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Radical scavenging and Antioxidant activities of three extracts of *Lavandula coronopifolia* grown in El-Hoggar, Tamanrasset, Algeria

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ABSTRACT

The genus Lavandula (common name Lavender) involves 39 species of flowering plants in the Lamiaceae family. The present study deals with the antioxidant activity of three different solvent extracts of Lavandula coronopifolia that grows in Hoggar (Tamanrasset) south of Algeria. The radical scavenging potential was determined on basis of the activity of trapping the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The values of IC_{50} (mg/mL) were found to be 0.0347g/L, 0.0414g/L and 0.0910g/L for diethyl ether, ethyl acetate and n-Butanol extracts respectively, compared to the ascorbic acid (VC) and the tocopherol acid (VE) which were 0.0088g/L and 0.0154 g/L respectively. Moreover, the FRAP (Ferric reducing antioxidant power) assay was carried out and revealed a better efficacy of ethyl acetate extract than the other extracts. By using VCEAC (Vitamin C Equivalent Antioxidant Capacity), *it is found that the reduction percentages are: 0.90 g/L, 0.680 g/L and 0.304 g/L for Diethyl ether, Acetate ethyl and n-Butanol respectively. The ascorbic acid (VC) considered, as a positive test has a VCEAC of 1.223g/L.*

Key words: Lavandula coronopifolia, flavonoid, extraction, antioxidant activity.

INTRODUCTION

Due to their numerous properties, the use of aromatic and medicinal plants and their active principles provide important environmental, economic and social benefits, in addition to being a wide field of application of the food, pharmaceutical, cosmetic and perfumer industries [1].

Algeria involves a tremendous amount of native plants distributed throughout its vast territory and used to cure and to relieve pain, as ingredients in some gastronomies, as spices and flavors, and so on.

Hoggar is among the Algerian sites which are famous for their variety of bioactive plant species used by local population for different purposes. It involves different phyto-sociological groupings, which intrigued since long time several researchers [2,3]. Touareg people (local tribe) made use of this patrimony since ancient times. They knew how to benefit from their active extracts and their noteworthy pharmacological properties [4].

The lavender of the central Sahara (*Lavandula coronopifolia*), the subject of our work, is a true vulnerary plant [5], Known by its antibacterial and disinfectant properties. It is known as well to slacken the nerves, decrease the muscular tension, and relieve flatulence and colics.

MATERIALS AND METHODS

2-1-Preparation of the extracts

The preparation of the extracts from *Lavandula coronopifolia* is carried out using liquid-liquid extraction based on the principle of solubility in organic solvents. Three organic solvents of increasing polarities were used namely diethyl ether, ethyl acetate and *n*-butanol.

2-2-total flavonoids

The quantification of the total flavonoid content in the various fractions of *L. coronopifolia* is carried out according to the method of the Aluminum Trichloride [8]. To 1 mL of each extract, 1 mL of the Aluminum Trichloride solution ($AlCl_3$, 2%) in methanol is added. The mixture is left to react during 10 min then the absorbance is read at 430 nm. The concentration of the flavonoid in the extracts is calculated based on calibration range established with the quercetin (1-25 µg/mL) and expressed in milligrams of quercetin equivalent per gram of total dry material (mg QE /g of TDM).

2-3-DPPH assay

The hydrogen atoms or electrons donation ability of the corresponding extract was measured from the bleaching of purple colored MeOH solution of DPPH. This spectrophotometric assay uses stable radical 1,1-Diphenyl-picrylhydrazyl (DPPH) as a reagent [9]. 50 µL of each extract in MeOH at various concentrations were added to 5 mL of a 0.004% MeOH DPPH solution. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Percent Inhibition of free radical DPPH was calculated according to the formula:

$$\text{Inhibition \%} = [(\text{A blank} - \text{A sample})/\text{A blank}] \times 100$$

Where A blank is the absorbance of the control reaction (containing all reagents except the tested compound) and A sample is the absorbance of the tested compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as inhibition percentage against extract concentration. All tests were carried out in triplicate. vitamin C and Vitamin E were taken as positive control.

