

Study on Antioxidant Property of *Syzygium aromaticum* (Clove)

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ABSTRACT

This paper is aimed at evaluating the antioxidant properties of *Eugenia Caryophyllata* (*Syzygium aromaticum*), 100% aqueous and 20:80% (water and alcohol) for hydroethanolic and hydromethanolic extracts were prepared as working solutions for the studies. About 5 different antioxidant examinations of *Syzygium aromaticum* (Clove) extracts were carried out which include total phenolic content, antioxidant assay, free radical's DPPH scavenging activity, scavenging hydrogen peroxide and reducing power assay, which was estimated spectrophotometrically by the use of butylated hydroxyanisole (BHA) as standard, and tocopherol used as the standard for reducing power test. Hydromethanolic extract exhibited higher free radical scavenging activity (62.12%), at the highest concentration of 1000 µg/mL followed by hydroethanolic and aqueous (58.80% and 48.32% respectively), less reducing power properties were observed with high absorbance value in the highest concentration of hydromethanolic extract (0.198 ± 0.001 A) and also hydromethanolic extract shows highest scavenging hydrogen peroxide (76.99 ± 0.09).

INTRODUCTION

Antioxidants are substances capable to clean up free radicals and prevent them from causing cell damage which can be found in the natural plant, fruits, vegetables, spices, est.[12]. Free radicals are responsible for causing a wide number of health problems which include cancer, ageing, heart diseases, and gastric problems est. [12]. Antioxidants cause a protective effect by neutralizing free radicals, which are toxic by-products of natural cell metabolism. The human body naturally produces antioxidants, but the process is not 100% effective in case of overwhelming production of free radicals and that effectiveness also declines with age. Antioxidant intake can increase disease prevention and lower health problems. Research is increasingly showing that antioxidant-rich foods and herbs reap health benefits. Foods may enhance antioxidant levels because foods contain a lot of antioxidant substances [14]. Fruits and vegetables are loaded with key antioxidants such as vitamin A, C, E, beta-

carotene and important minerals, including selenium and zinc. According to [13]. Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds. Natural products, mainly obtained from dietary sources provide a large number of antioxidants. Phytoconstituents are also an important source of antioxidants and are capable to terminate the free radical chain reactions [8].

Clove biologically is an important medicinal plant due to the wide range of pharmacological effects consolidated from traditional use for centuries and reported in the literature review of several scientific reports that the most important biological activities of clove and eugenol are antioxidant properties [14]. Recently, the United States Department of agriculture in collaboration with universities and private companies create a database with the polyphenol content and antioxidant activity of different kinds of foods. Based on this database, [5] classified the 100 richest dietary sources of polyphenols and the results indicate

that the spice plants are the kind of food with higher polyphenol content followed by fruits, seeds and vegetables. Among spices, clove showed a higher content of polyphenols and antioxidant compounds. In another work published by [9]. The main phenolic compounds in 26 spices were identified and quantified by high-performance liquid chromatography, followed by the in vitro antioxidant activity analysis by the ABTS method. Results showed a high correlation between the polyphenols content and the antioxidant activity.

Clove (buds) was the spice presenting higher antioxidant activity and polyphenol content, (168.660 & 0.024) tetraethyl ammonium chloride (mmol of Trolox/100g dried weight) and (14.380 & 0.006) g of gallic acid (equivalents/100g of dried weight) respectively. The major types of phenolic compounds found were phenolic acids (gallic acid), flavonol glycosides, phenolic volatile oils (eugenol, acetyl eugenol) and tannins. It highlighted the huge potential of clove as a radical scavenger and as a commercial source of polyphenols [13].

This research work aims to examine and evaluate the total antioxidants property of three different extracts of *Syzygium aromaticum* (Clove) with the objectives to prepare aqueous, hydroethanolic and hydromethanolic extracts as the main working solution and evaluating the antioxidant properties of *Syzygium aromaticum* (clove) by both qualitative and quantitative methods.

MATERIALS AND METHODS

Chemicals use

All chemicals used in this research work were of analytical reagent grade and were obtained from the Biotechnology Department laboratories of Sharda University, Greater Noida, Uttar Pradesh (UP), India.

Source of Plant Material

The clove was purchased from Spencer Ansal Plaza, Greater Noida, Uttar Pradesh, India and authenticated by the Botanist of Life Science Department

Determination of the Antioxidant Properties of *Syzygium aromaticum* (clove)

Total Phenolic Content

The total phenolic content of each of the three different extracts of clove was determined spectrometrically according to [10]. In this technique, 1mL of Folin Ciocalteu's reagent, previously diluted (1:20), was then added to 1mL of each of the three samples of the respective extracts [(100, 200, 400, 600, 800, 1000) µg/mL] and mixed thoroughly. To the mixture, 4 mL of sodium carbonate (10 g/50 mL) and 5 mL of distilled water were added and mixed well.

The mixtures were allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000 rpm for 5 minutes and the absorbance of the supernatant was taken at 750nm by spectrophotometer. A standard curve was obtained using various concentrations of gallic acid. And the results were expressed as a percentage of gallic acid equivalents (GAE) per 100 grams of fresh mass.

Antioxidant assay

To get free radical scavenging activity of 1-1-diphenyl-2-picrylhydrazyl (DPPH). The free-radical scavenging activity of each of the three different extracts of clove was measured by a decrease in the absorbance of methanol solution of DPPH [11].

