A study of antioxidant potential of Perilladehyde

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A study of antioxidant potential of Perilladehyde

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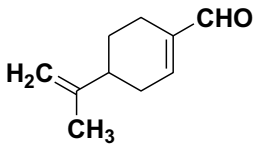
Abstract. The use of plants as food, medicine is credited to a biological property of their secondary metabolites. These naturally occurring secondary metabolites are found to have great importance in controlling the formation of free radicles. These antioxidants are capable to catch the free radicles present in the body and maintain its balance. Antioxidant activity and potency of Perillaldehyde using various *in vitro* biochemical assays were studied. The assay involves various levels of antioxidant action such as free radical scavenging activity through DPPH, reducing power determination, nitric oxide scavenging ability, metal chelation power, scavenging of hydrogen peroxide, membrane stabilizing activity, and lipid peroxidation study.

1. Introduction

The safety of synthetic antioxidants is always questioned and stimulated the need of naturally occurring antioxidants. Perillaldehyde (4-(prop-1-en-2yl) cyclohexa-1-enecarbaldehyde) is a monoterpenoid abundantly found in annual herb Periilla. Perillaldehyde is commonly used as food additive and in perfumery.



4-(prop-1-en-2-yl)cyclohex-1-enecarbaldehyde

Free radicals are the chemical species having odd number of electrons in their valence shell thus are highly reactive and unstable[1]. These can either be organic (quinones) or inorganic molecules (O_2^-) [2,3] . Activities of these are vital in normal functioning of various biological systems. Physiologically they are produced by the catalytic action of various enzymes[4,5], electron transport chain of cellular respiration (ATP biosynthesis)[6], and immunological defense (oxygen & nitrogen reactive species)[7]. Besides the physiological processes exposure to ionization radiation, radiations from mobile phone[8], several environmental agents (drugs capable of free redox cycling, xenobiotic capable of forming free radical) cause free radical generation in the body (OH^-)[9]. These free radicals undergo secondary reactions with the cellular oxygen or macromolecules.

This leads to cell death, fetus or germ cells teratogens[8], and carcinogenesis [10]. Recently, researchers have proved that their implication into the number of pathophysiology of different dreadful diseases such as diabetes[11], neuropathy[4], cardiovascular diseases[12], cancer[13,14] and aging[15,16].

Antioxidant is an exogenous or endogenous compound that inactivates an oxidant [17,18]. The defensive action is given by one or more mechanisms [19]. Also, it donates an electron to end the ever searching of electron cascade of free radical. It produces inducing effect to the glutathione peroxidase, catalases to remove the free radical. It helps through transition metal ion chelation responsible for free radical generation. It induces the repair machinery of the cell along with the clearance system (enzyme Methionine sulfoxide reductases) to remove the damaged biomolecules before they reach alarming threshold to alter the cellular metabolism and viability. Thus bringing in the need to develop and access the compounds having anti- oxidant property in order to develop an effective and efficient therapeutics to deal with oxidative stress[20].

2. Experimental

The test sample perillaldehyde was purchased from Sigma-Aldrich with purity of 99.99%. DPPH, $FeCl_2$, Ferrozine, H_2O_2, sodium nitroprusside, Naphthylethylenediaminedihydrochloride, sulfanilamide (Griess Reagent), potassium ferricynaide, trichloroacetic acid were used for the study. All the chemicals were bought from Sigma-Aldrich and are of analytical grade. Methanol (purity 99.99%, AR grade) and phosphate buffer were used as solvent. Phosphate buffer was prepared according to I.P. guidelines.

2.1 Scavenging of hydrogen peroxide

A stock solution of perillaldehyde (test sample) and ascorbic acid (standard) having concentration 1000µg/mL was prepared and diluted successively to get the final concentration of 10, 25, 50, 75 and 100 µg/mL. A 40mM solution of hydrogen peroxide in phosphate buffer (pH 7.4) was prepared. 0.6 mL of the respective test/standard sample was added to 3.4 mL hydrogen peroxide solution. The resulting mixture was mixed well and incubated for 10 minutes at 37°C. Absorbance of the mixture was measured at λ 230 nm in UV-visible spectrophotometer (UV 1601, SHIMADZU). The hydrogen peroxide solution alone was used as control. All the experiments were run in triplicate. The percentage scavenging activity was measured as follows.

