. africana* was estimated by using Folin- Ciocalteu reagent. A volume of 100 μl of the extract solutions at different concentrations are added to 1.5 ml of Folin-Ciocalteu reagent (10%). After 5 min, 1.5 ml of sodium carbonate (6%) are added. The mixture is allowed to react for 90 minutes at room temperature and then the reading is made at 725 nm. Gallic acid (0.03 -0.3 mg/ml) is the standard used to establish the calibration curve, from which the concentration of the total polyphenols in the extracts is calculated. The result is expressed in mg equivalents of gallic acid per gram of extract (mg/g). [8] (Figures 1-7).

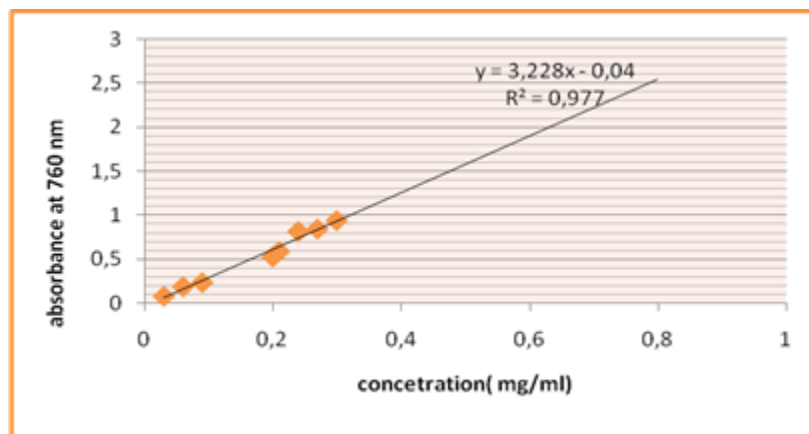


Figure 1: The standard curve of gallic acid.

Total flavonoid content

Total flavonoid content in crude extract and *S. africana* fractions was estimated using the aluminum chloride method. 0.5 ml of a 2% ethanol AlCl_3 solution was added to 0.5 ml of extract. After 30 min of incubation at ambient temperature, the absorbance was measured at 430 nm and the results were expressed in mg of quercetin equivalent per gram of dry weight deplant (mg EQ/g). [9]

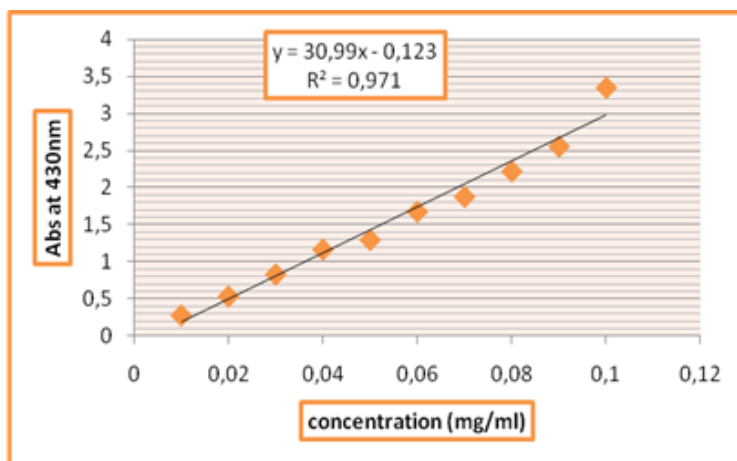


Figure 2: The standard curve of quercetin

Determination of anti-oxydant activity

The free radical scavenging activity of *S. africana* extracts was measured in terms of hydrogen donation or radical scavenging ability using the stable free radical DPPH according to the method by Zhou and Yu [22]. Different concentrations of the extract were added to 3.0 ml of 0.1 mM DPPH solution in ethanol. After vigorous mixing the tubes were incubated in the dark. After 30 minutes, the absorbance was read at 517 nm. The IC₅₀ value (the concentration required to trap 50% of free radicals of DPPH) was calculated from the curve of concentration versus trapping activity. Ascorbic acid has been used as a positive control [10]. The ability to trap the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [A_0 - A_1 / A_0] \times 100$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample. The 50% inhibitory concentration of the DPPH (IC₅₀) root activity is calculated for each extract of the inhibition curve (% I) against the concentration of extracts.

