STUDY OF ANTIOXIDANT ACTIVITY OF CLOVE AND CINNAMON OIL USING UV-VISIBLE SPECTROPHOTOMETER

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ABSTRACT: -Antioxidants are the first line defence against free radical damage and are critical for maintaining optimum health and well-being. Damage to healthy cells due to free radicals is believed to play a central role in aging process and in disease progression. There are many factors which leads to the production of free radical in our body. When these free radicals are overproduced, it leads to the emergence of diseases. Hence antioxidants should be included in everyday diet. In this study, antioxidant activity of cinnamon and clove oil was evaluated using DPPH radical. The clove oil showed higher antioxidant property as compared to cinnamon oil.

Keywords: **- DPPH, clove oil, IC 50, reactive oxygen species, eugenol**

INTRODUCTION: -'Antioxidants' are substances that neutralize free radicalsor their actions. Nature has endowed each cellwith adequate protective mechanisms against any harmfuleffects of free radicals: superoxide dismutase (SOD),glutathione peroxidase, glutathione reductase, thioredoxin,thiols and disulfide bonding are buffering systems in everycell (TPA Devasagayam et al, 2004).Antioxidants are capable of stabilizing,ordeactivating, free radicals before they attack cells. Antioxidantsare absolutely critical for maintaining optimal cellular and systemichealth and well-being (Dr. Mark Percival, 1998).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 (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage. ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as:

1) A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.

2) Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.

3) Xenobiotic metabolism, i.e., detoxification of toxic substances (Dr.Marck Percival, 1998).

In popular scientific/biomedicalliterature the term 'free radical' is used in a broad sense andalso includes related reactive species such as 'excited states'that lead to free radical generation or those species that resultsfrom free radical reactions (TPA Devasagayam et al, 2004).

As several metabolic diseases are associated with oxidative processes in the human body, the antioxidant properties of spices have attracted considerable attention among researchers and consumers (HanisMastura et al, 2017). The antioxidants not only eliminate ROS but also adjust the cellular redox state and enable redox signal transduction. They act by inhibiting the initiation and propagation steps, leading to the termination of the reaction and delaying the oxidation process. The mechanism of antioxidants may involve the scavenging of free radicals (IlhamiGulcin, 2011).

The inhibition process can be best explained by the following reactions: -

Initiation: **RH →R• + H•** ----------(1)

Propagation: **R• + O_2→ROO•** ---------- (2)

ROO• + RH →ROOH + R•----------(3)

Termination: **ROO• + ROO• →ROOR + O_2**----------(4)
ROO• + R• →ROOR } *non- radical products* ---------- (5)
R• + R• →R-R ----------(6)

In the first reaction, an initiation step occurs wherein an alkyl radical (R•) is formed from an unsaturated fatty acid (RH). Once formed, an alkyl radical (R•) reacts very rapidly with oxygen from a peroxylradical (ROO•) (Reaction 2). The second step of propagation, the abstraction of a peroxyl radical(ROO•) to generatehyperoxide (ROOH), and another radical (R•), is the rate determining step(Reaction 3). Propagation steps may be more complicated than the simple transfer, and additionsteps. The reactions terminate when radicals react with each other, forming more stable productsthat are not capable of propagating the chain reactions (Reactions 4- 6) (Anca Maria Juncan, 2011).

Spices are plant products which include bark, leaves, flowers, roots, seeds or whole plants. They are generally added to food and contribute to the flavour, taste and colour of a dish. These characteristics are associated with the phenolic compounds contained in the spices. In addition, spices were reported as top five foods with the highest phytochemical content in the form of polyphenols. Spices have been widely used since long time ago for culinary, health benefits as well as preservatives, especially in Mediterranean countries and Asia. In India, spices have been used as traditional medicine, for example, turmeric for jaundice, basil to protect heart and ginger for relieving nausea and indigestion (HanisMastura et al, 2017).

Different methods can be employed to understand the antioxidant property. These methods are broadly classified into two classes: - direct method and indirect method. Direct methods are the methods in which 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) is measured. Indirect methods are the methods in which reagent concentrations, time of incubation, solvents used, matrix of sample, among other factors may be determinant on the results obtained which may vary strongly. This results in incomparable data.Still indirect methods commonly used, such as DPPH and ABTS methods, despite of their poor repeatability and the lack of certitude for the capability of samples to inhibit oxidative processes(Jaqueline Badanai et al, 2015).

In this study % scavengingactivity of cinnamon and clove oil is determined using DPPH radical by UV spectrophotometer. DPPH method was used in this study because the method is very simple, sensitive, requires little sample material (Olivera Politeo et al, 2006). In addition to this, DPPH radical is more stable than ABTS and has a simple sample preparation step.DPPH is stable since it is a nitrogen centred free radical that can accept an electron or hydrogen radical so as to become a stable diamagnetic molecule (IlhamiGulcin et al, 2012).

