Studying the Antioxidant Activity of Some Plants Extracts Tested by Hydroxyl Radical Scavenging

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Abstract. This investigation was carried out to study four types of herbs and spices, which were cumin fruits (cuminum cyminum), Black pepper seeds (Piper nigrum), Sage leaves (Salvia officinalis) and Pinus needles (Pinus halipensis). Three kinds of extracts namely Aqueous, Ethanolic and essential oil extracts for each plant were prepared and examined. Commercial antioxidant BHA (Butylated hydroxyl anisole) and BHT (Butylated hydroxyl toluene) were also employed. The antioxidant activity of the extract were estimated by determination the ratio hydroxyl radical scavenging. Results showed that some plant extracts test possessed effective antioxidant high excelled for antioxidants Industrial (BHT) and (BHA). Recorded the highest rate to hydroxyl radical scavenging for alcohol leaves sage extract (SE), which amounted to 49.97%, followed by the value of scavenging to extract alcohol for the fruits of cumin which was 47.88%, which exceeds the proportion scavenging recorded by antioxidants industrial BHA and BHT follows reached 36.77 and 46.97% respectively. Ranged values hydroxyl radical scavenging for the rest of aqueous extracts and a alcoholic and essential oil of these plants between (40.8% and 23.91), while the less extracts efficiency in hydroxyl radical scavenging was for an alcoholic extract of black pepper seeds (BE) as the percentage of 2.95% scavenging.

Keywords: Scavenging, Antioxidant, Plants extracts, Hydroxyl Radical.

1. Introduction

Free radicals can be described as chemical species that have an unpaired electron. The reactivity of free radicals varies from relatively low, as in the case of the oxygen molecule itself, to very high as in the case of the short-lived and highly reactive hydroxyl radical (OH) [1]. Free radicals induced oxidative stress damages cellular component leading to many human diseases plant-derived antioxidant compounds have become a profitable alternative to prevent oxidative stress in cell [2]. An imbalance between free radical formation and radical scavenging capacities causes oxidative stress that extensively damages all components of cell including protein, lipids, and DNA, ultimately leading to many diseases such as cancer, atherosclerosis, cardiovascular, ageing and inflammatory diseases [3], [4]. Addition of antioxidant is effective in delaying the oxidation and extending the shelf life of food [5]. In the food industry, the oxidative deterioration of fats and oils is prevented by synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT). Although preventing oxidation, only a few synthetic compounds are currently approved for use in the food industry because of their potential toxicity and carcinogenic [6]. Currently used synthetic antioxidant have been suspected to cause or promote negative the health effects, hence stronger restrictions have been placed on their application and there is a trend substitute them with naturally occurring antioxidants[7]. To satisfy consumers’ preference for natural food additives over synthetic ones, there is increasing importance searching for natural antioxidant from herbs, fruits, vegetable and spices as a less harmful alternatives to synthetic antioxidant [8]. Thus many essential oil water extract or plant extract have been qualified as natural antioxidant and would have obvious advantages in relation to certification and use in food [9], [10]. The current investigation was carried out to study the antioxidant activity to four types of

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herbs and spices, which were cumin fruits (cuminum cyminum), Black pepper seeds (Piper nigrum), Sage leaves (Salvia officinalis) and Pinus needles (Pinus halipensis), as a natural antioxidant agent by hydroxyl radical scavenging.

2. Materials and methods

2.1. Raw materials

Been buying spices from local markets in the city of Baghdad and collected leaf pine needle trees in Jadriya near Baghdad University and classified samples in lush national-General Authority for examination and certification of seeds-the Ministry of Agriculture-Abu-Ghraib-Baghdad. Grinding each individual plant in a small electric grinder and put each plant powder in a sealed glass case.

2.2. Preparation of plant extracts

Attended the aqueous extract each individual plant water extract, alcoholic extract and essential oil extract, water extract was attended by way Sakai and his group [11] The extraction was conducted at 1: 5 (Powder plant: distilled water), while alcoholic extract was attended by [12] the extraction was conducted at 1:2 (Powder plant: alcoholic extract 99.9%).