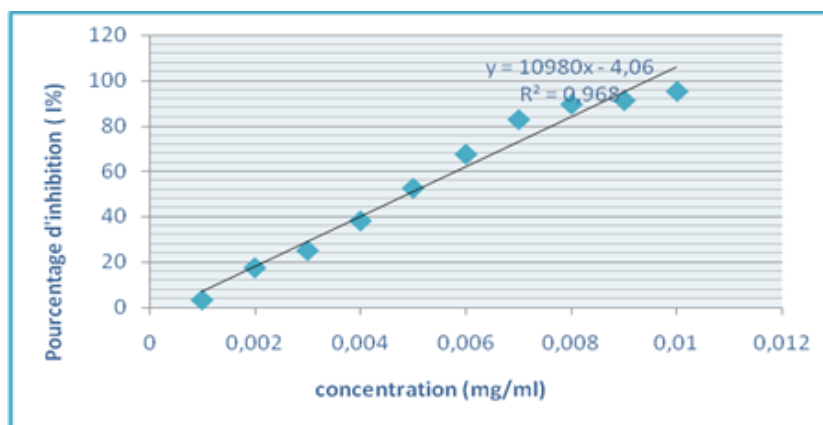


Figure 3: The standard curve of ascorbic acid.

Determination of total antioxidant activity

The total antioxidant capacity (TAC) of the extracts is evaluated by the method of phosphomolybdenum from [23]. A volume of 0.3 ml of each ethanolic extract is mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes are screwed and incubated at 95°C for 90 min. After cooling, the absorbance of the solutions is measured at 695 nm against the white which contains 3 ml of the reagent solution and 0.3 ml of ethanol and is incubated under the same conditions as the sample. The total antioxidant capacity was expressed as mM equivalent ascorbic acid [11].

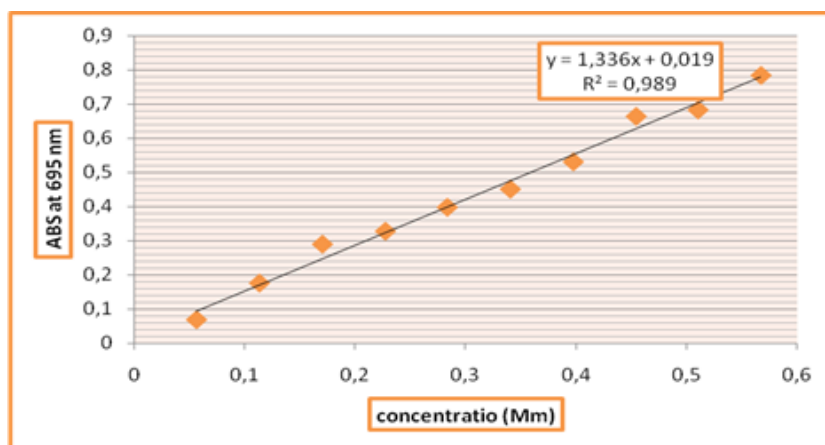


Figure 4: The standard curve of ascorbic acid

Antibacterial activity

Antibacterial studies have been evaluated by method of discs-diffusion [12].

Bacterial strains

Three references bacterial strains were obtained from the Pasteur laboratory in Algeria two Gram negative strains (*Escherichia coli* ATCC25921, *Pseudomonas aeruginosa* ATCC29733), and a third Gram-positive strain (*Staphylococcus aureus* ATCC25923).

Preparation of extracts

Melted 0.1 g of each extract in 1 ml of DMSO, and then diluted each with specific concentrations.

Sterilization: Before starting work, the work surface was cleaned with bleach water; the work is near a benzene beak. We have prepared Whatman 3 paper diameter 6mm discs which have good absorption of extracts, the whole of (forceps + these discs) is sterilized at 120°C for 20 min in autoclave.

Anti-biograms

Seeding: Bacteria are removed with a sterile swab of physiological saline, spread over the surface of the medium zigzag, taking care to cover the entire surface with bacterial colonies.

