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Research Article

CHEMICAL KINETICS STUDY AND EVALUATION OF ANTIOXIDANT ACTIVITY IN CLOVE, CUMIN, CINNAMON AND CARDAMOMOILS USING DPPH RADICAL

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ABSTRACT

There are a great number of aromatic, spicy, medicinal, and other plants which contain peculiar chemical compounds. These chemical compounds exhibit antioxidant properties. Antioxidants are very much essential for leading a healthy life. In this study along with evaluation of antioxidant activity of essential oils like clove oil, cinnamon oil, cardamom oil and cumin oil using DPPH$^{\bullet}$ radical, a chemical kinetic experiment was also carried out to understand the optimum time required for the completion of reaction. The antioxidant activity or scavenging activity revealed that the clove oil has the highest antioxidant property amongst cardamom, cinnamon and cumin oil.

INTRODUCTION

Antioxidants are the compounds which inhibit the oxidation reactions caused by free radicals. Free radicals which are also called as Reactive Oxygen Species (ROS) or Active Oxygen Species (AOS), are produced during various metabolic cellular processes (Abdul Rasheed Md *et. al*, 2013). Antioxidants containing a phenolic group play the major role in foods for to avoid food degradation, and they play an important role in preventing many lifestyle related diseases and ageing, being closely related to the formation of reactive oxygen species (ROS) and to lipid peroxidation. Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease (O. Turgay and Y. Esen, 2015). Reactive oxygen species, which are generated in human body during various metabolic activities, are also generated during irradiation by UV light, X-rays, gamma rays, metal catalysed reactions and are byproducts of mitochondria catalyzed electron transport reactions and other mechanisms.

In addition, human beings produce oxygen free radicals (superoxide and hydroxyl radicals) and other oxygen reactive species (hydrogen peroxide, nitric oxide, peroxynitrile and hypochlorous acid) due to diverse physiological and biological processes as a result of aerobic metabolism. These free radicals may be responsible for oxidative damage of lipids, proteins and nucleic acids, which can lead to pathologies such as rheumatoid arthritis, diabetics, cancer, atherosclerosis and cardiovascular diseases among other chronic and degenerative diseases (Jaqueline Badanai *et. al*., 2015). When these ROS's are overproduced in the body, it leads to the appearance of these diseases.

Oxidative stress has been defined as an imbalance between the production of ROS and antioxidant defence. Because of the disturbance in the equilibrium state of prooxidant-antioxidant reaction, ROS are overproduced to induce oxidative stress, which inhibits normal functions of cellular lipids, proteins, DNA, and RNA (IlhamiGulcin, 2011). Oxidative stress has also been linked to cell injury via a range of by-products such as 8- hydroxydeoxyguanosine (8-OHdG), protein carbonyl and lipid peroxides (Choo Suet Cheng Candy *et al*, 2013). The antioxidants not only eliminate ROS but also adjust the cellular redox state and enable redox signal transduction. The mechanism of antioxidants may involve the scavenging of free radicals (IlhamiGulcin, 2011). Thus, antioxidants may be said to have defensive properties. Due to antioxidant activity of naturally occurring substances in higher plants, attention has increased on the protective activity of these natural antioxidants against chronic disorders caused by oxidative process (Eugenio José Garcia *et al*, 2012).

Antioxidants act by: removing O_2 or decreasing local O_2 concentrations, removing catalytic metal ions, removing key

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ROS, e.g. $O_2^{\bullet-}$ and H_2O_2, scavenging initiating radicals, e.g. $OH^{\bullet}$, $RO^{\bullet}$, RO_2^{-} , breaking the chain of an initiated sequence, quenching or scavenging singlet oxygen, enhancing endogenous antioxidant defences by up-regulating the expression of the genes encoding the antioxidant enzymes, repairing oxidative damage caused by radicals, increasing elimination of damaged molecules and not repairing excessively damaged molecules in order to minimise introduction of mutations (IlhamiGulcin *et al*, 2012). At present, a variety of synthetic antioxidants are commonly used. However, the use of these compounds has been restricted by legislation because of doubts over their toxic and carcinogenic effects. Plant foods are potential sources of natural antioxidants, such as vitamin C, tocopherol, carotenoids, flavonoid, and phenolic acids that prevent free radical damage. All of this has given impetus to attempts to explore natural sources of antioxidants (IlhamiGulcin, 2011).