Percentage scavenging activity = [(absorbance of control – absorbance of sample)/absorbance of control] x 100

2.2 Metal chelating activity

Stock solution of sample and standard having concentration of 1000µg/ml were prepared and diluted to get different concentrations of 10, 25, 50, 75 and 100 µg/mL, 5 mL of the test/standard solution was mixed with 0.1 mL of 2.0 mM $FeCl_2$ solution. To this solution 0.2 mL of 5mM ferrozine solution was added. The resulting mixture was incubated under shaking condition at room temperature for 10 minutes to complete the reaction. The absorbance was measured in a UV-Visible spectrophotometer (UV 1601, SHIMADZU) at λ 562 nm. All the reactions were run in triplicate. The percentage inhibition of ferrozine-Fe^{2+} complex formation which is directly proportional to activity was calculated as follows.

Percentage of inhibition = [(absorbance control- absorbance sample)/absorbance control] x100

2.3 Determination of reducing power

Oyaizu's method was followed for determination of reducing activity (potential) of the essential oil and compared with ascorbic acid as standard. Stock solution of test and standard was prepared having concentration of 1000µg/mL in ethanol and diluted consecutively to get concentrations of (test/standard sample) 10, 25, 50, 75 and 100µg/mL. 1.0 mL of the ethanolic solution of different concentrations of sample/test were mixed with 2.5 mL of 0.2M phosphate buffer (pH 6.6) followed by addition of 2.5mL of 1% (W/V) of potassium ferric cyanide. The resulting solution was incubated for 20-30 minutes at 50°C. 2.5mL10% (W/V) trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes. Then, 2.5mL of upper layer was pipetted out and diluted with 2.5 mL of water. 0.5mL of 0.1% (W/V) freshly prepared ferric chloride was added. Absorbance was measured at λ700 nm in UV-Visible spectrophotometer (UV 1601, SHIMADZU) to estimate the formation of prussian blue coloration and indirectly concentration of Fe^{2+}. All the reactions were carried out in triplicate. The following formula was used to find out the percentage reducing potential.

Percentage reducing potential = [(absorbance of control – absorbance of sample)/absorbance of control] x 100.

2.4 Nitric Oxide scavenging activity

Stock solution with concentration 1000µg/mL was prepared and diluted successively to get different concentration of 10, 25, 50, 75 and 100µg/mL of test/standard sample. Sodium nitroprusside is used to generate nitric oxide[21] and measured through nitrite accumulation, formed due to reaction between oxygen and nitric oxide, by using Griess reagent[22]. 0.5mL of various concentrations of test/standard sample is mixed with 2mL of 10mM sodium nitro prusside in phosphate buffer (pH 7.4) and the mixture is incubated for 150 min at room temperature. 0.5 mL is taken out from the mixture after incubation and 1mL of sulfanilic acid (33% in 20% glacial acetic acid) is added. The mixture is again incubated for 5 minutes at room temperature. To this mixture 1mL of naphthylethylenediamine dihydrochloride (NED) (0.1% W/V) was added and re-incubated for 30 minutes at room temperature. All the reactions were carried out in triplicate. Absorbance was measured in UV-Visible spectrophotometer (UV 1601, SHIMADZU) at λ 540 nm to estimate pink chromophore generated due to diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED. The percentage scavenging activity was calculated as follows.

Percentage scavenging activity = [(absorbance of control – absorbance of sample)/absorbance of control] x 100.

2.5 DPPH radical scavenging assay

1,1- diphenyl-2-picryl hydrazyl radical (DPPH) is a stable organic nitrogen free radical thus used widely for studying free radical scavenging activity of test sample. Stock solution having concentration 1000µg/mL was prepared and diluted to generate various concentrations 10, 25, 50, 75 and 100µg/mL of test/standard sample. 1mL of various concentrations of test as well as standard sample was mixed with 4mL of 100 µM ethanolic DPPH solution. The solution was mixed thoroughly and incubated in dark for 15 minutes at room temperature. Absorbance was measured in UV-Visible spectrophotometer (UV 1601, SHIMADZU) at λ 517 nm. All the reactions were carried out in triplicate. Percentage scavenging activity was calculated as follows.

Percentage scavenging activity = [(absorbance of control – absorbance of sample)/absorbance of control] x 100.

3. Result and Discussion

3.1 H_2O_2 scavenging activity

The percentage scavenging activity of Perillaldehyde is slightly diverged from that of ascorbic acid. The scavenging was relative to the concentration of the test substance. The antioxidant compound donates electron to hydrogen peroxide thus forming a water molecule[23]. As the concentration hydrogen peroxide decreases, the absorbance of the assay mixture falls down. Hence, hydrogen peroxide scavenging activity of Perillaldehyde is marginally lower than ascorbic acid.