Place disks and incubation: Each batch of disks is dipped into the extracts. The disks so drenched are dried in room temperature, by a sterile forceps are deposited in petri dishes and close them. The Petri dishes are finally placed in a 24-hour incubator at 37°C.

Read the results: The larger the zone of inhibition, the greater is the sensitivity of the bacterial strain tested vis-à-vis the extract studied. Each zone can be measured by various means: ruler, compass, vernier caliper. The circular inhibition zone is measured by the diameter in mm and then it will be possible to calculate the MIC of the extract for the strain examined. Inhibition concentration (MIC) is defined as the lowest concentration of the extract to inhibit the growth of microorganism and was determined on the basis of micro-well dilution method [13].

MIC: is the lowest concentration that completely inhibits growth of the organism in the tubes as detected by the unaided eye. The MIC was determined by macrodilution broth method [14]

The study of anticorrosion activity

The Tafel method (polarization curves)

Preparation of sample: The sample to be studied is cylindrical, (1.6 cm × 0.75 cm) XC52 steel. This sample is scraped before each experiment by glass paper degraded (400, 600, 800, 1000, 1200, 1500 and 2000).

Used equipment and tools

* Potentiostat **PGZ 301** connected to a computer with software (Volta master 4)

* Electrochemical cell of the glass **Pyrex** double-wall thermostatic capacity of 500 ml, provided with three slots allows the insertion of electrodes.

* **Electrodes:** Working electrode (**ET**): is X52 steel. Auxiliary electrode (**CE**): Is a platinum cylinder polished 1cm² surface, its role is to ensure the passage of electric current in the electrochemical cell and the reference electrode (**ER**): which is a saturated calomel electrode noted ECS in potassium chloride (KCl), constituted by the Hg₂/Hg system; Cl/KCl [15]

Preparation of solutions

A. Corrosive medium: The study medium chosen is (HCL 1M)

B. Inhibitory solutions: The various concentrations of inhibitor which represents the crude ethanol extract (1–25 ppm), solution and without the presence of inhibitor solution (1 M HCL) are taken as blank for comparison.

The Tafel curves were obtained by changing the electrode potential automatically from –300 to +300 mV versus the open circuit potential at a scan rate of 0.2 mV/s.

The corrosion yield as well as the surface coverage density was calculated from the two relationships [16].

$$R\% = \frac{i_{corr} - I_{corr}}{i_{corr}} \quad \theta = R / 100$$

i_{corr} : Corrosion current in the absence of inhibitor.

I_{corr} : Corrosion current in the presence of inhibitor.

$R\%$: Inhibition yield.

θ : Surface coverage ratio.

E : potential

The plot of the intensity-potential curves allows the determination of the potential of corrosion (E_{corr}), the polarization resistance (RP), the corrosion current density (I_{corr}), the cathodic (bc) and anodic (ba) slopes of Tafel and consequently the rate of inhibition under the operating conditions used [20].

RESULTS AND DISCUSSION

Preliminary data showed the presence of most chemical families in the crude ethanol extract, indicating that the plant being studied is rich in secondary metabolites, particularly tannins, comarines and terpenes, giving a rather distinct color dark.

Extraction yield, total phenolic, flavonoid, DPPH scavenging, and total antioxidant activity

The yield of the extraction : The yield of the extraction of the petroleum ether and the butanol extract very close and they were the highest yield (0.4684% and 0.4633% respectively), while the acetate and chloroform were the least yielding 0.3877% & 0.3659% respectively) . In general, the yield is somewhat weak, which explains the plant's poverty quantitatively rather than qualitatively. Total phenolic of *S. africana* extracts expressed as gallic acid equivalent per gram dry weight (mg GAE/g DW), ranged from The highest content was found in n-butanol and AcOEt, the lowest content was registered in Petroleum ether and Chloroform of the crude extract of *S. africana*.

