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Research Article

Total Phenolic Compounds and Antioxidant Potential of Rokrose *(Cistus salviifolius)* Leaves and Flowers Grown in Tunisia

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ABSTRACT
This study was undertaken in order to examine the potential antioxydant of Tunisian *Cistus salviifolius* (leaves and flowers) and their composition of antioxidants including total polyphenol, flavonoids, proanthocyanidins content, The research was conducted on five extracts (ethanol, butanol, ethyl acetate, dichloromethane and water extract) in order to identify new sources of antioxidants. The total phenolic, flavonoid and Proanthocyanidin were determined spectrophotometrically using Folin - Ciocalteu colorimetric method as described by Singleton et al, Zhishen method and Broadhurst method. The different extracts were subjected to the scavenging tests of DPPH, ABTS and FRAP radicals.
The aqueous extract of *C. salviifolius* leaves exhibited the highest quantity of total phenolic (56.03 g GAE.100 g^{-1} of DW), flavonoid (28.35 g catechin eq. 100 g^{-1} of DW) and Proanthocyanidin content (3.70 g catechin eq. 100 g^{-1} of DW). The obtained results showed also that the aqueous and ehanolic extract were detected with best antioxidant capacity (IC 50 = 2.18 and 3.52 µg.mL^{-1}). A positive relationship between antioxidant activity (DPPH, ABTS and FRAP) and polyphenolic compounds of the tested *C. salviifolius* leaves and flowers was found. High positive linear correlation was found between DPPH and total phenolic in the flowers (r^2 = 0.9966). The results clearly showed that utilization of polar solvent enable extraction of significant amounts of phenolics and flavonoids. The richness of *C. salviifolius* in antioxydant allow justifying the traditional uses of this plant in the treatments of rheumatism, and inflammatory diseases. Therefore, *C. salviifolius* could be as a good source of natural antioxidant.

Keyword: *Antioxidant capacity assays, Total phenolic, Proanthocyanidin content, Catechin*

INTRODUCTION

Antioxidants present in all parts of plants, play an important role in the protection of cells and organisms[1], they possess a wide spectrum of biological effects, including inflammatory, antitumor, antibacterial and antiviral activities[2-4]. The use of natural antioxidants from plants is medically helpful and least detrimental with very few side effects as compared to synthetic ones. As indicated by various studies, the beneficial effects of antioxidant have been attributed to the Secondary metabolites such phenolics, flavonoids and tannins compounds[5]. The cistaceae is a plant family used in traditional medicine for treatment of various diseases such as rheumatism, antimicrobial, antiulcerogenic, anti-diarrheal and vasodilator remedies[6,7]. This work was undertaken to explore the potential antioxydant and antibacterial of Tunisian *Cistus salviifolius* of the family the *cistaceae* and of the genus *Cistus.* The genus *Cistus* is a major source of labdanum gum and labdanium[8].This Resin (labdanium) was used since ancient times for its aromatic and pharmaceutical properties [9-11]. *Cistus salviifolius*, popularly known as Tunisian “Melliya”, is one species of the genus Cistus. They are used as infusion for the treatment of diarrhea. Previous studies have revealed that the arial parts of *C. salviifolius* were rich in flavonoids compounds such as flavonoid aglycones (kaempferol, quercetin, myricetin) , flavonoid glycosides , steroid aglycone (β-sitosterol) and steroid glycosides[12]. These researchs proved that *C. salviifolius* may contain various potential compounds that are beneficial to health. The composition of essential oil of *Cistus salviifolius* was well studied. Fatty acids, esters, neophytadiene and pentacosane were identified in several Greek and Tunisian populations of *C. salviifolius*[13,14], besides, antioxidant activity of Tunisian C. salviifolius was not investigated. The purpose of this study was to evaluate total phenolic (TPC), total flavonoid (TFC) and total proanthocyanidin content (TCT) in alcoholic and aqueous extracts of leaves *Cistus salviifolius* (CSL) and Flowers (CSF) using spectrophotometer methods. In this investigation we used three in vitro-antioxidant capacity assays such as Free Radical-Scavenging Activity (DPPH), ABTS radical cation decolorization assay (2, 2′-azinobis (3-

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Table 1. Total phenolic, flavonoid, and Proanthocyanidin content in different extracts from *Cistus salviifolius* (Leaves and flowers)