2-4-FRAP assay

The ferric reducing antioxidant power (FRAP) assay provides a measure of the reducing ability of the plant extracts. This method was established by Benzie and Strain [10]. The FRAP reagent was freshly prepared by mixing together 10mM of 2,4,6-tripyridyl triazine (TPTZ) and 20mM of Ferric Chloride in 0.25M of Acetate Buffer, pH 3.6. 100µL of test sample was added to 300µL of distilled water followed by 3mL of FRAP reagent. The absorbance was read at 593 nm after 5 min of incubation at room temperature against a blank. The standard curve of tocopherol was previously plotted. Antioxidant activity could be determined from the standard curve as Vitamin E Equivalent Antioxidant Capacity. Antioxidant activity of each sample was compared with standard vitamin C. Presented data are average of three replications

RESULTS AND DISCUSSION

3-1. Quantitative analysis

The flavonoid extraction yield is based on the degree of solubility of *L. coronopifolia leaves* in organic solvents. The results revealed that the ethyl acetate extract has the highest yield (13.4%) followed by butanolic extract (8.4%) and finally diethyl ether extract (7.02%).

- **total flavonoids**

The fractions representing the total flavonoids (TF) obtained from the collected extracts are given in mg of quercetin equivalent per gram of total dry matters (TDM). The TF were quantified by the method of Aluminum Trichloride ($AlCl_3$). The quercetin is the flavonoid taken as reference for the calibration curve (R^2 = 0.999) (figure 1 and table 1).

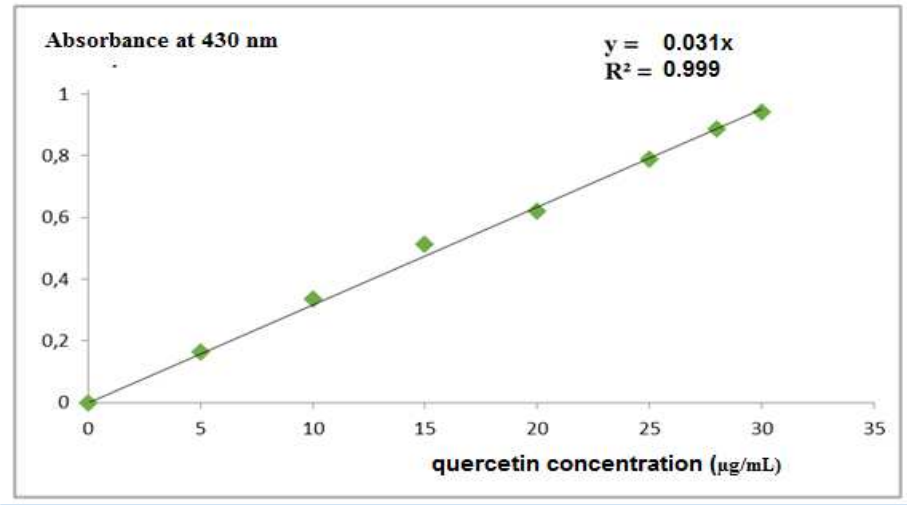


Figure 1: Standard Curve of quercetin

Table 2. Total flavonoids of *L. coronopifolia.*

Extracts	Total flavonoids (mg/g of TDM)
Diethyl Ether	0.74 ± 0.10
Ethyl acetate	0.92 ± 0.02
n-Butanol	2.86 ± 0.32

3-2. Radical DPPH scavenging activity

DPPH assay remains one of the most used methods for assessing the radical scavenging activity. The assay is based on the measurements of the antioxidants ability to scavenge the stable nitrogen-centered free radical DPPH. Our results clearly highlight the potential of *L. coronopifolia* extracts in scavenging free radicals. As usually observed in similar studies, the inhibition percentage increases with increasing concentration (Figures 2,3,4,5,6). Calculation of extract concentration inducing 50% inhibition (table 2), showed that there was a difference between the extracts and positive tests (ascorbic acid (VC) and tocopherol (VE).