Table 1: Hydrogen peroxide scavenging activity

Concentration (µg/mL)	% scavenging activity (ascorbic acid)	% scavenging activity (perillaldehyde)
10	10.217±0.314	7.317±0.210
25	36.853±0.503	11.541±0.246
50	51.101±0.203	20.067±0.164
75	55.860±0.279	38.524±0.091
100	60.703±0.445	47.730±0.209

- Data is represented in terms of mean of the triplicate with standard deviation (as ±)

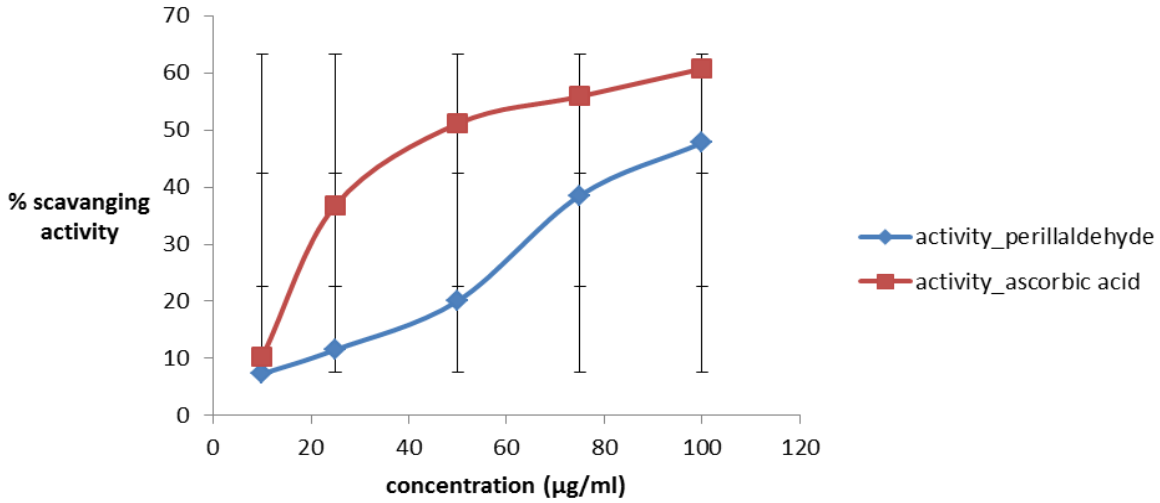


Figure 1. Percent hydrogen peroxide scavenging activity of Ascorbic acid and Perillaldehyde at various concentrations

Scavenging activity of Perilladehyde is proportional to concentration, but it doesn't vary in perfect linear fashion. There is no significant difference in the mean of ascorbic acid / perillaldehyde and these have a good correlation.

3.2 Ferrous ion chelation activity assay

Ferrous ion is the main cause of free radical generation in the body by Fenton reaction[24,25]. Ferrous ion and ferrozine react quantitatively to form a co-ordinate complex. Presence of chelation complex interferes with the formation of this red colored ferrous ion – ferrozine complex and thus brings down the intensity of red color that resulted from ferrous ion –ferrozine complex[26]. (Apetrei, Tuchilus et al. 2011). By virtue of this chelating property of test substance is quantified.

Table 2: Percent ferrous ion chelating activity of ascorbic acid and perillaldehyde

Concentration (µg/ml)	% chelating activity of ascorbic acid	% chelating activity of perillaldehyde
10	36.697±0.140	10.612±0.371
25	52.080±0.296	25.78±0.275
50	70.795±1.063	66.575±0.140
75	74.924±0.769	70.306±0.191
100	87.431±0.195	85.810±0.195

