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ANTIOXIDANT POTENTIAL OF CINNAMALDEHYDE; AN *INVITRO* STUDY

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Abstract: Antioxidants are the agents that are capable of effectively neutralizing the deleterious effects of free radicals. Therefore, the need for the search of antioxidants from natural origin has been greatly felt in the recent years. Cinnamaldehyde (CA) exert antimicrobial, anti tumor, anti inflammatory, anti diabetic effects as well as anti gastric properties. CA is potential against different free radicals which result in oxidative damage leading to various diseases. Current work was carried to evaluate the radical scavenging activities using different invitro models like DPPH, Superoxide, Nitric Oxide, H_2O_2 scavenging activity and Reducing power. Total antioxidant capacity expressed as equivalents of 160µg of Vitamin E / g of CA. CA has shown potent antioxidant activity in these methods with IC_{50} values of 377, 391, 437, 390 and 462µg/ml, respectively. The antioxidant activity of CA was compared with the standards like Ascorbic acid (AA) and Butylated hydroxy toluene (BHT). The results obtained in the present study indicate that the CA can act as a potential source of antioxidant scavenging free radicals and preventing oxidative stress leading to various diseases.

Keywords: Antioxidant Activity, DPPH, Free radical scavenging activity, Cinnamaldehyde, Ascorbic acid, Butylated hydroxy toluene.

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INTRODUCTION

Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive Oxygen species (ROS) [20]. The role of oxygen radicals has been implicated in several diseases, including cancer and diabetes mellitus. Antioxidants are vital reducing substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [21]. Reactive oxygen species including DPPH, Superoxide, Nitric Oxide, and H_2O_2 exert oxidative stress in the cells of human body rendering each cell to face about 10,000 oxidative hits per second [6,11].

Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [3]. In recent years, there is an increasing interest in finding antioxidant phytochemicals because they can inhibit the propagation of free radical reactions, protect the human body from diseases.

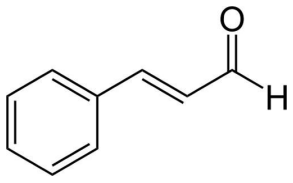

Figure 1 Cinnamaldehyde

Cinnamaldehyde (3-phenyl-2-propenal) is the major constituent of the essential oil of cinnamon bark isolated from Cinnamomum trees. Cinnamaldehyde has been widely used as a component in perfumes, a fungicide, and a flavouring agent in foodstuffs such as chewing gum, ice cream, candy and beverages. Cinnamaldehyde is alpha, beta-unsaturated carbonyl derivative with mono-substituted benzene ring [18]. Cinnamaldehyde has been used as a filtering agent, rubber reinforcing agent and is used as a brightener in electroplating processes, as an animal repellent, insect attractant and antifungal agent. Several biological activities such as peripheral vasodilatation, anti tumor, cytotoxic and mutagenic/ anti-mutagenic activities have been studied for Cinnamaldehyde [22].

The evaluation of antioxidant activity will establish the quality and pharmacological standard for these compounds and also it will identify the compound having maximum activity. This will also help in their commercial gradation. Thus, the present work is aimed at testing of the target compound for free radical scavenging activity by using DPPH, Superoxide, Nitric Oxide, and H_2O_2 free radical scavenging methods.

Furthermore, literature survey of CA compound revealed that no research has yet been reported for invitro antioxidant activities of this compound. It is worth investigating on the invitro antioxidant activities of CA.

Materials & Methods

Cinnamaldehyde were purchased from Sigma- Aldrich. All other chemicals used are of analytical grade.

Determination of total antioxidant Assay

The total antioxidant activity was evaluated by the method of Prieto[16]. An aliquot of sample solution (100 - 500µg/ml was combined with reagent solution (0.6 M Sulfuric acid, 28 mM Sodium Phosphate and 4 mM Ammonium molybdate). In case of blank, methanol was used in the place of the sample. The tubes were capped and incubated in a boiling water bath at 95^0C for 60-90 min. The samples were then cooled at room temperature and the absorbance was measured at 695 nm against the blank in spectrophotometer. The experiment was conducted and the values are expressed as equivalents of Vitamin E per g of Cinnamaldehyde.

Total reducing ability

The reducing power of the CA was determined according to the method of Oyaizu[14]. Different concentrations of the CA (100-500µg/ml) in 1.0 ml of deionised water were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min and later 2.5ml of trichloroacetic acid (10%) was added to the mixture and centrifuged at 3,000rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm.