Were conducted stirring for extractors of water and alcohol in shaker incubator (28 C for 24 hours) were filtering by Buchner funnel on filter paper type Wattman (number 1) and then concentrate on rotary evaporator at 40 C. Were conducted drying in an electric oven at temperatures of 40 C until get a dry powder.

Essential oil extracted by Clevenger distillation using water (Hydro distillation), described by, [13]. Extracts collected and placed each sample in a glass tube sealed and stored in a freezer at -18 until use.

2.3. Hydroxyl radical scavenging method

Used this method as described by[14]. And adopted in measuring the capture or remove the radical hydroxyl to compete happening between ribose penta sugar Loose oxygen (Deoxyribose) and plant extract, the radical hydroxyl emerging from a system composed of ferric Fe +3 / Ascorbate / EDTA / Pure hydrogen oxide H2O2. The free radical which free from penta sugar loose oxygen (Deoxyribose) due to conform Thiobarbaturic reactive substances(TBARS)[15].

Attended 0.04% concentrate of plant extracts and that by weighing 0.0012 mg of each of the plant extracts, as well as attended 0.02% concentrate of (BHA) and (BHT) and complete solution of the reaction mixture to the size of 3 ml, which consists solution reaction mixture of 3 mM penta sugar Loose oxygen (Deoxyribose), 0.1 mM Molar ferric chloride FeCl3, 0.1 mM Molar EDTA, 0.1 mM of ascorbic acid , 1 mM peroxide hydrogen and 20 mM solution phosphate buffer (Phosphate buffer) at No. pH (pH = 7.4) so that the final size of each extract with the reaction mixture by 3 ml after incubating the tubes in an incubator temperature 37 ° C for a period of one hour. Take 1 ml of reagent TBA (Thiobarbituric acid) (1)% and (0.1) ml of tricoloric acetic acid (TCA) added 2.8% to test tubes containing extracts and the reaction mixture is placed in a water bath at 100 C for (20) minutes. After cooling absorbance is measured at a wavelength of 532 nm against the blank, which included penta sugar (Deoxyribose) and buffer solution (Buffer) only. The extent of inhibition on the basis of penta sugar crash loose oxygen (Deoxyribose) percentage is calculated as follows:

S.P=[A0-(A1–A2)]*100/A0
S.P. =Percentage of hydroxyl radical scavenging
A0=Blanka bsorbency
A1 = Control absorbency present penta sugar crash loose oxygen (Deoxyribose)
A2 = Sample (extract) absorbency with penta sugar crash loose oxygen (Deoxyribose)

3. Results and Discussion

Fig.1. showed the effectiveness of plant extracts tested concentration (0.04)% in scavenging Hydroxyl Radical depending on the percentage of the curb or scavenge , the extracts have antioxidant activity in biological systems compared with antioxidants industrial concentration 0.02% BHT and BHA. Where the
results showed the viability of plant extracts tested and components in curbing hydroxyl radicals in particular in focus (0.04) %, while at least did not show the focus (0.02) % of such susceptibility. Outweigh extract sage alcohol (SE) and extract cumin alcohol (CE) and concentration (0.04)% antioxidants Industrial (BHT and BHA) concentration (0.02)% in curbing roots hydroxylated rate reached braking (Scavenging Percentages) for the latter (46.97 and 36.77) % And, respectively, while the extracts (SE and CE) is (49.97 and 47.88)%, respectively, as of the same shape that the rest of the plant extracts and concentration (0.04)% showed susceptibility curb less than antioxidants industrial curb radical hydroxylated and can order of preference in curbing the radical hydroxyl of plant extracts concentration (0.04)% and compared with the industrial antioxidants (BHT and BHA) concentration (0.02)% as follows:

BE <BO <BW <SO <CO <CW <SW <PW <BHA <BHT <CE <SE .