2. PRINCIPLE OF DPPH• FREE RADICAL SCAVENGING ACTIVITY: - In this assay, the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) 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).

3. MATERIALS AND METHODS

3.1 Materials: -The essential oils of spices like cinnamon and clove were used to study the antioxidant activity. The essential oils were obtained by hydro distillation. 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) fromSisco 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).

3.2 Methods: -The essential oils of cinnamon and clove were extracted by hydro distillation using conventional distillation apparatus.The antioxidant study was carried out using Perkin Elmer Lambda XLS+ Spectrophotometer. To understand whether the method is functional and suitable or not,many trial experiments were carried out. Two different concentrations of Eugenol standardwere mixed with 10ppm and 20ppm DPPH reagent. 10ppm DPPH solution faded very quickly as compared to 20 ppm, 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.

3.2.1 Antioxidant study

3.2.2.1 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.

3.2.2.2 Antioxidant Study: -From the trials it was clear that the calibration curve could not be plotted beyond 20 ppm Eugenol because DPPH color faded very quickly and absorbance was very low. Hence, the final calibration curve selected was 0.5, 1.0, 2.0, 10.0 and 20.0 ppm. Different concentrations of essential oils were preparedand analyzed. These different concentrations of essential oils were mixed with 20ppm DPPH solution and the absorbances were recorded after 30 mins.

For clove oil, 0.1, 1.0, 2.5 and 5 ppm solutions andfor cinnamon50, 100, 200, 500 and 1000 ppm solutions were prepared and analyzed. Upon analysis of cinnamon oil, these concentrations showed a non-liner graph with very low

linear regression. Hence a higher range of cinnamon oil i.e. 1000 ppm, 5000 ppm, 10000 ppm, 25000 ppm and 50000 ppm were prepared and analyzed.

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

Scavenging effect DPPH• (%) = [1- ($A_{518\text{-}S}/A_{518\text{-}C}$) x 100] = {($A_{518\text{-}C}$ - $A_{518\text{-}S}$) x 100} / $A_{518\text{-}C}$ ……equation 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 Mdet al, 2013; Jaqueline Badanaiet 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).

4. RESULTS AND DISCUSSIONS

The absorbances for clove oil and cinnamon oil were recorded for different concentrations. The % scavenging activity of DPPH radical was calculated using the equation 1 and it is tabulated as follows: -

Table 1 different concentrations of clove and cinnamon oils and their % scavenging activity of DPPH radical

Sr. no.	Name of essential oil	Concentration of essential oil in µg/ml	% Scavenging activity of DPPH•
1	Clove	0.1	6.41
		1.0	23.93
		2.5	40.60
		5.0	62.93
		10.0	91.89
2	Cinnamon	1000	13.16
		5000	17.18
		10000	22.93
		25000	32.71
		50000	42.86

From the above table it is clear that there is a direct relation between the clove oil concentration and the antioxidant activity/scavenging activity. The highest scavenging activity of clove oil is seen at 10 ppm.Cinnamon showed antioxidant activity at higher concentrations as compared to clove oil.

4.1Inhibition Concentration50 (IC50): - IC 50 is defined as "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). To calculate the IC50 value of clove oil and cinnamon oil, concentration of essential oil versus % scavenging activity of DPPH graphswas plotted. IC 50 for clove oil was found to be 6.85 µg/ml and for cinnamon oil 6.45 % was determined.