Superoxide anion scavenging activity

The scavenging of superoxide anion was measured as described by Hyland[7]. The reaction mixture contained various concentrations of CA (100-500µg/ml), nitroblue tetrazolium and alkaline DMSO. The blank consisted of DMSO. The absorbance was read at 560 nm using a UV-Visible double beam spectrophotometer.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured by Green[4]. 3 ml of reaction mixture (CA) containing sodium nitroprusside in PBS and sample was incubated at 25°C for 150 minutes. Controls were kept without test compound in an identical manner. After incubation, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds.

DPPH radical scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the CA [10]. Different concentrations of CA (100-500µg/ml) were added, at an equal volume of methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. BHT and Ascorbic Acid were used as controls.

Hydrogen Peroxide radical scavenging activity

The Hydrogen peroxide-scavenging activity of extract was determined by the method

of Ruch[19]. The CA was dissolved in 3.4 ml of 0.1M phosphate buffer (pH 7.4) and mixed with 600 µl of 43 mM solution of hydrogen peroxide. The absorbance of the reaction mixture was recorded at 570nm. for each concentration, a separate blank sample was used for background substraction.

Statistical Analysis & Calculations

Calculations of IC_{50} Values and graph were done using Graph Pad Prism Software 6.0.

Results & Discussion

Free radicals are known to play a definite role in a wide variety of pathological conditions such as pain, inflammation, cancer, diabetes, Alzheimer, hepatic damage etc. Antioxidants scavenge free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or increasing the antioxidant defense mechanisms. Dietary component which can either sacrificially scavenge ROS/RNS to stop radical chain reactions are considered as primary chain breaking antioxidants or free radical scavengers (FRS), or it can inhibit the reactive oxidants from being formed in the first place are considered as secondary or preventive antioxidants [9]. Primary antioxidants, when present in trace levels, respond to oxidants or free radicals. Therefore, no single assay accurately reflects the mechanism of action of all radical sources or all antioxidants in a complex system [17]. At least two methods should be employed in order to evaluate the total antioxidant activity [8], due to various oxidative processes. The antioxidant activity of a compound has been attributed to various mechanisms viz. prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging ability.

Natural antioxidants that are present in spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. In the present work, we have evaluated the free radical scavenging activity of CA and compared it with Butylated hydroxytoluene & Ascorbic Acid.

Total Antioxidant Assay

Total antioxidant capacity by Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of Vitamin E. Cinnamaldehyde showed potent total antioxidant capacity. Total antioxidant capacity was found to be 160 µg of Vitamin E / g of CA.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was detected and compared with AA & BHT. Fig 2 (a) shows the % inhibition values of CA, AA & BHT. The IC_{50} value of CA was 377, 279

for AA & 323 for BHT µg/ml, respectively. CA was effective as the standards namely, AA and BHT. DPPH is a stable nitrogen-centered free radical and the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [2]. It was found that the radical-scavenging activities of CA was dose dependent. IC_{50} for DPPH radical-scavenging activity is reported in Table 1. DPPH radical has been widely used to test the radical scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids [15].

Nitric Oxide Radical Scavenging Activity

The compound showed good nitric oxide scavenging activity between 100 – 500 µg/ml. The % inhibition increased with increasing concentration of the CA. Concentration of CA, AA and BHT required for 50% inhibition are found to 437, 342 and 310 µg/ml, respectively. Fig 2 (b) shows CA has good antioxidant activity when compared with AA and BHT standards. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions [12]. Sodium nitroprusside serves as a main source of nitric oxide radicals.CA, AA and BHT, scavenges the NO formed from the sodium nitroprusside by inhibiting the chromophore formation and hence the absorbance decreases as the concentration of the CA increases.

Superoxide Radical Scavenging Activity

CA was found to exhibit inhibition of superoxide radical with an IC_{50} value of 391 µg/ml, respectively. They were compared with reference standard AA and BHT (IC_{50} – 315, 353 µg/ml: Table 1). The % superoxide scavenging activities of the CA, AA and BHT at concentration of 100 - 500 µg/ml is shown in Figure 2 (c). Superoxide anion, which is a reduced form of molecular oxygen, has been implicated in initiating oxidation reactions associated with aging [23]. It plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA [1]. The decrease in absorbance in the presence of these compounds indicates the consumption of superoxide anion in the reaction mixture.

Reducing Power Assay

CA is found to possess significant reducing power; CA exhibited reducing power. The IC_{50} values of CA, BHT and AA are 462, 298 and 390 µg/ml, respectively. Fe (III) reduction is often used as an indicator of electrondonating activity, which is an important mechanism of phenolic antioxidant action [12]. In the reducing power assay, the presence of antioxidants in the samples would result in reducing of Fe3+ to Fe2+ by donating an electron. Amount of

Fe2+ complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reducing ability. Fig 2 (d) shows dose-response curves for the reducing powers of the samples. It was found that the reducing power of all the compounds increased with the increase in concentrations. CA had shown good reducing power that was comparable with AA and BHT.