Fig. 1: Antioxidant activity of plant study extracts with 0.04% concentrate compared with industrial antioxidant BHA and BHT with 0.02% concentrate by the percentage of hydroxyl radical scavenging (S.P) %.

Showed Fig. 2. portability aqueous extracts of plants tested concentration 0.04% to rein in radicals hydroxyl and clear top efficiency aqueous extract of leaves pine needle by 40.8%, followed by aqueous extract of leaves sage by 31.71% and then extracts cumin and black pepper water (28.65, 30.58) on respectively and are less efficient than the industrial antioxidant BHT and BHA concentration (0.02)%.

Efficiency can be explained by pine extract water (PW) high between aqueous extracts, for its high materials from Al-taninat without rest aqueous extracts as well as it contains flavonoids and this is what was understood by examining chemical evidence of active compounds to study plants by[16].

Showed Figure3. portability extracts aromatic plants study in hydroxyl radical scavenging compared with industrial antioxidants. Cumin essential oil extract(CO) superiority and was more active on that of black pepper (BO) in hydroxyl radical scavenging with value (29.72 and 23.91)% respectively, these values less efficient from industrial antioxidants in hydroxyl radical scavenging as show flow: BO<CO<BHA<BHT

The results obtained are consistent with[17] who indicated that the essential oil of cumin and black pepper has effective anti-oxidant are almost high as it contains chemical composition of the compounds phenolic compound and phenols multiple that have the ability to link with hydroxyl radical and curbed and thus minimize the reaction of oxidation in biological systems.

Fig. 4. showed the impact of alcoholic extracts of test plant as anti oxidant, as seems high efficiency of two alcoholic extracts : cumin (CE) sage (SE) in curbing or scavenging percentage (47.88 and 49.97)%, respectively, while less scavenging percentage was for black pepper (BE) by and was ranked efficiency compared with industrial antioxidant as follows:

BE <BHA <BHT <CE <SE.

Fig. 5. The efficiency of cumin fruit extracts in scavenging hydroxyl radicals (S.P.) as superiority cumin alcoholic extract on cumin others, amounting to efficiency values curb hydroxyl radicals compared with industrial antioxidants where the sequence preference results in braking is as follows:

CO <CW <BHA <BHT <CE
Also appears to outweigh the alcoholic cumin fruit extract on other cumin extracts antioxidants by measuring the proportion of scavenge the hydroxyl radicals amounted values (S.P.) cumin extracts and efficiency in the order above compared with antioxidants industrial namely: (29.72, 30.58, 36.77, 46.97, 47.88) %, respectively.

Fig. 2: Antioxidant activity of water extracts with 0.04% concentrate compared with industrial antioxidant BHA and BHT with 0.02% concentrate by the percentage of hydroxyl radical scavenging (S.P) %.

Fig. 3: Antioxidant activity of essential oil extracts with 0.04% concentrate compared with industrial antioxidant BHA and BHT with 0.02% concentrate by the percentage of hydroxyl radical scavenging (S.P) %.

Fig. 4: Antioxidant activity of alcoholic extracts with 0.04% concentrate compared with industrial antioxidant BHA and BHT with 0.02% concentrate by the percentage of hydroxyl radical scavenging (S.P) %.
Fig. 5: Antioxidant activity of Cumin fruit extracts with 0.04% concentrate compared with industrial antioxidant BHA and BHT with 0.02% concentrate by the percentage of hydroxyl radical scavenging (S.P) %.

From the top the aqueous extract of the seeds of black pepper efficient on the other black pepper extracts in scavenging hydroxyl radical due that to its content of flavonoids which proved detect chemical initial plant the (BE) was the least efficient among extracts pepper However, the efficiency of black pepper extracts are all lower than those for industrial antioxidants ,so that agree with [18],[19] said that the black pepper extracts have a limit antioxidant activity.

Fig. 6: Antioxidant activity of Black pepper extracts with 0.04% Concentrate compared with industrial antioxidant BHA and BHT With 0.02% concentrate by the percentage of hydroxyl radical scavenging (S.P)%.

