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The Antioxidative Activity of Aqueous and Ethanolic Extracts of Rosemary and Green Tea Leaves: A Comparative Study

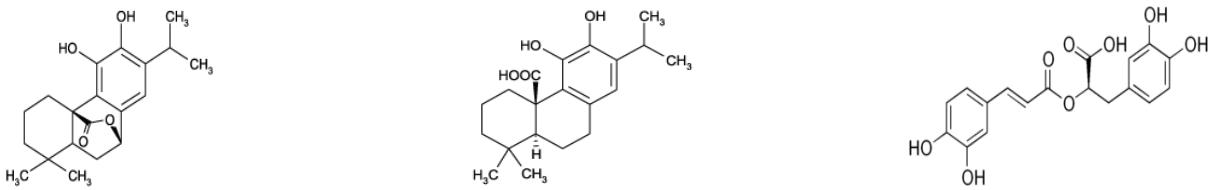
*N. M. Tariq, S. U. Wisam, H. M. Faik and T. H. Mayson
Basic Science Section, College of Agriculture, University of Baghdad, Baghdad-Iraq
E-mail: *Tariq_nm2000@yahoo.com

ABSTRACT
The antioxidant activities of rosemary and green tea leaves, aqueous and ethanolic extracts, have been studied by using two different methods (reducing power and chelating ability). It was found that the total phenolic compounds in aqueous and ethanolic extracts of rosemary and green tea leaves were 13.44, 18.75, 39.38 and 48.44 mg/ 100 mg dry extract respectively. The flavonoids (which is a part of the phenolic compounds) were found to be 9.54, 12.65, 17.69 and 22.70 mg/ 100 mg dry extract in aqueous and ethanolic extract of rosemary and green tea leaves respectively. The ethanolic extract shows high content of phenolic compounds and in turn highly antioxidative activiy for both rosemary and green tea leaves as compared with aqueous extract.The aqueous and ethanolic extracts of rosemary and green tea leaves show high reducing power ability comparing with their abilities as chelating agents. Although, the phenolic compounds of green tea leave almost about 3-fold as compared with rosemary leave in both aqueous and ethanolic extracts, their extracts show extremely the same mode of action in both methods of determination (the reducing power and chelating ability). Therefore, we are fully recommended the rosemary leave extracts as a potent food preservative.

Keywords: Rosemary & green tea, leaves extracts, antioxidants

1. INTRODUCTION
Oxygen free radicals induce damage due to peroxidation to biomembranes and also to DNA, which lead to tissue damage. Antioxidants neutralise the effect of free radicals through different ways and may be prevent the body from various diseases. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have recently been reported to be dangerous for human health. Thus, the search for effective, non-toxic natural compounds with antioxidative activity has been intensified in recent years [1]. About 5% or more of the inhaled oxygen (O_2) is converted to reactive species (ROS) such as O_2^-, H_2O_2 and OH by univalent reduction of O_2 [2]. Antioxidants can act by scavenging reactive oxygen species, by anhibiting their formation (e.g. by blocking activation of phagocytes), by binding transition metal ions and preventing formation of OH and /or decomposition of lipid hydroperoxides by repairing damage (e.g. α-tocopherol repairing peroxyl radicals and so terminating the chain reaction of lipid peroxidation) or by any combination of the above [3].

Rosemary (*Rosamarinus officinalis*), is a woody, perennial herb with fiagrant, evergreen, needle-like leaves and white, pink, purple or blue flowers, native to Mediterranean and Asia regions. It is a member of the mint family (Lamiaceae). The name rosemary drives from the latin (*Rosamarinus*), which means dew of sea [4]. Rosemary extracts contain several compounds which have been proven to exert antioxidative functions. These compounds belong mainly to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes [5]. The principal antioxidative components of rosemary extracts are the phenolic diterpenes carnosol, carnosic acid (the most abundant) and rosamaric acid (Fig. 1).carnosol and carnosic acid exert potent anti-inflammatory and anti-carcinogenic properties [6]. They impair the proliferation of several cancer cell lines and induce apoptosis [7-12].