- Values are presented as mean and standard deviation(±) of the triplicate

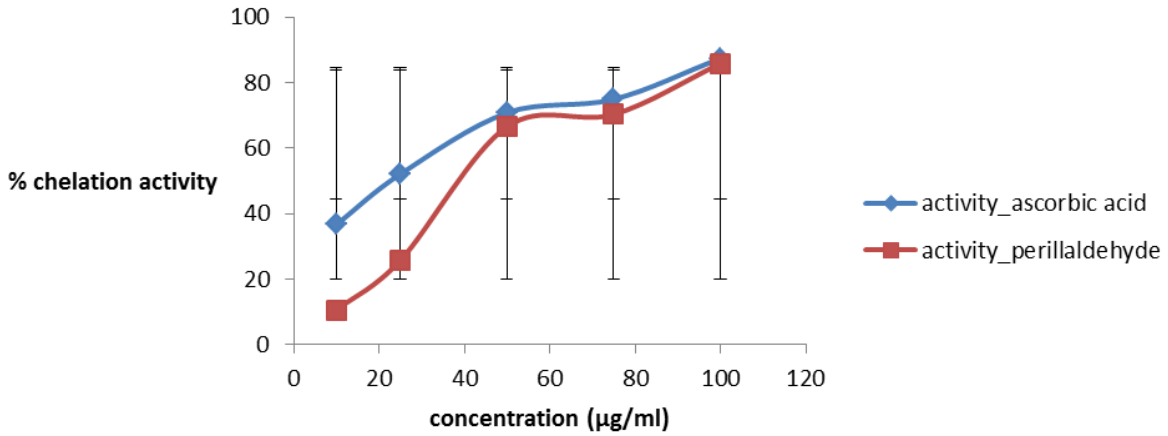


Figure 2. Chelation activity of ascorbic acid and perillaldehyde at different concentrations

Chelation activity of ascorbic acid and perillaldehyde is concentration dependent but don't have perfect linear relation. Activities of both compounds are at par with low standard deviation and good linear correlation.

3.3 Nitric oxide scavenging assay

A dose dependent elevation of scavenging activity of nitric oxide by ascorbic acid as well as perillaldehyde was witnessed. The rise in activity with concentration was uniform having diminutive deviation from standard mean. Nitric oxide scavenging activity of perillaldehyde lags in comparison to ascorbic acid with small margin.

Table 3: Percent nitric oxide scavenging activity of ascorbic acid and perillaldehyde

Concentration (µg/ml)	% scavenging activity of Ascorbic acid	% scavenging activity of perillaldehyde
10	23.000±0.110	13.176±0.970
25	45.598±0.115	33.713±0.087
50	57.793±1.247	44.488±1.642
75	73.093±1.225	49.570±1.670
100	85.732±3.190	67.641±1.581

- The data are exhibited in form of mean and standard deviation (±) of triplicate

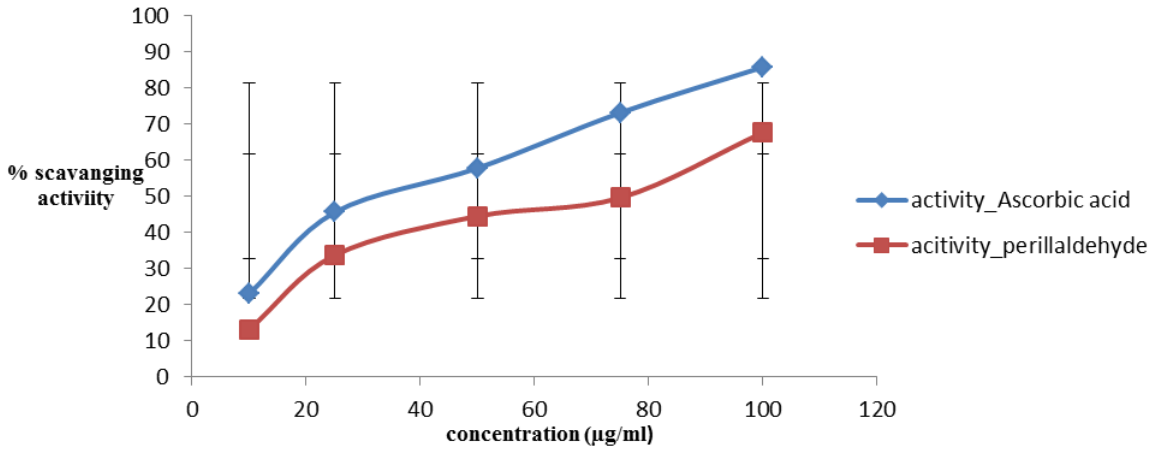


Figure 3. percent nitric oxide scavenging activity of ascorbic acid and Perillaldehyde at different concentrations

Nitric oxide is a vital biochemical agent required for the maintenance of normal physiological homeostasis [27]. It has wide range of distribution and carries out plentiful functions i.e. acts as hormone, secondary messenger[28], neuromuscular transmission and conduction[29], regulation of blood pressure[30,31]. Thus, it is evident that compounds which can scavenge nitric oxide in vivo are of having great medical importance.