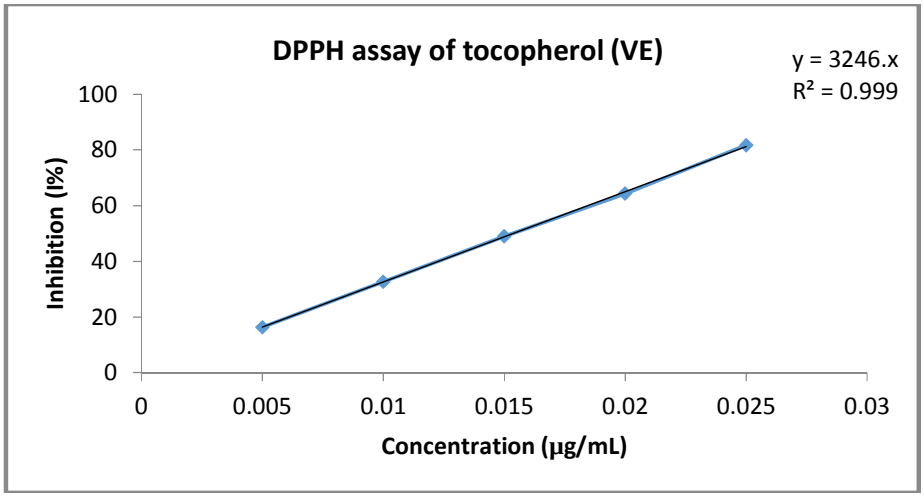


Figure 2. DPPH essay of tocopherol(VE)

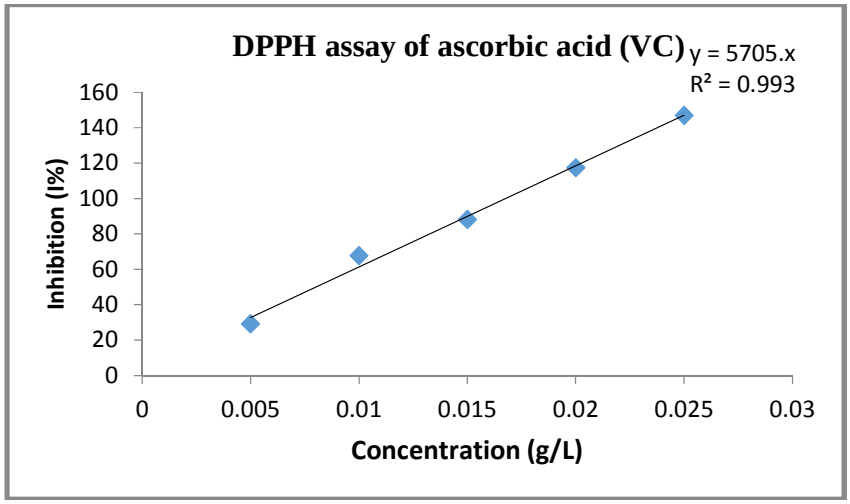


Figure 3: DPPH essay of ascorbic acid (VC)