The methods to understand the antioxidant property are broadly classified into two classes: - direct method and indirect method. Direct methods are based on the effect of the sample with potential antioxidant activity on the oxidative degradation of a tested substrate (individual lipids, lipid mixtures, proteins, nucleic acids, lipid containing biologically relevant species such as blood plasma, lipoproteins of low density, biological membranes, among other substrates). Generally indirect methods are mostly used, such as DPPH and ABTS methods, although their poor repeatability and the lack of certitude for the capability of samples to inhibit oxidative processes. Reagent concentrations, time of incubation, solvents used, matrix of sample, among other factors may be determinant on the results obtained which may vary strongly, do not permitting compare results (Jaqueline Badanai *et al*, 2015).

In this study chemical kinetic study is carried out to understand the optimum time the reaction required to complete. In this study antioxidant property of different spice oils like clove, cumin, cardamom and cinnamon is evaluated using DPPH radical by UV spectrophotometer. DPPH radical was used in this study to understand the antioxidant property because the method is very simple, sensitive, requires little sample material (Olivera Politeo *et al*, 2006), DPPH radical is more stable than ABTS and has a simple sample preparation step.DPPH is stable nitrogen centred free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule (IlhamiGulcin, 2012).

Principle of DPPH$^{\bullet}$Free Radical Scavenging Activity

In this assay, the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) is reduced by antioxidant/reducing compounds to the corresponding pale-yellow hydrazine. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 518 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution (Aline AugustiBoligon *et al*, 2014). Eugenol is believed to have an aromatic ring. This phenolic group stabilized a radical formed on a-carbon with conjugation in the eugenol molecule. It is well known that phenolic groups stabilize a radical formed on phenolic carbon with their resonance structure (IlhamiGulcin, 2011). IllhamiGulcin proposed a mechanism between eugenol and DPPH$^{\bullet}$ radicals and the formation of dehydrodieugenol which can be seen in below figure 1.

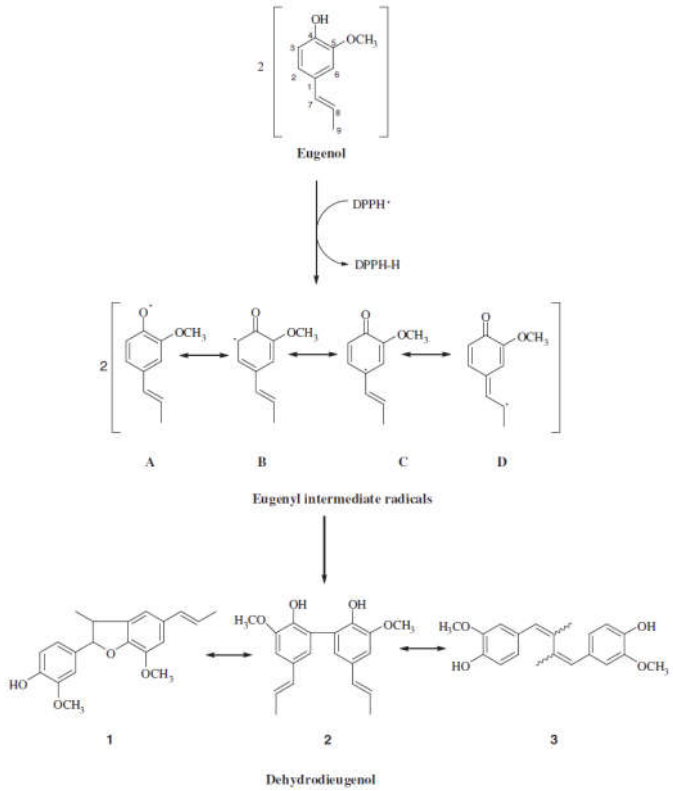

Figure 1 proposed mechanism of eugenol and DPPH$^{\bullet}$ radicals and the formation of dehydrodieugenol. Source: - IlhamiGulcin, 2011.

MATERIALS AND METHODS

Materials**: -**The essential oils of spices like cardamom, cinnamon, clove and cumin. The essential oils were obtained by hydro distillation using steam distillation apparatus. 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) from Sisco Research Laboratories (Purity – 95%) as scavenging reagent, Eugenol from Research Fine chemicals (Purity – 99%) as standard and methanol from S D Fine Chemicals as a solvent (HPLC grade).