3.4 DPPH scavenging activity

The violet color of DPPH changed to pale yellow color of picryl group[32] attributable to its reduction by receiving an electron or hydrogen ion from the test sample. DPPH scavenging activity of ascorbic acid and perillaldehyde were concentration dependent. Scavenging activity of perillaldehyde was relatively lower than that of ascorbic acid. Although small deviation from the mean was noticed data had good linear correlation.

Table 4: Percent DPPH scavenging activity of ascorbic acid and perillaldehyde

Concentration (µg/ml)	% scavenging activity of Ascorbic acid	% scavenging activity of perillaldehyde
10	45.450±0.238	28.264±2.995
25	60.315±1.727	39.245±1.470
50	71.003±1.287	60.242±0.147
75	83.535±1.868	76.512±0.984
100	94.980±0.751	88.408±1.011

- Values are organized as mean and standard deviation (±) of triplicate

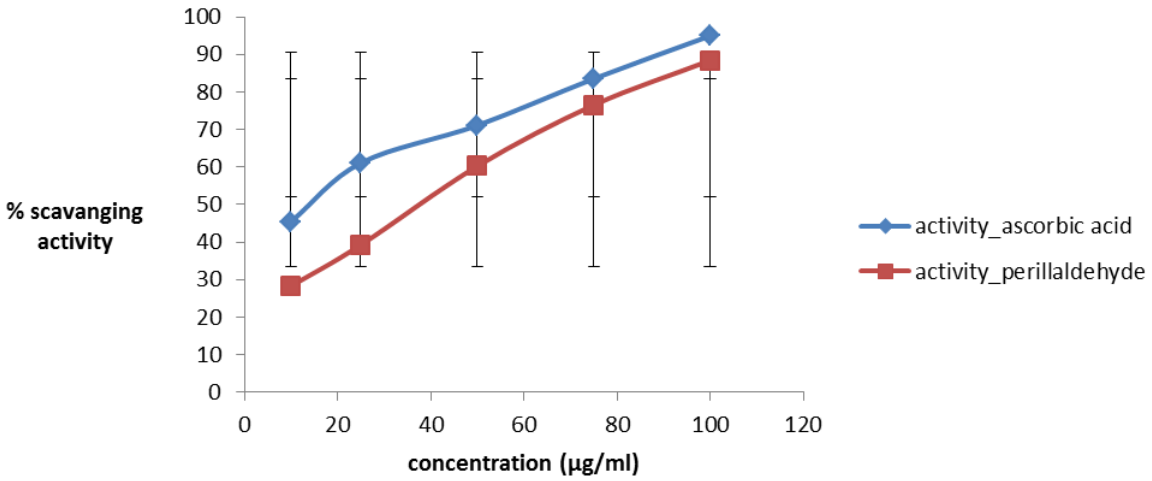


Figure 4. percent DPPH scavenging activity of ascorbic acid and perillaldehyde at different concentrations

3.5 Reducing power

In the reducing power assay, the yellow colour of the test solution changed to various shades of green and that depends on the reducing power of the sample. The antioxidant radicles convert the Fe^{3+}/ferricyanide complex to ferrous form. Increase in the absorbance at wavelength 700 nm with increasing concentration of ascorbic acid and perillaldehyde is observed. This indicates reducing (electron donating) ability of test sample, which is function of concentration of sample. Perillaldehyde was observed to have good reducing activity.

Table 5: Percent reducing activity of perillaldehyde

Concentration(µg/ml)	% Reducing activity of Perillaldehyde
10	33.717±0.345
25	37.130±0.429
50	38.378±0.624
75	54.219±0.581
100	59.511±0.100

- Data are shown as mean and standard deviation (±) of triplicate

3.6 Statistical analysis

The percentage activity of ascorbic acid and Perillaldehyde was analyzed at 95% confidence level (SPSS, version 16.0, SPSS Inc., Chicago, IL, USA) and represented as mean ± standard deviation of the triplicate run. Two way ANOVA was run to check the change in activity due to difference in concentration of compounds i.e. ascorbic acid and Perillaldehyde and experimental method.

4. Conclusion

Perillaldehyde exhibited significant antioxidant property but comparatively lower activity than ascorbic acid. Studies on perillaldehyde showed better activity above 50µg/mL concentration and in some of the assays at par with ascorbic acid. In future use of Perillaldehyde as an antioxidant agent in health and medicine as well as food and flavor sector is plausible.

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