	T.phenolics ^{a*}		T. flavonoid ^{b*}		T.proanthocyanidin^{c*}	
	Leaves	**Flowers**	**Leaves**	**Flowers**	**Leaves**	**Flowers**
E1	53.62±0.38	38.2±0.17	27.84±0.02	26.40±0.35	1.35±0.00	0.75±0.13
E2	12.11±0.15	7.83±0.29	3.50±0.63	4.23±0.70	0.90±0.41	0.11±1.25
E3	11.96±0.14	8.43±0.75	2.06±0.35	2.45±0.04	0.54±0.53	0.04±1.05
E4	47.40±1.63	27.50±0.0	27.80±0.33	25.03±0.55	2.20±1.01	0.48±0.70
E5	56.03±0.06	41.67±0.45	28.35±0.01	26.31±0.01	3.70±1.23	2.54±0.04

E1: Ethanol Extract, E2: Ethyl acetate extract, E3: Dichloromethane extract, E4: n-butanol extract, E5 : water extract
a: gram gallic acid eq.100g^{-1} of dry matter*
b: g catechin eq. 100g^{-1} of dry matter*
c:gram catechin eq. 100g^{-1} of dry matter*

Table 2: Antioxidant activities obtained using the DPPH, ABTS and FRAP methods on *Cistus salviifolius*

	DPPH IC 50 (µg.mL^{-1})		ABTS IC $_{50}$ (µg.mL^{-1})		FRAP IC $_{50}$ (mmol Fe^{2+}/g)	
Gallic acid	2,24		2.61		40.34	
Ascorbic acid	6,25		8.21		14.38	
	Leaves	**Flowers**	**Leaves**	**Flowers**	**Leaves**	**Flowers**
E1	3.52	11,79	10.97	15,35	54.50	35.00
E2	>52,38	>52,38	>52	>52	17.80	18.01
E3	>52,38	>52,38	>52	>52	14.25	16.70
E4	20,74	29,16	2.73	16,44	47.20	48.54
E5	2,18	8.40	8.77	7,24	77.68	59.60

Values are presented as means ± S.E.M (n = 3)

ethylbenzothiazoline-6-sulfonic acid) and ferric reducing antioxidant power (FRAP).

MATERIALS AND METHODS

Chemicals and reagents

Folin- Ciocalteus's phenol reagent, DPPH (2, 2-Diphenyl picrylhydrazyl), Gallic acid, Catechin, Ascorbic acid, Isoquercetin and Trolox (6- hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) standards were obtained from Sigma-Aldrich Co (St. Louis, MO, USA). Sodium carbonate is from Fluka Biochemika (Switzerland), concentrated hydrochloric acid (37%) and absolute methanol was purchased from Panereac Quimica Sa (Barcelone). Absolute ethanol (≥ 99.8 % purity) is from Scharlab S.L (European Union).

Plant materials

The plant of *Cistus salviifolius* L were collected in June 2012 from Sidi Mechreg (Bizerta). The plant was botanically identified by comparison with already identified herbarium specimens collection at the herbarium of the Faculty of Science (Bizerta), a voucher specimen (Cis-0014) has been deposited in the Faculty of Sciences, Bizerte (Tunisisa). Samples were stored in a dry place until analyzed.

Preparation of extract

The leaves and flowers of *Cistus salviifolius* (200 g) were extracted by percolation with ethanol (EtOH) at 95% for 24 hours. The EtOH extracted was concentrated to dryness. Subsequently, the EtOH extracted was performed a liquid-liquid partition successively with dichloromethane, n-butanol, ethyl acetate and water.

Determination of total phenolics, total flavonoids and total condensed tannins

Total phenolics

The total phenolics content was determined using Folin-Ciocalteu colorimetric method as described by Singleton et al, with gallic acid as standard[15]. Total phenolic content was expressed as g gallic acid equivalents (GAE). 100 g $^{-1}$ of dry weight. 1 mL of each sample extract was diluted to 5 mL with distilled water, 1 mL of sodium carbonate (20 %) and 1 mL of Folin - Ciocalteu (9 %) reagent was added. The mixture was mixed and allowed to stand in a water bath, in the dark, at 40°C. After 30 min, absorbance was measured at 760 nm using ultraviolet-visible spectrophotometry.

Total flavonoids

The total flavonoids content in leaves (CSL) and flowers (CSF) of *Cistus salviifolius* was determined using g a colorimetric method as described by Zhishen et al[16], with catechin as standard. The results were expressed as g catechin eq. 100g $^{-1}$ of dry weight (DW). 125 µL of the sample extract was added to a 100 µL of a $NaNO_2$ (5%), at 6 min, 150 µL of aluminium trichloride (10%) was added, then kept in dark for 5 min, 750 µL of NaOH (1M) was added and the mixture was diluted with 1375 mL of distilled water. The solution was mixed and the absorbance was measured at 510 nm against a blank sample (methanol) after 15 min.