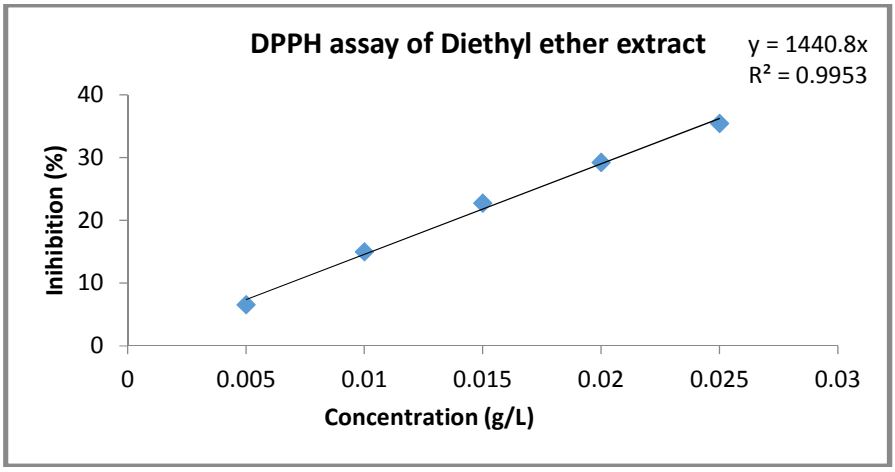
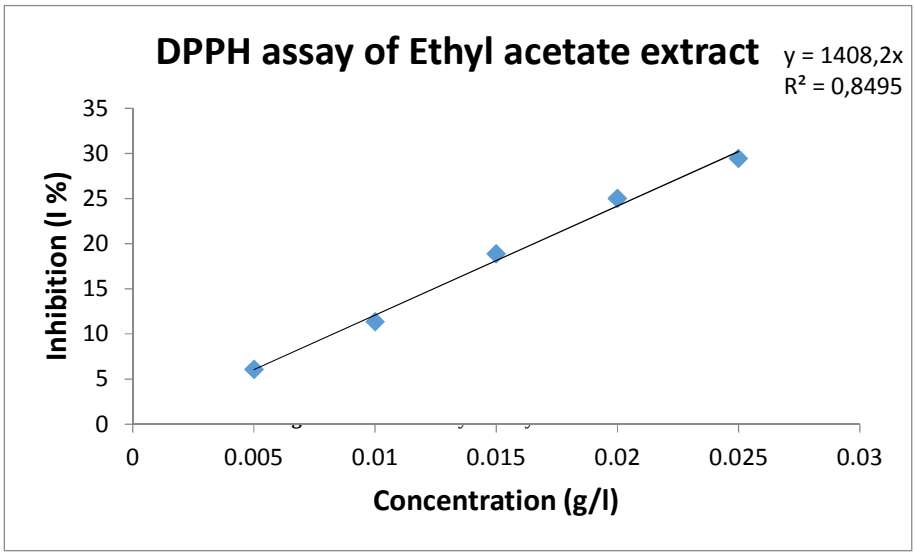


Figure 4: DPPH essay of Diethyl ether extract



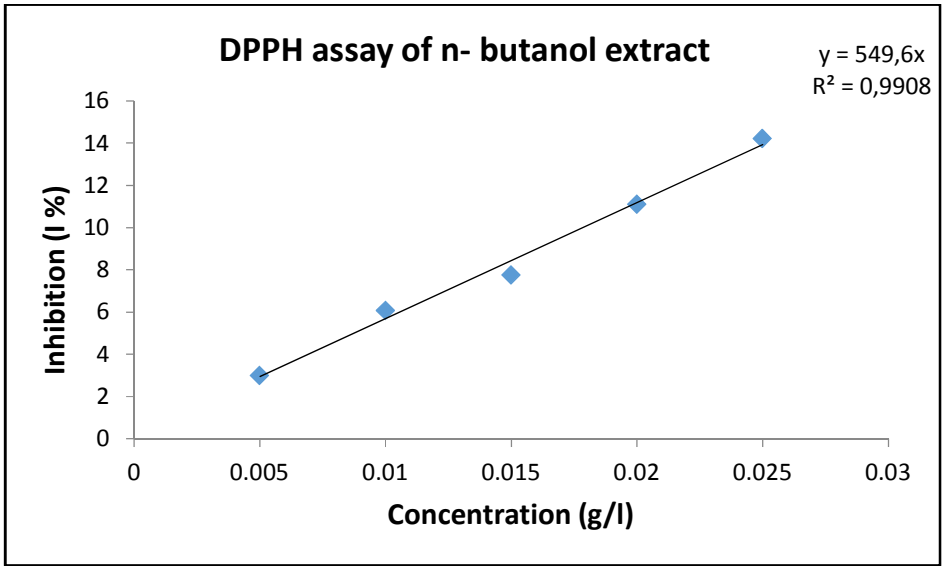


Figure 6: DPPH essay of n-butanol extract

Table 2: IC_{50} for the tested extracts

Extract	Diethyl ether	Ethyl acetate	n-Butanol	Vitamin C	Vitamin E
IC_{50} (g/L)	0.0347	0.0414	0.0910	0.0088	0.0154