Carnosic Acid Carnosol Rosamarican Acid

Fig-1: Chemical structure of the three major antioxidative compounds in rosemary extracts

Tea (*Camellia sinensis*) refers to the aromatic beverage prepared from cured leaves by hot or boiling water [13]. Tea is the second most popular drink in the world [14]. The green tea is relevant in the terms of preventive effect on metastasis of lung, breast cancer [15], prevention of inflammation and thrombosis [16], preventive effect on atherosclerosis and decreasing cholesterol concentration in the blood [17]. The antioxidant activities have been established for the green tea by the ability to bind and neutralize the free radicals [18]. Catechins which is a fraction of flavonoids are the basic phenolic compounds in green tea (especially the main compound, epigallocatechin-3-gallate, Fig-2) are responsible for antioxidant activities [19, 20].

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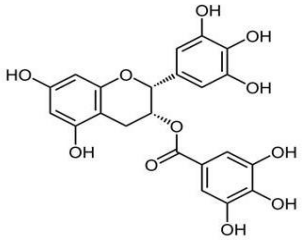

Fig-2: The epigallocatechin-3-gallate compound.

The present work is a comparative study between rosemary and green tea leaves throughout their abilities as antioxidants by using two different methods, and find out, which of them could be recommended as a natural preservative in foods.

2. MATERIALS AND METHODS

The rosemary and green tea leaves were locally obtained, cleaned and ground. 20 gr of ground material was extracted by 250 ml distilled water or ethanol 95% at boiling point, under reflux for 1 hr. The extractive wasfiltered and evaporated at 50^{o}C to the compete dryness.

2.1 *Determination of total phenolic compounds*

A Folin-ciocalteu's colorimetric method was used as described by Ayoola et al. (2008)[21]. To a 0.5 ml of (1 mg/ml) extract a 2.5 ml of a ten-fold diluted Folin-ciocalteu's reagent and 2ml of 7.5%.sodium carbonate solution were added before the reaction allowed standing for 30 min at room temperature. The absorbance was recorded at 760 nm by using UV/VIS Spectroscan 80 D spectrophotometer. The total phenolic compounds were determined according to gallic acid standard curve (0.01 to 1 mg/ml) (Fig. 3)

2.2 *Determination of flavonoids*

The total flavonoids in aqueous and ethanolic extracts were determined according to Rao et al, (2012) [22]. 1 ml extract solution (1mg/ml) was placed in 10 ml volumetric flask. 5 ml of distilled water and 0.3 ml of 5% $NaNo_2$ solution were added. After 5 min 0.6 ml of 10% $AlCl_3$ was added. 2 ml of 1M NaOH solution was added after another 5 min, and the volume was made up to 10 ml with distilled water. The mixture was mixed thoroughly and the absorbance was measured at 510 nm. The total flavonoids were expressed as µg catechin equivalents per gram dry matter according to catechin standard curve (Fig-4).