Total proanthocyanidin

The total proanthocyanidin content was estimated with the method by Broadhurst et al[17], catechin was used to make the standard calibration curve. The results were reported as mean ± standard deviation and expressed as g E. Catechin. 100 g $^{-1}$ of DW. 400 µL of CSL and CSF extract was mixed with 3 mL of solution vanillin (4%) and 1.5 mL of concentrated HCl. The solution was mixed and the

Table 3: **Relationship between antioxidant activities, total phenolic, flavonoid and proanthocyanidin content.**

		Correlation coefficients		
		$TEAC_{DPPH}$	$TEAC_{ABTS}$	$TEAC_{FRAP}$
TP	Leaves	Y=-0.882x+59.367 (R^2=0.9741)	Y=-0.8785x+58,446 (R^2=0.9362)	Y=0.7964x+2,5484 (R^2=0.8961)
	Flowers	Y=-0.7548x+47.99 (R^2=0.9966)	Y=-0.7205x+45.338 (R^2=0.9474)	Y=0.371x-1,4937 (R^2=0.7459)
TF	Leaves	Y=0.5306x+31,833 (R^2=0.9179)	Y=-0.5656x+33.063 (R^2=0.9764)	Y=0.4754x-2,1921 (R^2=0.8314)
	Flowers	Y=-0.5508x+33.862 (R^2=0.8878)	Y=0.4754x-2.1921 (R^2=0.8314)	Y=0.5823x-3,829 (R^2=0.7786)
TT	Leaves	Y=-0.0378x+2.7294 (R^2=0.5601)	Y=-0.042x+2,1849 (R^2=0.7549)	Y=0.0431x-0,083 (R^2=0.821)
	Flowers	Y=-0.0392x+2.1922 (R^2=0.631)	Y=0.0431x+0,083 (R^2=0.821)	Y=0.0547x-0,9609 (R^2=0.9634)

absorbance was measured at 500 nm against a blank sample (methanol) after 15 min of incubation nm in the dark using a ultraviolet-visible spectrophotometry.

Antioxidant Activity Determination

DPPH Radical Scavenging Activity

Free Radical-Scavenging Activity was measured using a method established by Brand-Williams et al[18]. Ascorbic acid (3 to 15 µg.mL^{-1}), gallic acid (0.5 to 5.0 µg.mL^{-1}) and Trolox (2.5 to 15 µg.mL^{-1}) were used as reference controls. Briefly, 50 µL of sample solution in different concentrations (2.5 µg/mL to 50 µg/mL) was mixed with 200 µL of methanolic containing DPPH radicals (0.070 mg/mL). The negative control consisted of 200 µL of DPPH solution and 50 µL of methanol. The mixture was incubated at 25 °C in the dark against a blank that was prepared with methanol. The assay was performed in 96-well black microplates. After 1 hour, the UV absorbance of sample extracts was recorded at 517 nm. All determinations were performed in triplicate.

The percentage of inhibition (%I) of free radical DPPH by Extract samples was calculated using the formula given below:

% of inhibition =

((Abs Control – Abs test) / Abs Control) ×1000

ABTS radical cation decolorization assay

The ABTS radical cation decolorization assay was performed according to a modified method described by Pellegrini et al[19]. ABTS radical A.B.T.S $^+$ solution was prepared as follows: 1 ml of ABTS (7mM) was mixed with 1 mL of potassium persulfate (4.9mM), the mixture was diluted at 1/20th in ethanol to a final absorbance between 0.800 and 1.000 nm. 200 µL of A.B.T.S $^+$ working solution with 50 µL of EtOH was used as a negative control. ascorbic acid, and gallic acid were used as positive controls in this assay, ethanol was used an blank. Sample solutions were prepared to mixing 50 µL of each concentration with 200 µL of ABTS reagent. The assay was performed in 96-well black microplates.The sample extracts was vortexed and incubated at 25°C for 30 min and its absorbance was later read at 734nm. All determinations were carried out in triplicate and the absorbance was removed and means values were calculated.