All the treated extracts have a less radical scavenging activity regarding the ascorbic acid and tocopherol used in food industry as conservatives. The maximum value of the antioxidant activity was found in the extracts of Diethyl ether with an IC_{50} of 0.0347g/L.

3-3. Reducing ability (FRAP)

Figures 8, 9, 10, 11 and 12 illustrate the different absorbance values of the studied extracts using the **FRAP** test.

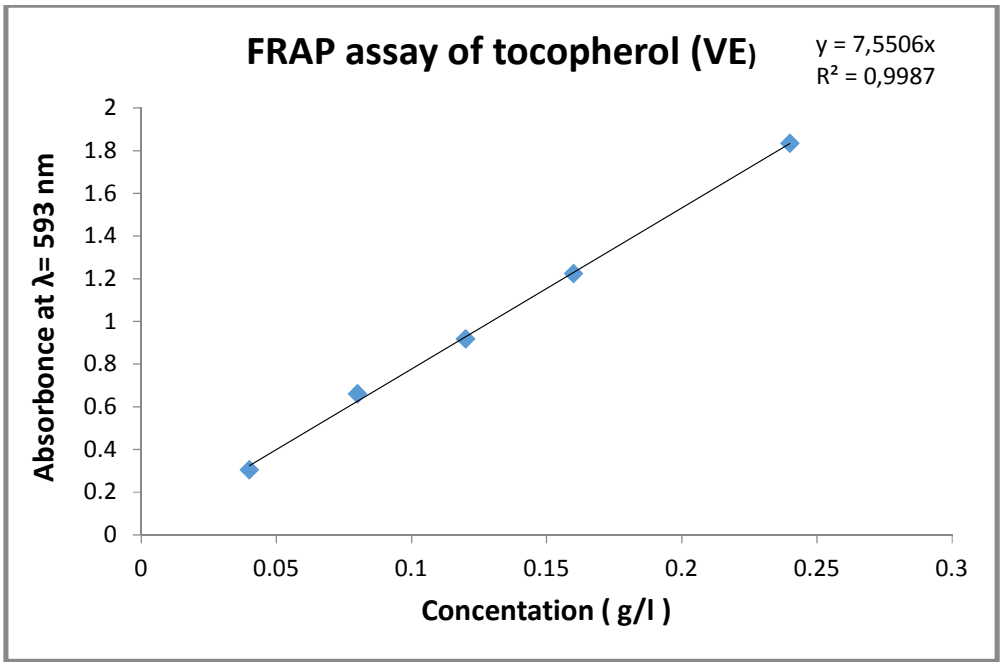


Figure 8: Standard FRAP assay of tocopherol.