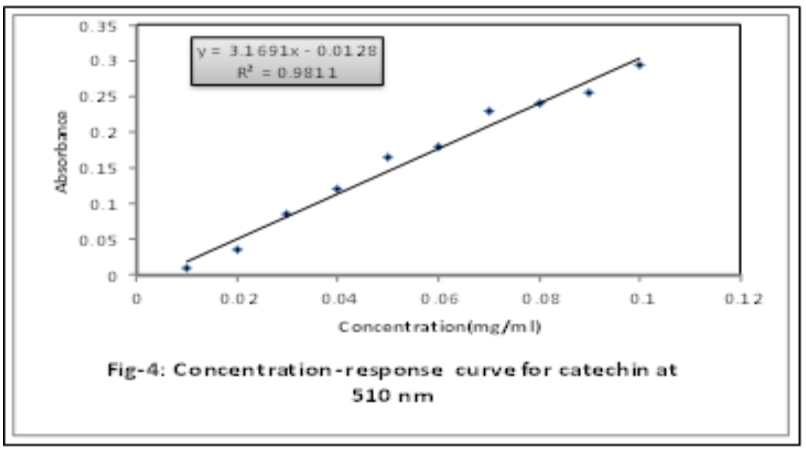

Fig-4: Concentration-response curve for catechin at 510 nm

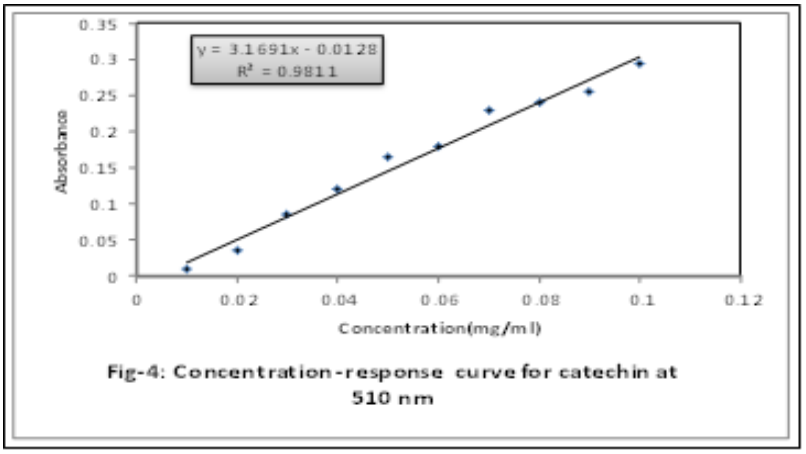

Fig-4: Concentration-response curve for catechin at 510 nm

2.3 *The assay of antioxidant activity*

2.3.1 *The reducing power*

The reducing power was estimated as described by Chou et al.(2009) [23]. 1ml extract of (2-10 mg/ml) was mixed with 2.5ml of 1% potassium ferric cyanide and 2.5ml of 0.2M (pH, 6.6) of sodium phosphate buffer, and incubated at 50c^{o} for 20 min. To stop the reaction, 2.5ml of 1% trichloroaceticacide (TCA) was added to the mixture and centrifuge for 10 min at 3000 rpm. 0.5ml of the supernatant was mixed with 1ml of 1% ferric chloride and stand for 10min.The absorbance was measured at 700nm. 0.02% of BHT used as reference.

2.3.2 *The chelating ability*

Chelating ability was determined according to Su et al. (2008) [24] with some modification. 1ml of (2-10mg/ml) extract was mixed with 0.2ml ferric chloride of 2mM and 0.2ml 8-Hydroxyquinoline (5mM). After 10min at room temperature, the absorbance was determined at 562nm.The EDTA-Na_2 was used as reference.

3. RESULTS AND DISCUSSION

Polyphenols are widely appreciated for their potential beneficial health effects, like antioxidant activity [25]. Table-1, shows the percentages of total phenolic compounds and flavonoids which are represent the main antioxidant compounds in aqueous and ethanolic extracts of rosemary and green tea leaves. The total phenolic compounds which expressed as gallic acid and flavonoids as catechins were determined according to standard curves, phenols were determined by Folin-Ciocalteu's colorimetric method and flavonoids by aluminum chloride colorimetric method. As shown, the total phenolic compounds in both, aqueous and ethanolic extracts of green tea leaves are higher than rosemary leaves which refer that, the antioxidative activity of the tea leaves will be more effective as compared with rosemary leaves for the both extracts. The high percentages of the total phenolic and flavonoids in alcoholic extract mean that, the ethanol as extracting solvent and according to the chemical composition of phenolic compounds are more effective than water [26].

Table-1: The total phenolic and flavonoid contents of rosemary and green tea leaves extracts (on dry-basis.