The percentage inhibition of The DPPH radical was calculated as follows:

% of inhibition =

((Abs Control – Abs test) / Abs Control) ×1000

The percentage of inhibition was converted into inhibition concentration (IC50 in µg/mL) using was the graph of percentage inhibition depending on the concentration.

Ferric Reducing/Antioxidant Power Assay (FRAP)

The FRAP assay was determined using the method developed by Benzie and Strain with slight modifications[20]. A fresh working solution was prepared by mixing 300 mM sodium acetate buffer (pH 3, 6), 10mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in HCl 40 mM and 20 mM $FeCl_3$, 6 H_2O in a proportion of 10: 1: 1. 50 µL of H_2O with 200 µL of FRAP reagent was used as blank, in the microplate reader. In the case of the sample solutions 50 µL of each concentration was added to 200 µL of FRAP reagent. The mixture was incubated at 40°C for 30 min in the dark and the resulting solution was measured spectrophotometrically at 593nm. The results were expressed as µmol Fe^{2+}/mL dry mass and compared with those ascorbic acid and gallic acid.

RESULTS AND DISCUSSION

Total phenolic, flavonoids and proanyhocyanidin compounds

Phenolic compounds are secondary plant metabolites that are ubiquitously present in fruit and vegetables[21]. Epidemiological studies have shown that the polyphenols exhibited a wide range of biological and pharmacological activities[22,23]. The total concentrations of phenolic compounds in the extracts are shown in table1. Results showed that the amount of phenolic, flavonoid and proanthocyanidin compounds in all extracts were higher in leaves than in flowers. The total polyphenol content of *C. salviifolius* ranging from 11.96 ± 0.14 to 56.03 ± 0.06 g GAE. 100 g -1 DM in leaves and from 7.83 ± 0.29 to 41.67 ± 0.45 g GAE 100 g $^{-1}$ DM in flowers. In both parts of plant, the maximum content was recorded in aqeuous extracts, the lowest polyphenols content was recorded in ethyl acetate flowers extract (7.83 ± 0.29 g GAE.100 g-1 of DW and in dichloromethane leaves extract (11.96 ± 0.14 g GAE.100 g-1 of DW). A similar tendency was observed for flavonoid and proanthocyanidin contents, aqueous extract being the richer in flavonoids (28.35±0.01 g GAE.100 g $^{-1}$ DM) and total condensed tannin (3.70±1.23 g E. Catechin. 100 g $^{-1}$ DM). Ethyl acetate and dichloromethane extracts contains considerably smaller

concentrations. These values showed considerable variations among different extracts tested in this study (Table 1). It can be seen that the total polyphenols compounds varied according to the polarity of solvent. These results clearly showed that utilization of polar solvent enable extraction of significant amounts of phenolics and flavonoids. The extracts obtained in this study have a higher total phenol content compared with those reported by Zidane et al[24] for the aqueous extract (83.31 ± 0.14 mg/g DM), méthanol extract (47.96 ± 0.13 mg/g DM) and ehanol extract (65.76 ± 0.1 mg/g DM) from Morocco leaves *C. ladanifer*. These results were totally consistant with those reported by Enrique Catalan et al., 2011, who indicated that polyphenols compouds were more abundant in *C. salviifolius* than in *C. ladanifer*[25]. Our study confirmed that *C.salviifolius* (leaves and flowers) are good sources polyphenolic compounds.

Antioxidant capacity

Published review articles demonstrated that the antioxifant activity of natural products has been determined by various different methods such DPPH, TRAP, ABTS, FRAP and ORAC method, but, there is no universal method that can mesure the antioxidant capacity. The different methods used to calculate antioxidant capacity of herbal sample, fruits and vegetables can give different results depending on the substrate, the solvent used, the oxidation conditions, interfacial phenomena and the specific free radical being used as a reactant and other compounds[26]. In this study, the antioxidant activity of *Cistus salviifolius* was evaluated by three different methods (DPPH, ABTS, and FRAP), results are showed in table 2. All polar extracts showed high antioxidant activities. The best reducing powers were obtained in the water extracts (2.18 µg. mL^{-1} (DPPH)) and n-butanol extract (2.73 µg. mL^{-1} (ABTS)) of *Cistus salviifolius* leaves. The aquous extract of leaves of *C. salviifolius* showed antioxidant potential stronger than standard gallic acid (2,24 µg. mL^{-1}). Leaves extract showed the better activity than flowers extract in DPPH and ABTS methods, with the exception of E5 in ABTS test. We can establish that the phenolic compounds and antiradical power of the extracts is strongly affected by the polarity of the solvent used. Fractions obtained with a high polarity solvent exhibited greater activity than apolar fraction. The aqueous and alcoholics extracts (E1, E4 and E5) of the *C. salviifolius* exhibiting greater TP and TF content, depicted also good radical scavenger activities in flowers and leaves. According to the previous studies, The reducing power is very much associated with their TP and TF content[27].The leaves have greater antioxidant activity than fruits, and aqueous, ethanolic and n-butanol extracts showed greater antioxidant activity than other. These results were in agreement with those obtained for polar extracts from Tunisa H. halimifolium (leaves and flowers) which belongs to the same family cistaceae[28]. Our results were consistent also with those reported by Zidane who found that the higher activities antioxydant in *C. ladanifer* and (97.8% for leaves and 97.3% for flowers) and in *C.libanotis* (96.9% for flowers and 93.4% for leaves)[24].