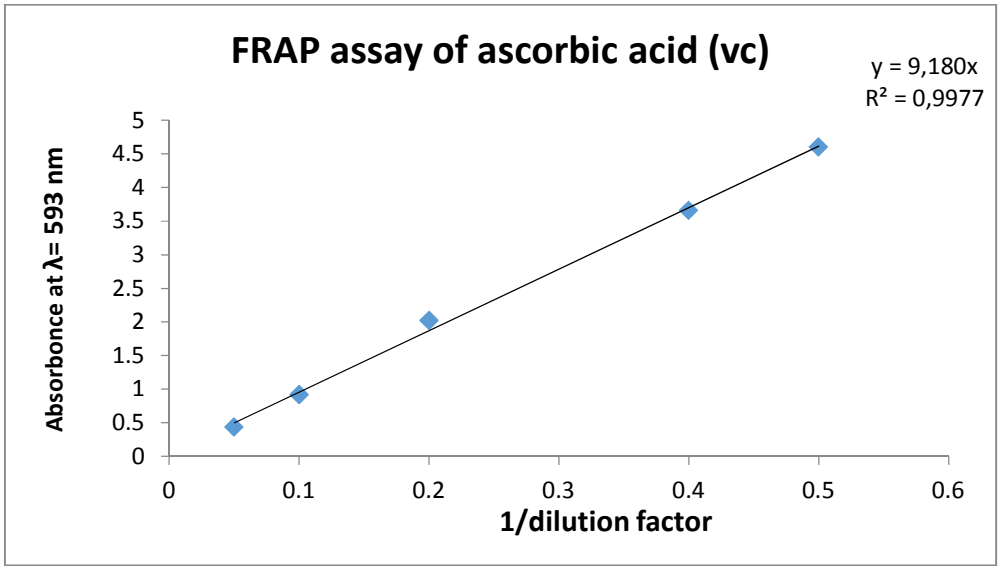


Figure 9: FRAP assay of ascorbic acid.

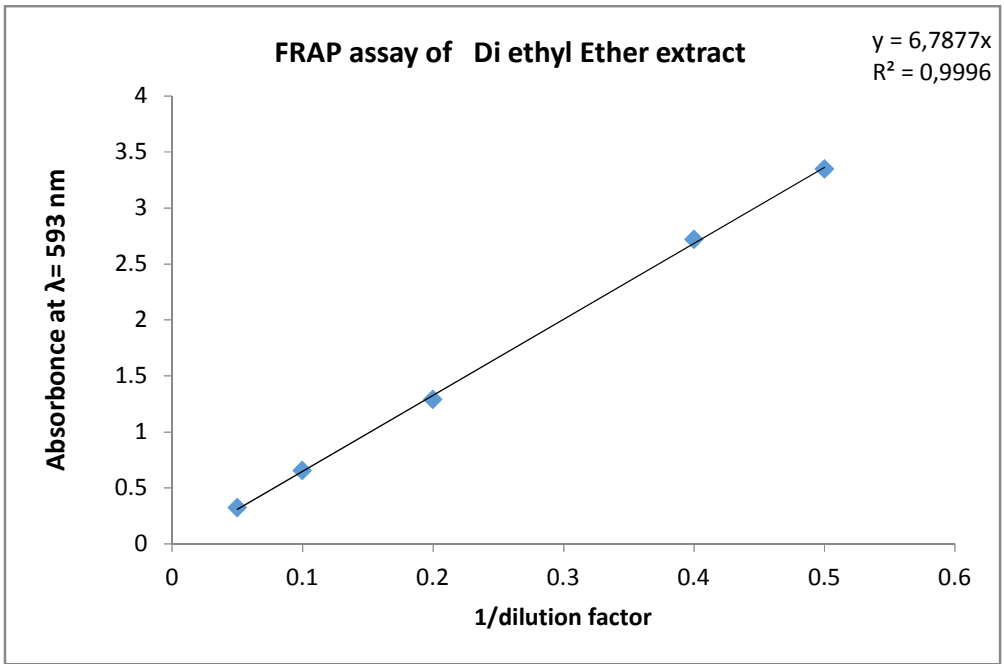


Figure 10: FRAP assay of Diethyl ether extract.