Total flavonoid of the extracts of *S. africana* expressed as quercetin equivalent per gram dry weight ($\mu\text{g QE/g DW}$). The highest amounts of flavonoids were present in the Petroleum ether and n-butanol extract, while the lowest were recorded in the acetate and chloroform extract. Polyphenols were generally found in the phases of polarity solvents such as acetate and butanol, which are interpreted as melting in these phases better than other non-polar or low-polar phases. The results of the antioxidant effect of the DPPH-test showed that the highest value of IC50 was for the n-butanolic phase followed by the acetate phase, then the petroleum ether phase and then the chloroform phase. IC50 ($\mu\text{g/ml}$)=ascroc acid $49.23 \pm 0.02 > \text{n-but } 45.90 \pm 0.573 > \text{AcOET } 38.80 \pm 1.201 > 26.13 \pm 3.112 \text{ Petroleum ether} > \text{Chloroform } 16.17 \pm 2.13 (\mu\text{g/ml})$.

These results correspond to the quantitative estimate of the presence of high phenols in the same phases which show high effectiveness against the free radicals. We know that many phenols are responsible for clearing free radicals, the rate of inhibition of the phase of the butanol is close to the inhibition ratio of the reference ascorbic acid and this explains why this phase contains donor groups of hydrogen atoms [17].

Total antioxidant activity TAC Expressed as absorbance in terms of the inverse of dilution factor. was recorded the highest in n-butanol extract, acetate and petroleum ether extract, the lowest antioxidant activity was recorded in the chloroform extract. These results are also consistent with the results of the quantitative estimation of polyphénols, it is also related to the results of the quantitative estimation of flavonoids where the n-betanol phase contains the second highest flavonoids after the phase of petroleum ether, this disparity in the rates of inhibition suggests that polar phases are always more likely to contain the particles that give the electrons than their non-polar counterparts (Tables 1 and 2).

Table 1: Result of total phenolic, flavonoid, DPPH scavenging, and total antioxidant activity result.

Variables	Yield %	total phenolic (mg GAE/g)	Total flavonoid (mgQE/g)	DPPH IC50 (µg/ml)	TAC (mM)
Petroleum ether	0.4684 ± 1.425	6.2855 ± 0.02	25.61 ± 0.858	26.13 ± 3.112	1.160 ± 1.3
Chloroform	0.3877 ± 0.01	5.3651 ± 0.6	6.3 ± 0.06	16.17 ± 2.13	1.045 ± 0.33
AcOET	0.3659 ± 2.7	43.6454 ± 0.2	1.71 ± 1.32	38.80 ± 1.201	1.130 ± 0.7
n-but	0.4633 ± 3.1	123,3875 ±	9.97 ± 0.573	45.90 ± 0.573	1.227 ± 0.02
Acide ascorbique	-	-	-	49.23 ± 0.02	-

Note through the curves results, the results clearly show that there is a positive relationship between the content of phenolic compounds and plant extracts and reticular effect on the DPPH root, in agreement with the results of many researchers. The mechanism of interaction between the antioxidant compounds and the DPPH root is its chemical structure as well as the number of hydroxyl groups it contains [21].