Fig. 7: Antioxidant activity of sage extracts with 0.04% concentrate compared with industrial antioxidant BHA and BHT with 0.02% concentrate by the percentage of hydroxyl radical scavenging (S.P) %.

Fig. 6. The efficiency of the black pepper plant extracts in scavenging hydroxyl radicals were as follows: BE <BO <BW <BHA <BHT

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Fig 7. showed the ability of sage extracts as antioxidant in scavenging hydroxyl radical the result were as follow: SO <SW< BHA< BHT <SE

Results were obtained in accordance with [14] who show that efficiency extract (SE) to have higher ability to scavenging hydroxyl radical and higher than (BHT) but we did not agree with him in that : were alcoholic sage extract not better than from essential oil sage extract (SO), and that due to alcohol extraction
method and the type of alcohol which used and its concentration and that is affected by polarity of phenolic compounds, especially multi them during the extraction process as he (Tepe) did not use ethyl alcohol in the extraction but methanol. The free radical is a single electron, very active could interaction with fat, sugar, peptide, poly peptide, nucleotides, organic acid, thiamine and quanosin and caused cell degradation [20]. These hydroxyl radicals can arise from the interaction of iron \( \text{Fe}^{2+} \) or \( \text{Cu}^{2+} \) with hydrogen peroxide (\( \text{H}_2\text{O}_2 \)).

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^-
\]

Through experiments chemical detection efficiency plant extracts in the inhibition of the oxidation and its role as anti oxidant concluded that extracts of certain plants experimental and in particular the SE and CE were the most efficient in the event due to the extract alcoholic and specially ethanolic extract have ability to dissolve active compounds such as Tannins and Polyphenol Filavonolat Flavonoid the aqueous extracts contain Tannins and Anthocyanins, a natural compounds very effective in curbing the hydroxyl radicals [21] which is effective materials related to the inhibition of oxidation in biological systems. As for the aqueous extracts of leaf pine is one of the most efficient aqueous extracts compared to the rest aqueous extracts of plants study and following efficiency extracts SE and CE as it contains dragons addition to flavonoids, while the rest of aqueous extracts contain flavonoids only by detecting chemical initial compounds and aggregates effective [16]. Any change in the order of efficient plant extracts as anti oxidant on the basis of effectiveness inhibitory antioxidant (Antioxidant agent) depending on the methods used to measure this event, due to the variety of ways first, and then the different material basis for each method where different effectiveness depending on these factors and material oxidation used. This is consistent with what the [22], who used two methods to measure the effectiveness of anti-oxidant tea extracts different is Beta-carotene bleaching, DPPM scavenging assay radical, where he was Order efficiency tea extracts as anti oxidant first method is green tea> tea fermented> tea is fermented> black tea while the sequence in the second method is green tea> black tea> fermented tea> tea is fermented. As well as between[23], different installation efficiency essential oil plant turmeric \textit{Curcum zedoaria} and its parts (Fraction) different methods of measurement. Terms used method of measuring the power of reductionism (Reducing Power) and hydroxyl radical scavenging (Scavenging effect), where he was Order efficiency way first F4.... Have been observed efficiency, an antioxidant industrial (BHT) and concentration (0.02)% antidote antioxidant oil sunflower when estimated on the basis of the value of acid AI thiaibarepettyoric acid (TBA) Malone Aldihyde / kg oil well amenability in sniper roots hydroxylated when measuring the percentage of sniper radical scavenging (%) compared with (BHA) and the rest of the plant extracts and agreed with[24], which between BHT and with 0.5 mg / g oil has the highest effectiveness inhibition in impaired antioxidant olive oil when storage oil for (80-10) on the temperature 45 C, where the figure was Alperoxide number 15.2 mile equivalent / kg oil after 80 days storage.

4. Conclusion

All study plant extracts have antioxidant activity some of them were better than industrial antioxidant BHA and BHT like SE and CE extract by scavenging hydroxyl radical, so we found new natural antioxidant agent we recommended to study the toxic dose of each plant extract which use in food preservative.

5. References