Methods**: -**The essential oils of cardamom, cinnamon, clove and cumin were extracted by hydro distillation. The chemical kinetic and antioxidant study was carried out using Perkin Elmer Lambda XLS+ Spectrophotometer. Before starting with the experiment some trials were carried out to understand whether the absorbance decreases or not and the method is suitable or not. For these two different concentrations of Eugenol standardppm were mixed with 10ppm and 20ppm DPPH reagent. 10ppm DPPH solution faded very quickly, hence 20ppm solution of DPPH reagent was used to carry out chemical kinetic and antioxidant study. All the solutions were prepared using methanol as a solvent.

Chemical Kinetic study**: -**To understand the optimum time required for the reaction, a time based, chemical kinetic study was carried out by using 20ppm and 50ppm Eugenol solutions. Equivolume solutions of the standards were mixed with 20 ppm DPPH reagent in the cuvette and absorbance was recorded with 1-minute interval from t0 min to t30 min.

Antioxidant study

***Method development*: -** Different concentrations of Eugenol standard solutions were prepared for plotting the calibration curve. Control absorbance (all reagents without standard and sample) was recorded every day before starting the experiment. Trials with different concentrations of essential oils were also carried out.

***Antioxidant Study*: -** The final calibration curve selected was 0.5, 1.0, 2.0, 10.0 and 20.0 ppm. Different concentrations of essential oils were prepared like for clove oil, 0.1, 1.0, 2.5 and 5 ppm solutions were prepared andfor cinnamon, cardamom and cumin oil, 50, 100, 200, 500 and 1000 ppm solutions were prepared. These different concentrations of essential oils were mixed with 20ppm DPPH solution and the absorbances were recorded after 30 mins.

The scavenging effect of DPPH free radical by an antioxidant was calculated using the following equation: -

$$\text{Scavenging effect DPPH}^{\bullet}\ (\%) = \left[1 - \frac{A_{518\text{-}S}}{A_{518\text{-}C}}\right] \times 100 = \frac{(A_{518\text{-}C} - A_{518\text{-}S})}{A_{518\text{-}C}} \times 100 \ldots\ldots \text{eqn 1}$$

Where, $A_{518\text{-}S}$ is the absorbance of the solution containing standard or sample measured at λ max 518nm and $A_{518\text{-}C}$ is the absorbance of the solution containing all reagents except the standard or sample measured at λ max 518nm (Abdul Rasheed Md*et al*, 2013; Jaqueline Badanai*et al*, 2015; IlhamiGulcin, 2011; IlhamiGulcin, 2012; Olivera Politeo *et al*, 2006; IdriesMuhsonAbeed Al. Mashkor, 2015;Oyas Ahmed Asimi *et al*, 2013; MałgorzataOlszowy and Andrzej L. Dawidowicz, 2016; Bhutkar Mangesh Anil and Bhise Satish Balkrishna, 2011; M.B. Hossain *et al*, 2008).

RESULTS AND DISCUSSIONS

The observed results for chemical kinetic study and antioxidant study are discussed in the following subsections.

***Chemical kinetic study*: -** The absorbances were recorded for both 20ppm and 50ppm Eugenol standard. The data for the concentrations are shown in the following table 1.

Table 1 shows the absorbances recorded for 20ppm and 50ppm Eugenol standard mixed with 20ppm DPPH from t0 to t30 min

Sr. no.	Time hh:mm:ss	Absorbance of 20ppm Eugenol A	Absorbance of 50ppm Eugenol A
1	00:01:00	0.164	0.190
2	00:02:00	0.157	0.157
3	00:03:00	0.152	0.138
4	00:04:00	0.146	0.124
5	00:05:00	0.142	0.115
6	00:06:00	0.138	0.106
7	00:07:00	0.135	0.098
8	00:08:00	0.132	0.092
9	00:09:00	0.129	0.088
10	00:10:00	0.126	0.084
11	00:11:00	0.124	0.081
12	00:12:00	0.121	0.077
13	00:13:00	0.118	0.073
14	00:14:00	0.116	0.071
15	00:15:00	0.115	0.069
16	00:16:00	0.112	0.066
17	00:17:00	0.111	0.064
18	00:18:00	0.109	0.063
19	00:19:00	0.108	0.061
20	00:20:00	0.104	0.059
21	00:21:00	0.104	0.058
22	00:22:00	0.102	0.056
23	00:23:00	0.102	0.055
24	00:24:00	0.100	0.054
25	00:25:00	0.098	0.053
26	00:26:00	0.097	0.052
27	00:27:00	0.097	0.051
28	00:28:00	0.097	0.050
29	00:29:00	0.095	0.049
30	00:30:00	0.092	0.048
31	00:31:00	0.093	0.048

The raw data of chemical kinetic study for 20ppm and 50ppm Eugenol is shown in the figure 2 and 3 respectively.