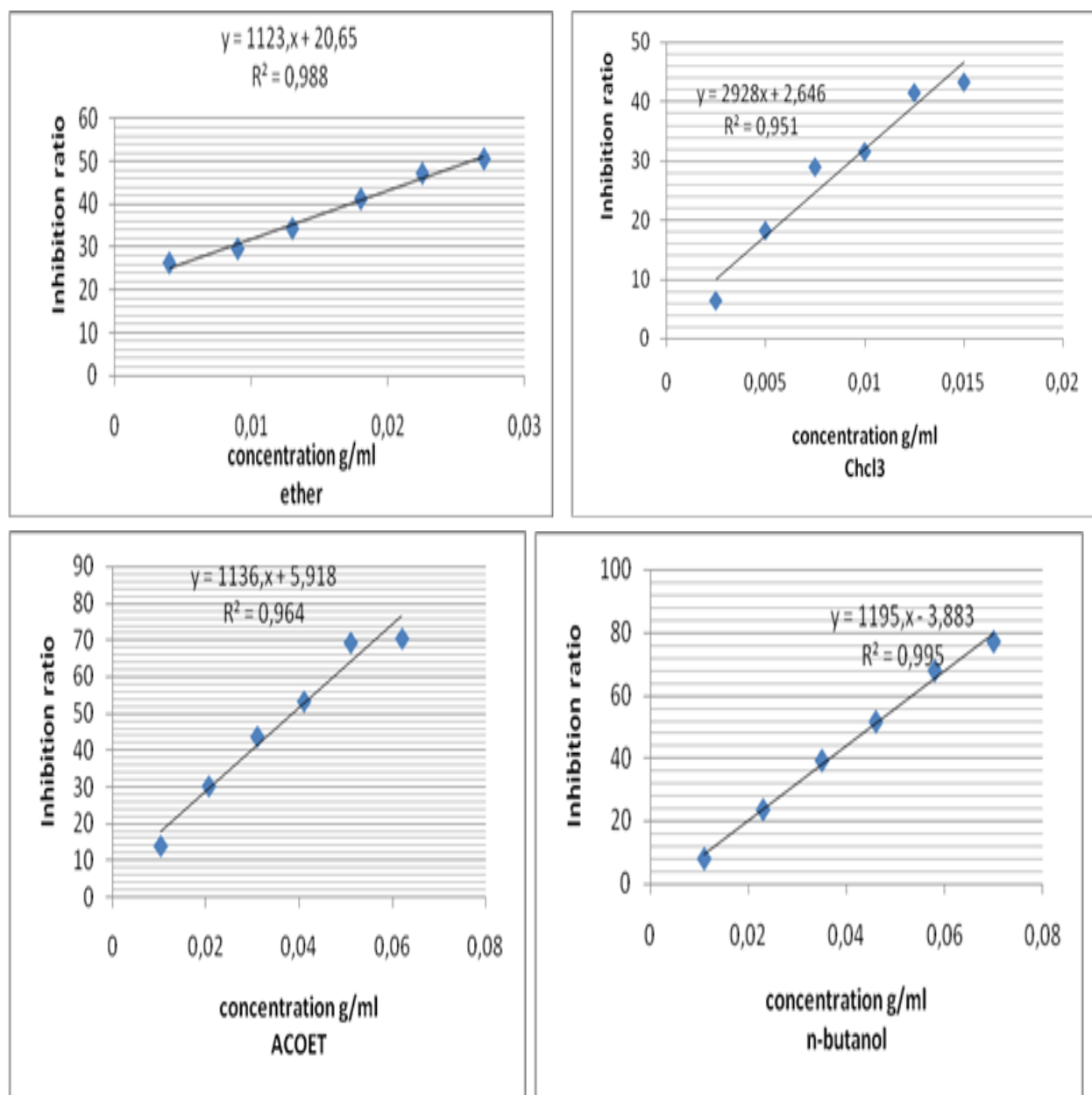


Figure 5: The inhibitory ratios curves (IC_{50}) of *Santolina* plant extracts.