Relationship between anti-oxidant activity and phenolic content

One of the objectives of this study was to consider the correlation between phenolic compounds and antioxidant activity of analyzed plants extracts. Correlation between the results of different antioxidant assays is shown in Table 3. A positive linear correlation between the values for the total phenolic content and antioxidant activity (DPPH, ABTS and FRAP) was observed in leaves ($r^2 \geq 0.8961$) and flowers ($r^2 \geq 0.7459$)). A good correlation was also observed for total flavonoid content ($r^2 \geq 0.7786$) in both parts for the plant, and for the three tests used. The ABTS and FRAP assays correlated with total condensed tannin. The lowest correlation coefficients were between DPPH values and total proanthocyanidin (R^2=0.5601) probably because reactions of TCT with DPPH radical are usually rapid. The polyphenols in extracts are probably responsible for the high antioxidant activity of polar extracts. However, this activity is not limited to phenolics content but also at the presence of other antioxidant secondary metabolites. These different correlation coefficients support the necessity to use multitude of methods to evaluate antioxidant activity of plants.

CONCLUSION

This work was undertaken to explore the potential of extract of *C. salvifolius* (leaves and flowers) of Tunisia as sources of natural antioxidants. The richness of leaves in bioactive compounds (phenolic acids, flavonoids and tannins) was noted. The leaves extract were found to possess strong antioxidant activity essentially the polar extracts (ethanol, water and butanol). The aqueous extract has a antioxidant activity quantitatively comparable to that of gallic acid. The phenolic compounds appear to be responsible for the antioxidant activity of extracts; a linear correlation of trolox equivalent antioxidant capacity (TEAC) versus the total phenolic content of *C. salviifolius* was established. The rich composition in antioxidant compounds and the higher antioxidant activity of aqueous extract of *C. salviifolius* can improve the use of these plants in various areas , and may replace synthetic antioxidant in food formulations.

REFERENCES

1. Boxin O.U., Huang D., Maureen H.W., Flanagan J.A., Elizabeth K.D. Analysis of antioxidant activities of common vegetables employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) assays: A comparative study. J. Agric. Food Chem. 2002; 50: 3122-3128.
2. Lepoivre M. Flaman J.M ., Bobé P., Lemaire G., Henry Y. Quenching of the tyrosyl free radical of ribonucleotide reductase by nitric oxide. J. Bio. Chem. 1994; 269: 21891-97.
3. Owen R.W., Giacosa A ., Hull W.E., Haubner R ., Spiegelhalder B., Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur. J. Cancer. 2000; 36 (10): 1235-1247.
4. Sala A ., Recio M.D., Giner R.M ., Manez S., Tournier H ., Schinella G., Rios J.L. Anti-inflammatory and

antioxidant properties of *Helichrysum italicum*. J. Pharm. Pharmacol. 2002; 54(3): 365-371.