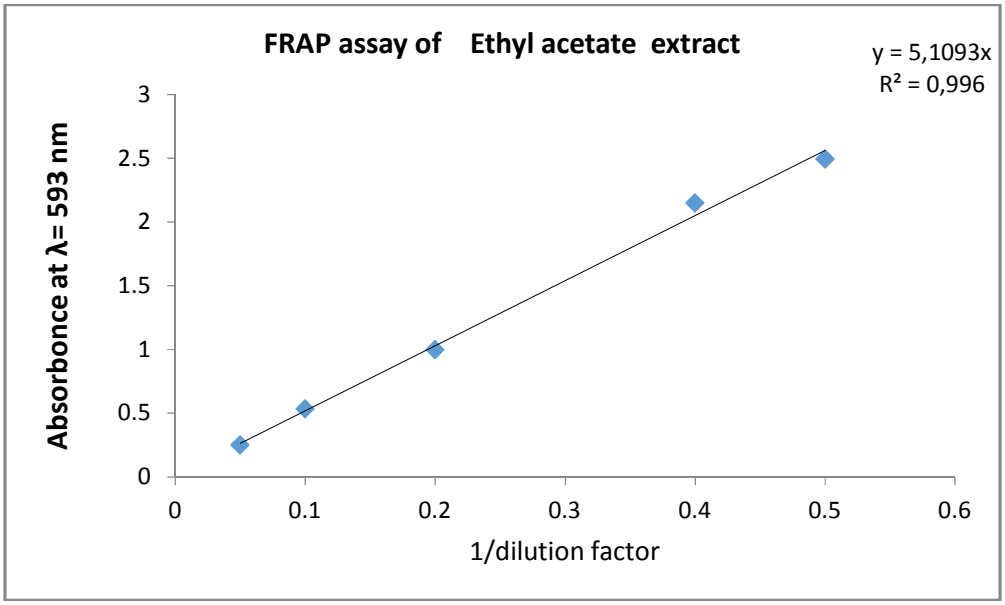


Figure 11: FRAP assay of Ethyl acetate extract.

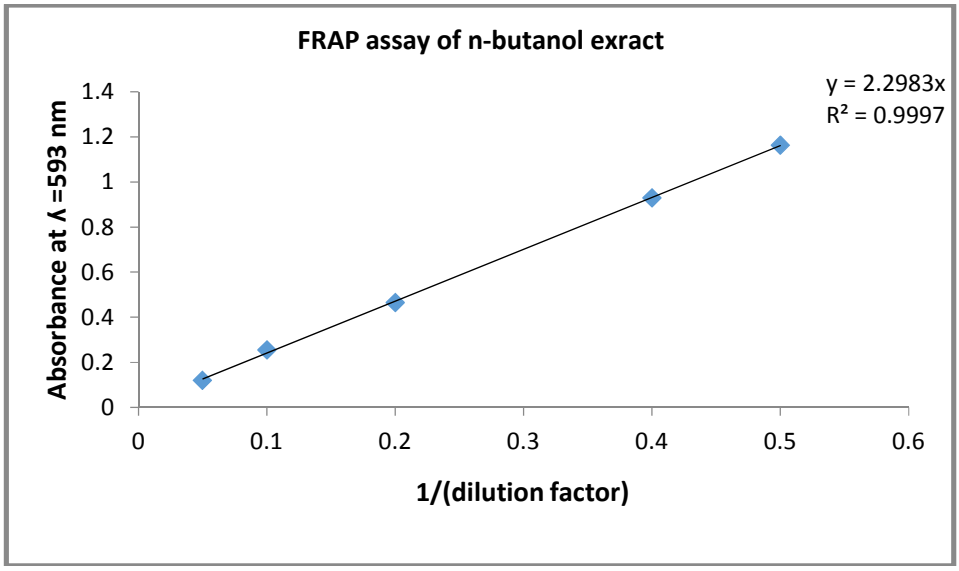


Figure 12: FRAP assay of n-butanol extract.

These results show that the acid ascorbic (VC) used in the food industry as an antioxidant agent is better than the extracts. While comparing the extracts, is found that the Diethyl ether extract with an antioxidant activity is better than the other extracts.

CONCLUSION

According to the results obtained and the experimental conditions, it was showed that all the extracts have an antioxidant activity. The highest value was recorded in the diethyl ether extract, but it is less than that found in the ascorbic acid to be used in food industry as a conservative.

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