The plant	Extract	% Phenolic compounds	% Flavonoids
Rosemary leaves	**aqueous**	13.44	9.54
	ethanolic	18.75	12.65
Green tea leaves	**aqueous**	39.38	17.69
	ethanolic	48.44	22.70

Free radicals are naturally formed in a wide range of biological as well as chemical systems. They are chemical stable atoms and molecules, which have one (or rarely more) free electron / electrons in the electron envelope [27, 28]. The free radicals are responsible for many pathological processes and cause important secondary damage to the biological systems and cells [29-32]. The antioxidant activity of the compound (or mixture of compounds) to inhibit oxidative reaction of various biomolecules (e.g. prevent the peroxidation of lipids). As shown in Figs 5 and 6, which are refer to the reducing power method for the determination of the antioxidative abilities of aqueous and ethanolic extracts of rosemary and green tea leaves (as compared with BHT as a reference), that there was a similarity in the way of how they are acting, in spite of, the total phenolic compounds in ethanolic extracts in both plants are more than that of aqueous extracts. This will depend on the kinds of phenols those were available in each extract at a certain concentration.

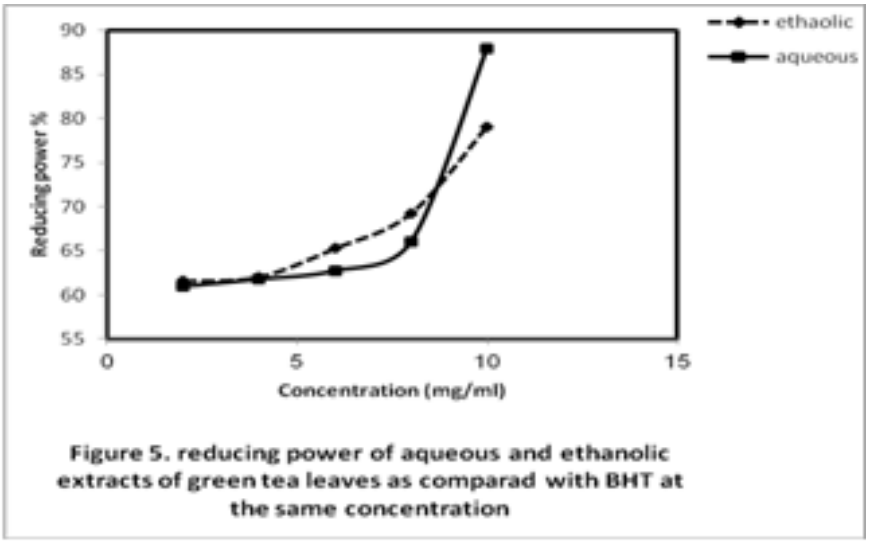

Figure 5. reducing power of aqueous and ethanolic extracts of green tea leaves as comparad with BHT at the same concentration

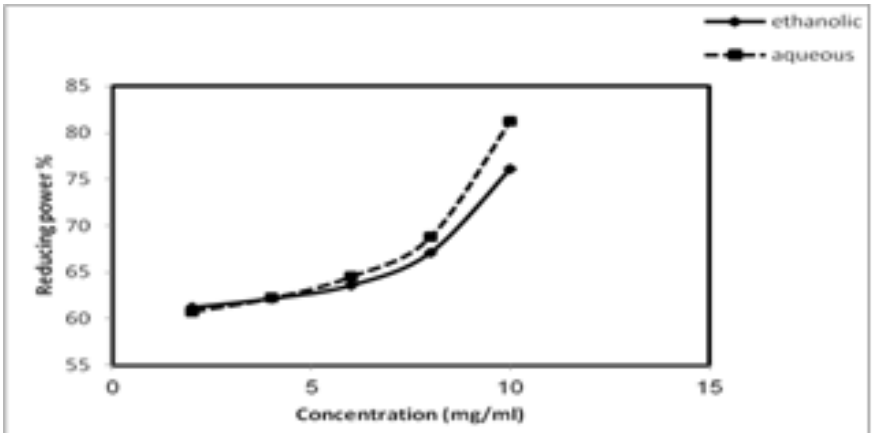

Figure 6. reducing power of aqueous and ethanolic extracts of rosmary leaves as comparad with BHT at the same concentration

Almost, the same mode of action is also associated with the second method of determination (the chelating ability, Figs 7 and 8). As shown in Figs 7 and 8, the abilities of aqueous and ethanolic extracts of rosemary and green tea leaves, as chelating agents (comparing with EDTA as a reference) are less than their abilities as reducing power.