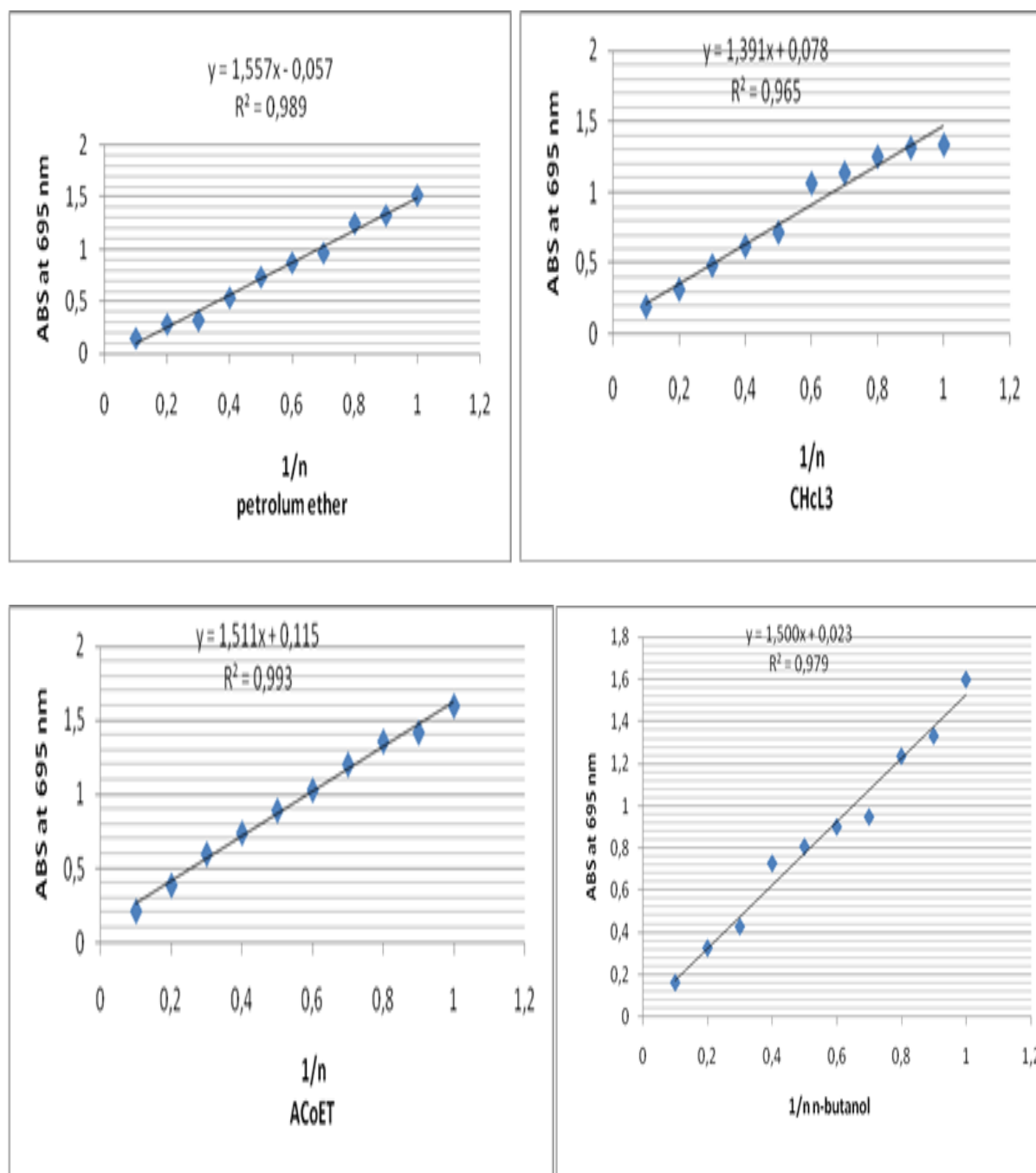


Figure 6: The curves of the total antioxidant capacity of *S. africana* extracts.

Results of anti-bacterial activity

Table 2: Antibacterial activity of *S. africana* extracts (Inhibition zones).

Inhibitor diameter (mm)			Concentration (mg/ml)	
<i>Escherichia coli</i> ATCC25921	<i>Staphylococcus aureus</i> ATCC25923	<i>Pseudomonas aeruginosa</i> ATCC29733		
-	4.5	-	A= 100	Raw extract
-	0.3	-	B=50	
-	-	-	C=25	
-	4	-	A=100	Aqueous extract
-	7	-	B=50	
-	5	-	C=25	
-	12	-	A=100	Petroleum ether extract
-	9	-	B=50	
-	9	-	C=25	
-	-	-	A=100	Chloroform extract
-	-	-	B=50	
-	-	-	C=25	
-	-	-	A=100	AcoEt extract
-	7	-	B=50	
-	9	-	C=25	
-	10	-	A=100	n-butanol extract
-	-	-	B=50	
-	-	-	C=25	

The results showed that the extract of petroleum ether gave the highest diameter inhibition between all extracts as much as (12 mm) at a concentration of 100 mg/ml against *Staphylococcus aureus* ATCC25923 bacteria followed by the extract of the water-based betanolic extract and then the acetate extract and then the water extract and the crude ethanol. As for the remaining two species, no extract from the extracts gave any resistance against these colonists (*Escherichia coli* ATCC25921, *Pseudomonas aeruginosa*).