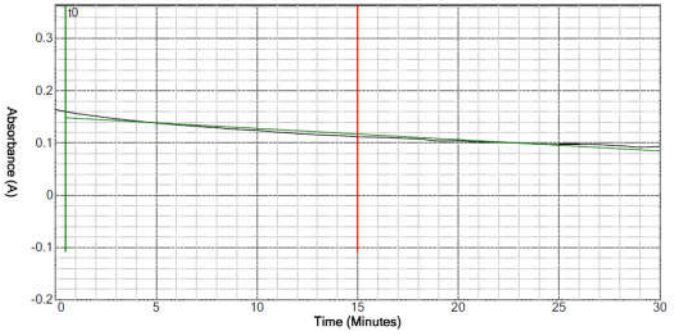

Figure 2 graph generated by Perkin Elmer Lambda XLS+ spectrophotometer for 20ppm Eugenol

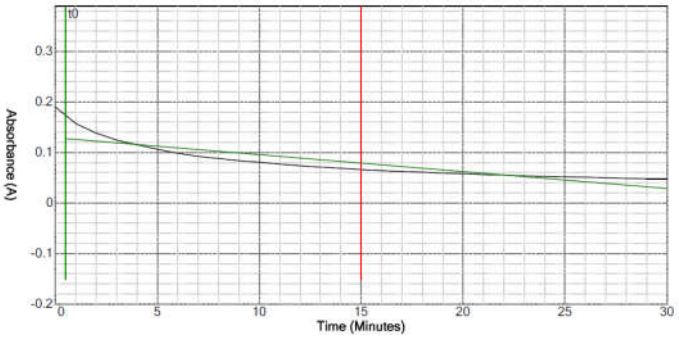

Figure 3 graph generated by Perkin Elmer Lambda XLS+ spectrophotometer for 50ppm Eugenol

From the graph it is very clear that after 25 mins the absorbances for both the concentrations were found to be stable. Hence the optimum time required to complete the reaction is 30 mins and antioxidant study was carried out by keeping the mixture of standards and essential oils with DPPH$^{\bullet}$ for 30 mins.

***Antioxidant study*: -**The absorbances for different concentrations of essential oils were recorded. Initially the concentration range for cardamom, cinnamon, clove and cumin was kept same. Upon performing analysis, the concentration range for cardamom, cinnamon and cumin was increased. The % scavenging activity of DPPH radical was calculated using the equation 1 and it is tabulated as follows: -

Table 2 different concentrations of different essential oils and their % scavenging activity of DPPH radical. Source for clove and cinnamon: - SulekhaGotmare and Esha Tambe, 2018

Sr. no.	Name of essential oil	Concentration of essential oil in µg/ml	% Scavenging activity of DPPH$^{\bullet}$
		0.1	6.41
		1.0	23.93
1	Clove	2.5	40.60
		5.0	62.93
		10.0	91.89
2	Cardamom	1000	7.14

		5000	12.41
		10000	4.14
		25000	-17.67
		50000	-37.22
		1000	13.16
		5000	17.18
3	Cinnamon	10000	22.93
		25000	32.71
		50000	42.86
		1000	1.50
		5000	25.94
4	Cumin	10000	38.72
		25000	58.65
		50000	77.44

From the above table it is clear that as the clove oil concentration increases its antioxidant activity/scavenging activity also increases. It showed a highest scavenging activity of 92% at 10 ppm. For cardamom oil, the correlation between concentrations and %scavenging activity was not clearly established. The scavenging activity was not seen even at 50000 ppm (5%) for cardamom oil. A directly proportional relationship between concentration and % scavenging activity for cinnamon oil and cumin oil was observed but at higher levels.

***IC50*: -** IC 50 is the concentration of the sample or essential oil needed to scavenge or decrease50% of DPPH (Jaqueline Badanai *et al*, 2015; G.A. Otunola and A.J. Afolayan, 2013; MałgorzataOlszowy and Andrzej L. Dawidowicz, 2016).IC 50 value is directly proportional to antioxidant capacity (M. Hossain *et al*, 2008). IC 50 value was calculated for clove, cinnamon and cumin oil (since cardamom oil proper response). In this study the IC 50 value for cardamom oil was not calculated because of the nonlinear graph. The clove oil showed a linear response for concentration of clove oil versus % scavenging activity. While the graph for cumin oil and cinnamon oil also showed a very nonlinear correlation between the concentration of oil against the % scavenging activity, the IC 50 was still calculated for cumin as well as cinnamon oil. The IC50 value was calculated using following graphs.