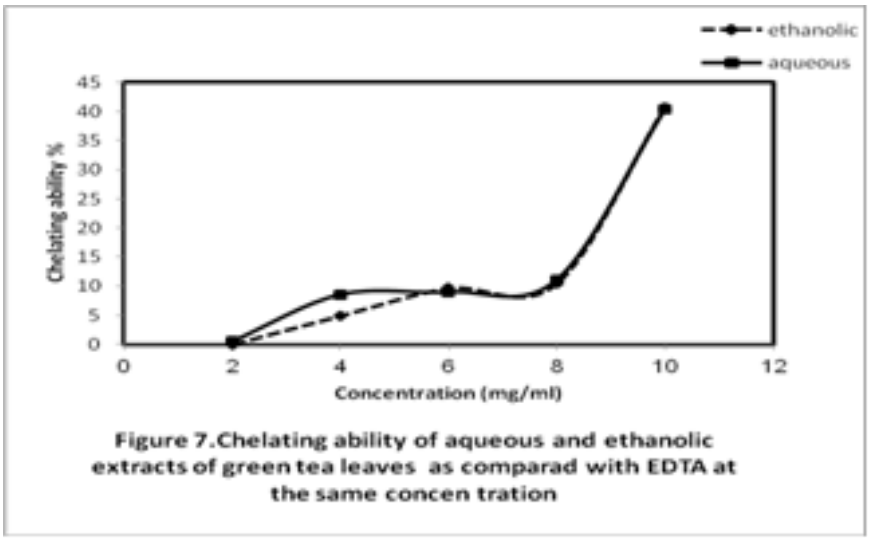

Figure 7.Chelating ability of aqueous and ethanolic extracts of green tea leaves as comparad with EDTA at the same concen tration

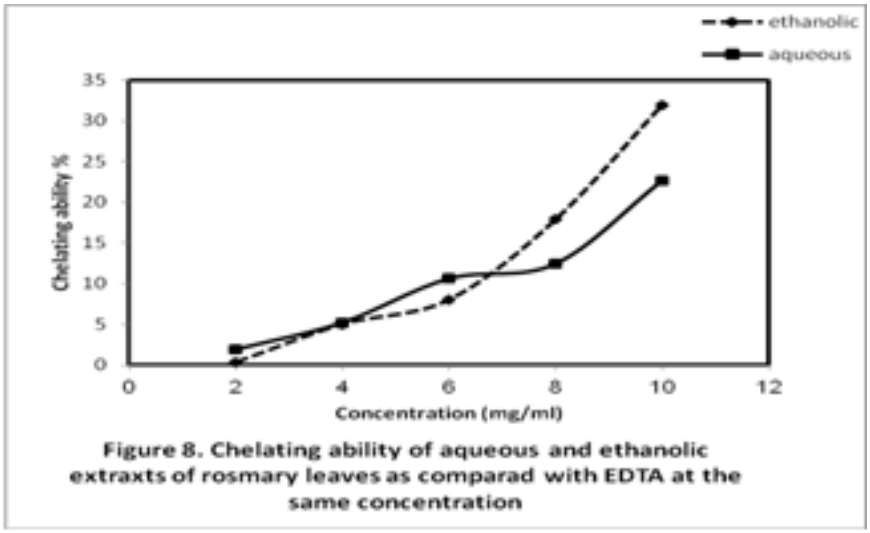

Figure 8. Chelating ability of aqueous and ethanolic extraxts of rosmary leaves as comparad with EDTA at the same concentration

Determination of the antioxidant activity is one of the ways how to biologically and nutritionally evaluate the quality of the fruit. It has been proved that antioxidant activity depends on the type of phenolics present in the plant, as some phenolic compounds exhibit higher antioxidant activity than others [33-38].