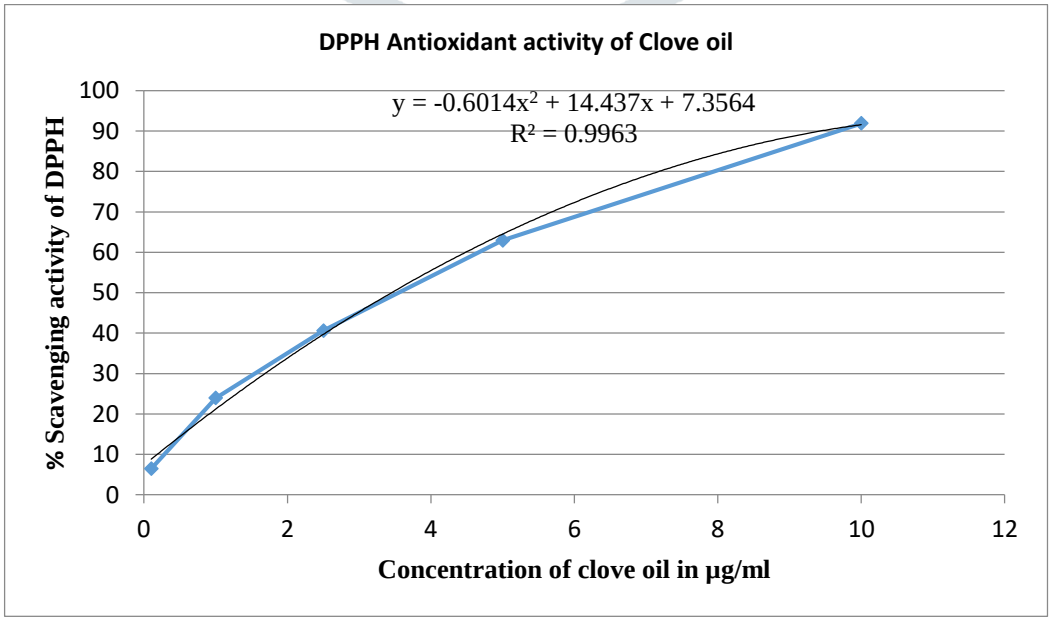

Figure 1 graph of different concentration of clove oil versus % scavenging activity of DPPH

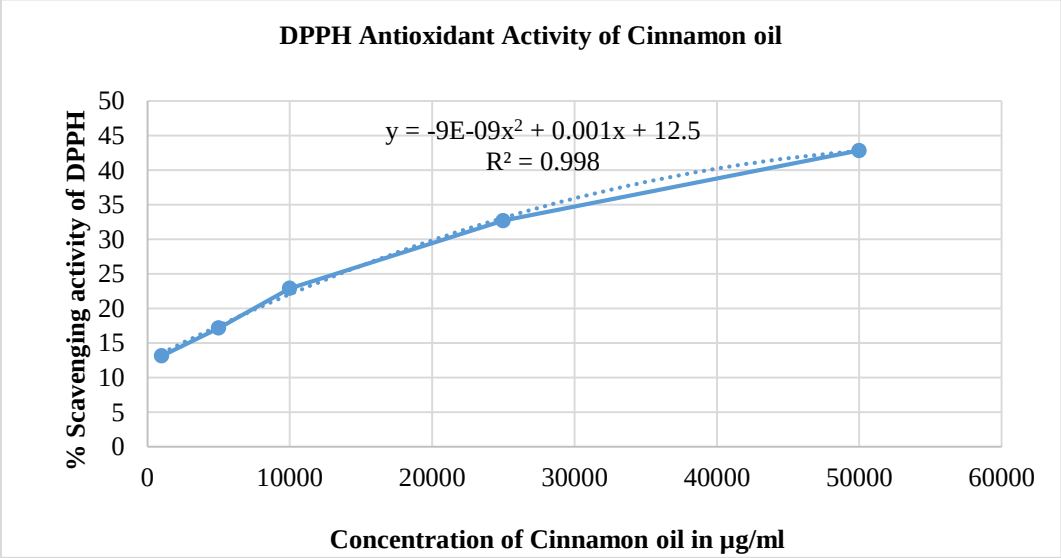

Figure 2 graph of different concentration of cinnamon oil versus % scavenging activity of DPPH

The IC 50 value and antioxidant activity are indirectly proportional to each other. Thus, the clove oil was found to possess very high antioxidant property while cinnamon showed very low antioxidant property.

4.CONCLUSION

Antioxidant activities of essential oils from aromaticplants are mainly attributed to the active compoundspresent in them. This can be due to the high percentageof main constituents, but also to the presence of other constituents in small quantities or to synergy among them (Olivera Politeo et al, 2006). DPPH radicals react with suitable reducing agents then losing colorstiochiometrically with the number of electrons consumed which is measured spectrometrically at 518nm (Bhutkar Mangesh Anil and Bhise Satish Balkrishna, 2011). The antioxidant activity ofclove essential oil is mainly due to the high content ofeugenol (Olivera Politeo et al, 2006).In this study spices which are commonly consumed in the diet were evaluated for their antioxidant property. The results showed that clove possess very high antioxidant property at 10 ppm, whereas cinnamon showed very low antioxidant property even at 5% concentration. Thus, it may also be concluded that clove contains more amount of phenolic compound as compared to cinnamon bark.

Emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure for the treatment of various infectious diseases. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries as approximately 80% of the world inhabitants rely on traditional medicine for their primary health care needs (Amrita Soni and Praveen Dahiya, 2014).

5. ACKNOWLEDGMENT

The authors are grateful and express our sincere regards to Dr. Ganapathy Ramakrishnan, Hon-Director SIES-ICS, Mr. Dipak Shetty, Laboratory Manager, SIES-ICS and Ms. Sneha Nair, Laboratory Chemist SIES-ICS for providing a friendly environment and research facilities.

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