Hydrogen Peroxide Radical Scavenging Activity

The hydrogen peroxide scavenging activity was detected and compared with AA and BHT Fig 2 (e). The CA is capable of scavenging hydrogen peroxide in a concentration dependent manner. IC_{50} for scavenging of hydrogen peroxide was 390 for CA, 275 for AA and 350 for BHT µg/ml as shown in Table 1, respectively. Although hydrogen peroxide itself is not very reactive, it can sometimes cause Cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing hydrogen peroxide is very important [13].

Conclusion

Invitro antioxidant potential of CA was carried out along with standards such an AA and BHT by DPPH free Radical Scavenging method, Superoxide method, Nitric Oxide method, Reducing power assay and Hydrogen peroxide. The IC_{50} value was determined for each compound. From the results, it found that CA compound displayed strong antioxidant activity towards DPPH, hydrogen peroxide and superoxide radicals. Further investigation on antioxidant potential can be carried to elucidate the mechanism of action.

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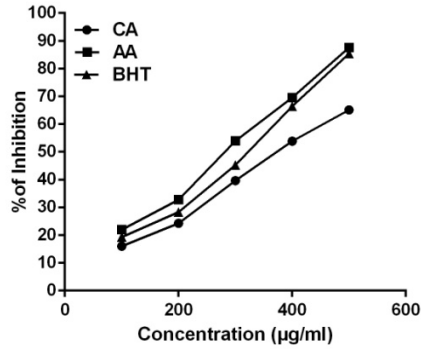
No conflict of interest.

Table 1: Comparison of antioxidant properties of Cinnamaldehyde, Ascorbic acid and Butylated hydroxytoluene.

S.No	Free radical scavenging Methods	IC_{50} Values(µg/ml)		
		Cinnamaldehye	Ascorbic Acid	Butylated hydroxytoluene
1	DPPH	377	279	323
2	Nitric oxide	437	342	310
3	Superoxide	391	315	353
4	Reducing Activity	462	298	390
5	Hydrogen peroxide	390	275	350

Figure 2: Free radical scavenging activity of CA, AA and BHT. (A) Scavenging of DPPH; (B) Inhibition of nitric oxide radical; (C) Inhibition of superoxide radical; (D) Reducing Activity; and (E) Inhibition of hydrogen peroxide. (CA- Cinnamaldehyde, BHT- Butylated hydroxytoluene and AA- Ascorbic acid).



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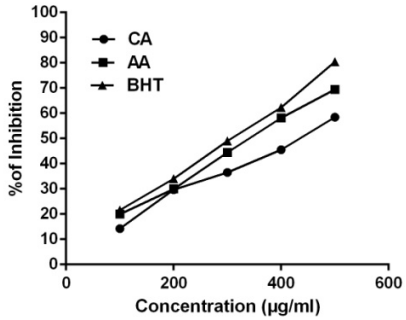

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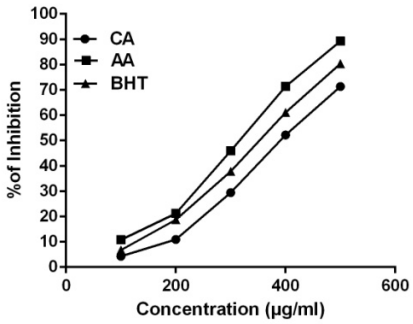

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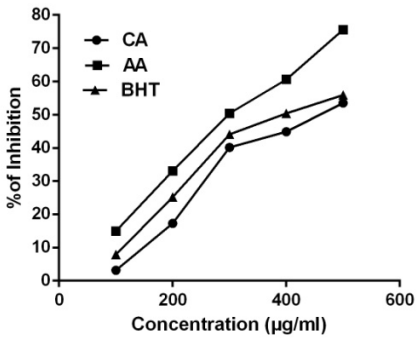

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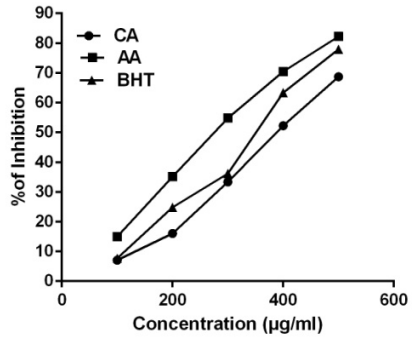

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