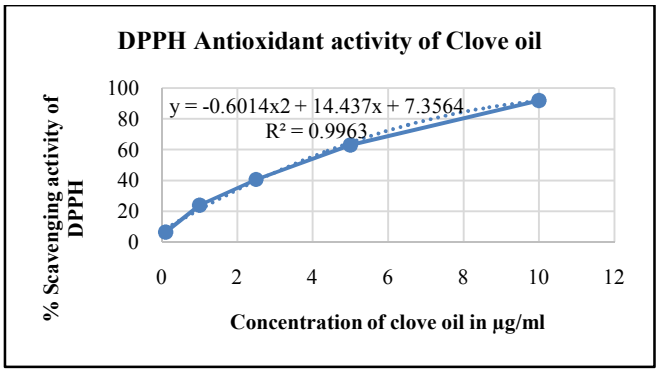

Figure 4 graph of concentration of clove oil and % scavenging activity of DPPH. Source: -SulekhaGotamre and Esha Tambe, 2018

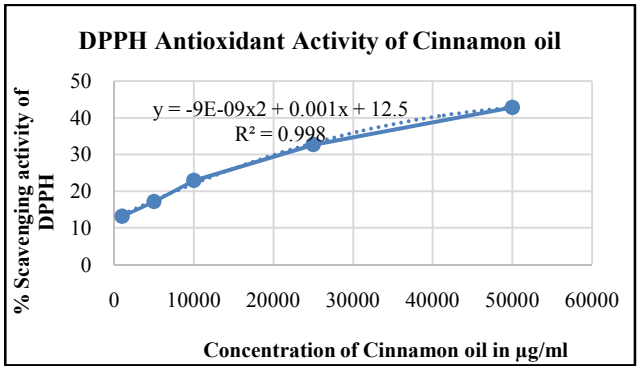

Figure 5 graph of concentration of cinnamon oil and % scavenging activity of DPPH. Source: -SulekhaGotmare and Esha Tambe, 2018

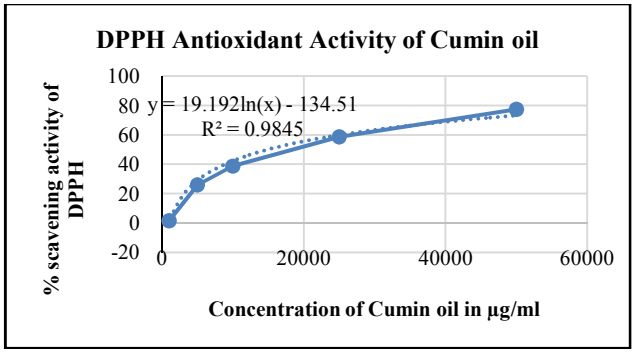

Figure 6 graph of concentration of cumin oil and % scavenging activity of DPPH

The IC 50 for the oils are tabulated as follows: -

Table 3 IC 50 value of clove, cinnamon and cumin oil. Source for clove and cinnamon: - SulekhaGotmare and Esha Tambe, 2018

Essential oil	IC50 value in µg/ml
Clove oil	6.85
Cinnamon oil	64,548.86 (6.45%)
Cumin oil	14,971.45 (1.50%)

The lower the IC 50 value, higher is the antioxidant capacity of the essential oil. In this study IC 50 value evaluated was found to be very less for clove oil. The ascending order of IC 50 value examined in this study is: - clove oil < cumin oil < cinnamon oil.

CONCLUSION

Spices are rich sources of polyphenolic compounds having strong antioxidant capacities and could potentially replace the synthetic antioxidants in food systems and offer additional health benefits. Consumption of spices has been implicated in the prevention of many chronic diseases such as cardiovascular diseases, cancer and inflammation etc (Virendra V. Panpatil *et al*, 2013). From this study it can be concluded that clove has the higher antioxidant property as compared to cinnamon and cumin oil. Cinnamon and cumin oil showed antioxidant property but at a high concentration. Cardamom oil does not possess antioxidant property even at high concentration. Thus, clove contains more amount of phenolics compound as compared to cinnamon and cumin, while cardamom oil does not have phenolic compound.

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