As shown in Fig.9, which refer to the activity of ethanolic extracts for the both plant leaves (rosemary and green tea), the ethanolic extract of green tea shows, to some extant, high reducing power ability as compared with rosemary, especially for the concentrations above 4 mg/ml. In Fig. 10, which represents the ability of aqueous extracts for the both plant leaves, the ability of aqueous extract of the rosemary leave shows high percentages of reducing power as compared with green tea, especially for the concentrations from 4 mg/ml to 8 mg/ml.

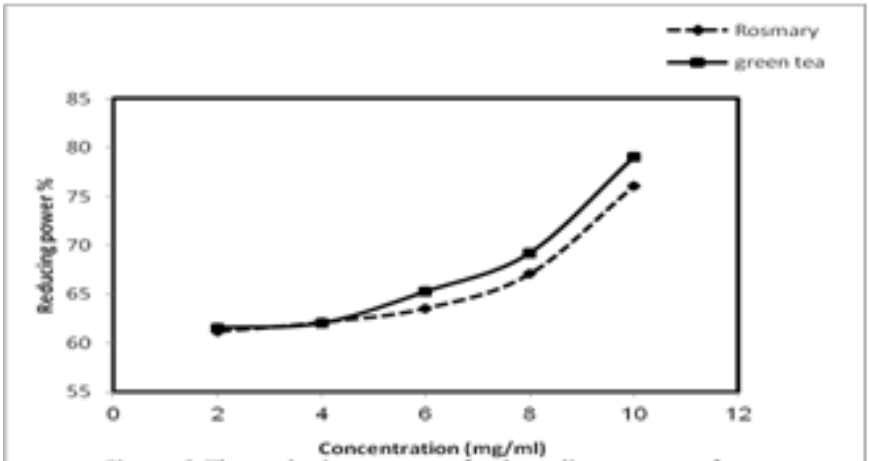

Figure 9:The reducing power of ethanolic extracts of rosmary and green tea leaves compared with gallic acid as a reference at the same concentrations

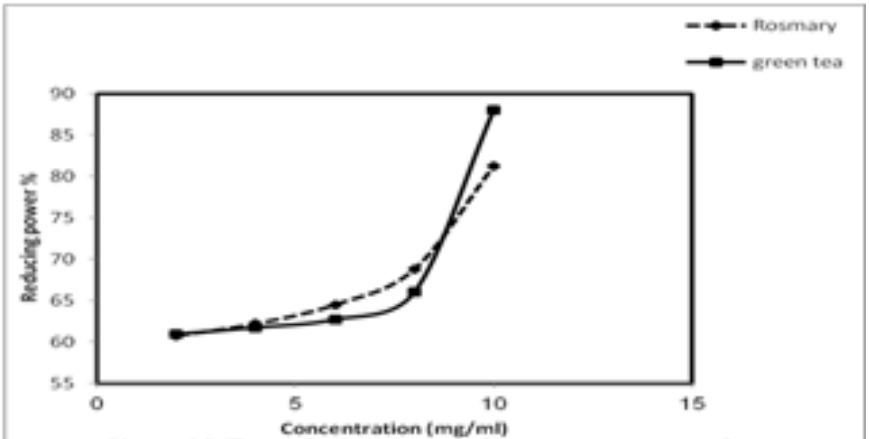

Figure 10:The reducing power of aqueous extracts of rosmary and green tea leaves compared with gallic acid as a reference at the same concentrations.

Figs 11 and 12 show the chelating abilities for the aqueous and ethanolic extracts of rosemary and green tea leaves. It is clear, that the active compounds in rosemary extracts (especially the ethanolic) having high ability as chelating agent comparing with green tea.