Corrosion inhibitory activity

Note that after the addition of the extract, the rate of corrosion decreases in the acid medium, accompanied by an increase in the corrosion yield R%, the decrease of current density is due to the adsorption of inhibitor molecules on mild steel surface to retard the corrosion reaction of electrode. From this study we observed that, on increasing the inhibitor concentration (1– 25 ppm) (Table 3).

Table 3: Effectiveness values of anti-corrosion and surface density

Concentration of extract (ppm)	E (i=0)mv	Rp (ohm. cm)	I (corr) (mA/cm ²)	Ba (mA)	Bc (mA)	V-corr (uA/V)	R %	Θ
0	-546.2	104.29	0.9726	186.5	-167.9	2.019	0	0
1 (C)	-529.9	86.75	0.7323	163.7	-155.2	8.565	24.7069	0.247069
2.5 (D)	-534.7	101.51	0.6177	160.2	-157.8	7.224	36.4898	0.364898
5 (E)	-534.6	91.28	0.553	162.6	-196.3	6.468	43.142	0.43142
10 (F)	-537.6	98.23	0.5125	144.3	-184.8	5.994	47.3061	0.473061
15 (G)	-524.6	91.26	0.4919	132.1	-184.1	5.753	49.4242	0.494242
20 (H)	-512.2	91.3	0.3938	116.8	-165.4	4.606	59.5105	0.595105
25 (I)	-481.5	132.91	0.3231	155	-195.2	3.779	66.7797	0.667797

The results of the table are translated into the following curves:

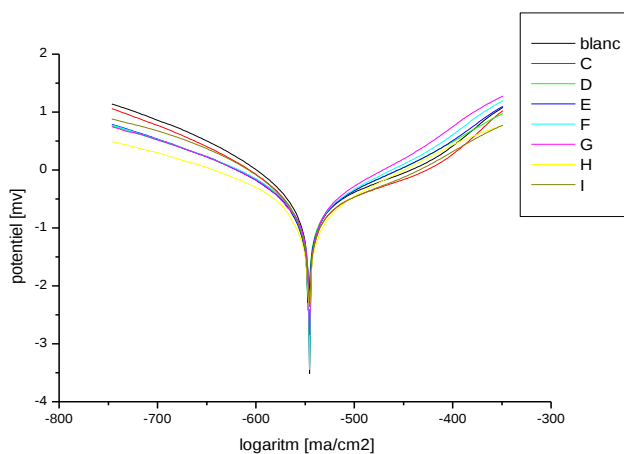


Figure 7: Polarization curves of X52 steel with and without addition of the extract of the *S. africana* in HCl at room temperature.

According to Ferreira and others [24], if the displacement of corrosion potential (E_{corr}) is greater than 85 mV with respect to the corrosion potential (E_{corr}) of the blank solution, the inhibitor can be seen as a cathodic or anodic type. In this study, the maximum displacement was 65.2 mV anodically with respect to the blank. This indicates that the studied inhibitor behaves predominantly as anodic type [18]. Our inhibitor reduces the anodic dissolution of steel (described by the reaction) ($\text{Fe} \leftrightarrow \text{Fe}^{+2} + 2\text{e}^-$) and delays the evolution of the cathodic discharge of protons $\text{H} + (2\text{H}^+ + 2\text{e}^- \leftrightarrow \text{H}_2)$.

The mechanism of this inhibition is made by forming a layer of the of the extract on the alloy steel, which is where prevent iron oxidation in middle of HCl . We can also say that this inhibitory force is due in part to the antioxidant activity of the flavonoids present in our synthesis according to one study [19].

CONCLUSION

In general, the plant of *Santolina africana* is rich in secondary metabolites, especially phenols and flavonoids, which show the effectiveness of resistance to free radicals as well as its resistance to the bacterium *Staphylococcus aureus* ATCC25923, as well as its anti-corrosion effect. The results obtained for the first time at the level of the extracts of *Santolina Africana*, according to our bibliographic research, as it was preceded by the level of volatile oils, promote the therapeutic benefits in traditional medicine for this type waiting for further studies in this plant *Santolina africana* joudr & fourr.

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