5. Cao G., Sofic E., Prior R.L. Antioxidant and prooxidant behavior of flavonoids:Structure –activity relationships. Free radical boil. Med.1997; 22: 749-760.
6. Lendeckel U., Arndt M., Wolke C., Reinhold D., Kdhne Th. and Ansorge S. Inhibition of human leukocyte function, alanyl aminopeptidase (APN, CD13) and dipeptidylpeptidase lV (DP lV, CD26) enzymatic activities by aqueous extracts of *Cistus incanus L.* ssp. Incanus. Journal of Ethnopharmacology.2002; 79 (2) :221 -227.
7. Attaguile G., Perticone G., Mania G., Savoca F., Pennisi G. and Salomone S. *Cistus incanus* and *Cistus monspeliensis* inhibit the contractile response in isolated rat smooth muscle. Journal of Ethnopharmacology. 2004; 92: 245-250.
8. Nicoletti M., Toniolo C., Venditti A., Bruno M., Ben Jemia M. Antioxidant activity and chemical composition of three Tunisian Cistus: *Cistus monspeliensis*, *Cistus villosus* and *Cistus libanotis*. Natural Product Research.2014; 12: 1-8
9. Demetzos C., Harvala C., Philianos S.M. A new labdane-type diterpene and other compounds from the leaves of *Cistus incanus* subsp. creticus. J Nat Prod. 1990; 53: 1365–1368.
10. Guy I, Vernin G. Minor compounds from *Cistus ladaniferus L.* essential oil from esterel. 2. Acids and phenols. J Essent Oil Res. 1996; 8: 455–462.
11. Hegnauer R. 1964. Chemotaxonomie der pflanzen. Band 3. Birkhauser Verlag, Bassel Switzerland. 429–431.
12. Perihan G., Lütfiye O.D., Zühal G., Ayşe K., Cavit K. Isolation and Structure Elucidation of Uncommon Secondary Metabolites from *Cistus salviifolius L.* Rec Nat. Prod. 2015; 9 (2): 175-183.
13. Demetzos, C., Angelopoulou, D., and Perdetzoglou, D. A comparative study of the essential oils of *Cistus salviifolius* in several populations of Crete (Greece). Biochem. Syst. Ecol.2002; 30, 651–665.
14. Loizzo, M. R., Ben Jemia, M., Senatore, F., Bruno, M., Menichini, F., and Tundis, R. Chemistry and functional properties in prevention of neurodegenerative disorders of five *Cistus* species essential oils. Food Chem. Toxicol.2013; 59, 586–594.
15. Singleton V. L., Rossi J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Americain Journal of Enology and Viticulture. 1965; 16 (3): 144–158.
16. Zhishen J., Mengcheng T., Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry.1999; 64 (4): 555–559.
17. Broadhurst R.B., jones W.T. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture. 1978; 48(3) 788–794.
18. Brand W.,. Cuvelier M. E, Berset C. Use of a free radical method to evaluate antioxidant activity. Food Science and Technology—Lebensmittel-Wissenschaft and Technologie.1995; 28(1): 25–30.
19. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine.1999; 26(9-10): 1231–1237.
20. Benzie I. F. F., Strain J. J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Analytical Biochemistry.1996; 239 (1): 70–76.
21. Razali, N., Razab, R., Mat Junit, S. and Abdul Aziz, A.. Radical scavenging and reducing properties of extracts of cashew shoots (Anacardium occidentale). Food Chemistry .2008; 111: 38–44.
22. Borneo, R., León, E.A., Aguirre, A., Ribotta, P., Cantero, J.J. Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their in vitro testing in model food system. Food Chem.2008; 112, 664-670.
23. Katalinic, V., Milos, M., Kulisic, T., Jukic, M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2007; 94, 550-557.
24. Zidane H. , Elmiz M., Aouinti F., Tahani A., Wathelet J., Sindic M., Elbachiri A. Chemical composition and antioxidant activity of essential oil, various organic extracts of *Cistus ladanifer* and Cistus *libanotis* growing in Eastern Morocco. African Journal of Biotechnology.2013; 12(34): 5314-5320.
25. Enrique B. C., Salvador F. A., Cristina R., Emilio G., Domingo S., Antonio S. C., Vicente M.A Systematic Study of the Polyphenolic Composition of Aqueous Extracts Deriving from Several *Cistus* Genus Species: Evolutionary Relationship. Phytochem. Anal.2011; 22: 303–312.
26. Antolovich M . 2002. Methods for testing antioxidant activity, Analyst, 127: 183.
27. Ghasemzadeh A ., Jaafar H. Z. E., Rahmat A. Antioxidant Activities, Total Phenolics and Flavonoids Content in Two Varieties of Malaysia Young Ginger (*Zingiber officinale Roscoe*). Molecules.2010; 15: 4324-4333.
28. Ahlem R., Souad I.B., Béatrice B.,Valérie M. L., Fathi M., Evelyne O., Jamila K.C., Malika T.A. Total Phenolic, Total Flavonoid, Tannin Content, and Antioxidant Capacity of *Halimium halimifolium* (*Cistaceae*). Journal of Applied Pharmaceutical Science .2014; 5 (01): 052-057.