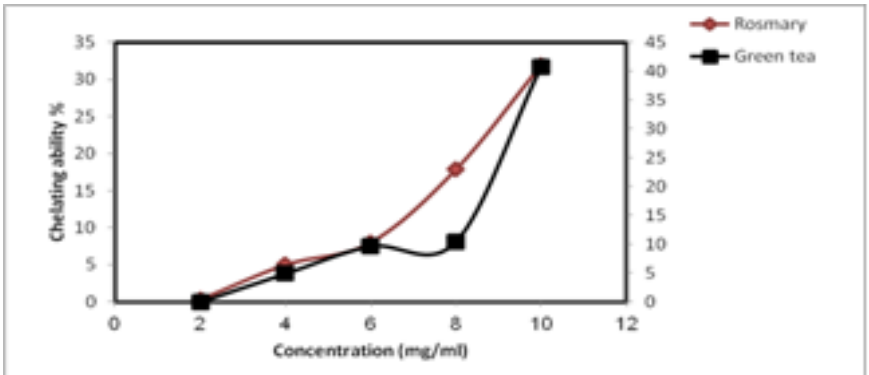

Figure 11:Chelating ability of ethanolic extracts of Rosmary and green tea leaves as comparadwith EDTA at the same concentrations.

Carnosic acid is the most abundant antioxidant substance found in the leaves of the rosemary plant and is the main compound responsible for its antioxidant activity [39]. Its radical scavenging activity follows a mechanism which is explained by the presence of two O-phenolic hydroxyl groups found at atoms C_{11} and C_{12} [40].
Rosemary can inhibit lipid oxidation, chelating metals and scavenge superoxide radicals. Nakatani (2003) [41] reported that phenolic diterpenes from rosemary are particularly antioxidative. The antioxidant activity of carnosic acid is more than twice that of any other phenolic diterpene. It has several times the antioxidative capacity of BHT and BHA [42]. Furthermore, carnosic acid and carnosol chelate iron and scavenge peroxyl radicals, especially in lipid-based systems [43].

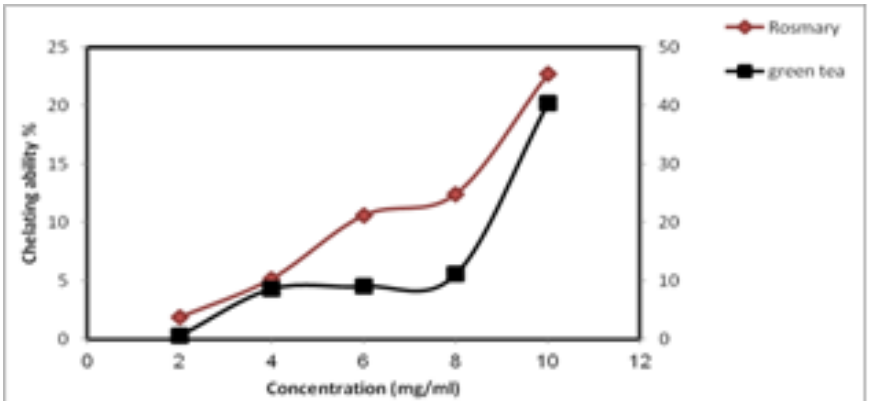

Figure 12:Chelating ability of aqueous extracts of rosmary and green tea leaves as comparad with EDTA at the same concentrations.

Green tea has substantial antioxidative activity, much of which appears to be due to natural flavonoids. Antioxidant activity of green tea infusions appears to be linearly related to phenol content [44]. Catechins, polyphenolic flavonoids in green tea, are particularly effective free radical scavengers [45]. The primary catechin polyphenol constituent and major peroxyl-radical-scavengeing compound is (-)-epigallocatechin-3-gallate [46, 47].

On the average, 65-70% of population is excessively impacted by oxidation stress caused by free radicals. Therefore, oxidative stress monitoring is an important part of reasonable health prevention [48-51].

4. CONCLUSION

In general, the ethanolic extracts of rosemary and green tea leaves are high in phenolic compounds as compared with aqueous extracts. The green content of phenolic compounds of both aqueous and ethanolic extracts about 3-fold comparing with rosemary. Although, there were differences in their phenolic content, rosemary and green tea leaves extracts gave almost similar mode of action as antioxidants (May due to the type of phenolic compounds in each plant). The phenolic compounds in rosemary leave (mainly, carnosic acid, carnosol and rosamaric acid) and green tea leaves (catechins, mainly epigallocatechin-3-gallate) gave high reducing power ability rather than chelating agents. As a result, we are fully recommended the extract of the both plant leaves, especially the rosemary, as a natural preservative in the food systems.

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