A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave an initial absorbance of 0.493, and 5mL of this stock solution was then added to 1 mL of each of the three different extracts of clove at different concentrations [0.1, 0.2, 0.4, 0.6, 0.8, 1.0(mg/mL)]. After 30 min, absorbance was measured at 517 nm and compared with standards (10-50 mg/mL). Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ Anti-radical activity} = \frac{\text{Control Abs} - \text{Sample Abs} \times 100}{\text{Control Abs}}$$

Scavenging of Hydrogen Peroxide

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (100, 200, 400, 600, 800, 1000 µg/mL) of each of the three different extracts of clove was added to a hydrogen peroxide solution (0.6 mL, 40mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of each of the three different extracts of clove and standard compounds were calculated using the following formula: % scavenged [H₂O₂] = [(A₀-A₁)/A₀] x 100. Where A₀ is the absorbance of the control, and A₁ is the absorbance in the presence of each of the three samples of clove and standards [4].

Reducing power assay

The reducing power of each of the three different extracts of clove was determined as reported by the method of [6]. Different concentrations of each of the three different extracts (100, 200, 400, 600, 800, 1000 mg/mL) in 1mL of methanol were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was also mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicates increased reducing power.

Total antioxidant capacity

Plant extract at different concentrations (0.1-1 mg/mL) were combined in Eppendorf tube with 1mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in thermal block at 95 °C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 595 nm against blank [1].

RESULTS AND DISCUSSION

In this study, the dry clove was analyzed for antioxidant evaluation using standard methods. Hydrogen peroxide (H₂O₂) is highly important because of its ability to penetrate biological membranes. H₂O₂ itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in the cells. Thus, removing H₂O₂ is very important for the protection of food systems. **Fig. 1** shows the H₂O₂ scavenging activity by 1000 µ/mL of Clove extract and comparison with 50 µm/mL of BHA. The percentage of H₂O₂ scavenging activity of clove extracts (aqueous, hydroethanolic and hydromethanol) and butylated hydroxyanisole or BHA (control) was found to (69.64%, 71.61% and 76.99% respectively) in comparison with the standard (96.42%).

DPPH assay

The free radical scavenging activity was evaluated by various in vitro assays. DPPH radical was used as a substrate to evaluate free radical scavenging activities of clove extract. It involves the reaction of specific antioxidants with a stable free radical 2, 2-diphenyl- 1-picrylhydrazyl DPPH. As a result, there is the reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH; this is detected by spectrophotometer at 490 nm. Fig. 2 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of extracts of *S. aromaticum* and BHA were used as standards.

The scavenging effect of clove extracts (Aqueous, hydroethanolic and hydromethanolic) on the DPPH radical was (48.32, 58.8 and 62.12% respectively) at a concentration of 1000 µg/mL. These results indicated that extract has a noticeable effect on scavenging the free radicals. In comparison with the BHA (control) of 92.94% scavenging activity of hydro methanolic was significantly higher than that of hydroethanolic and aqueous extract. Hydromethanolic extract showed significantly higher radical-scavenging activity than other extracts did. But in a lower concentration of 100µg/mL all the three different extracts of clove also showed scavenging activity as shown in Fig. 2.

According to this research, there is a significant difference between scavenging activities of BHA and clove extracts. These results revealed that clove ethanol extracts have the highest free radical-scavenging compounds, acting possibly as primary antioxidants. The radical-scavenging activity of the extracts is attributed to their hydrogen donating ability [5].

Reducing power

The antioxidant activity has been reported to be associated with reducing power [5]. Fig. 3 shows the reducing powers of different clove extracts using the potassium ferricyanide reduction method. At 750 nm the clove extracts obtained using aqueous, hydroethanol, and hydromethanol showed absorbance of (0.176, 0.186, and 0.198 respectively) at a concentration of 1000 µg/mL. The reducing power of clove hydromethaethanol and hydroethanol at 750nm absorption was significantly higher than Aqueous extracts. At a concentration of 100µg/mL also, hydromethanolic clove extract showed significantly higher reducing power than others.

The reducing properties are generally associated with the presence of reductions [2]. It is reported that the antioxidant action of reductions is based on the breaking of the free radical chain by donating a hydrogen atom [3]. Reductions also react with certain precursors of peroxide, thus preventing peroxide formation. The clove phenolic may act in a similar fashion as reductions by donating the electrons and reacting with free radicals to convert them to a more stable product and terminate the free radical chain reaction. An increased absorbance indicates increased reducing power.

Scavenging Hydrogen Peroxide of various extracts of *Syzygium aromaticum* (Clove)

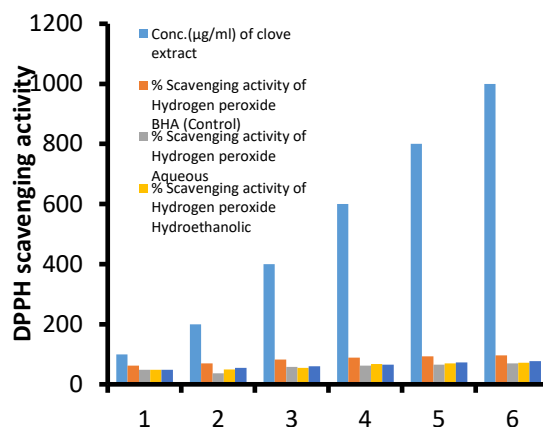


Fig. 1. DPPH scavenging activity of various extracts of *Syzygium aromaticum* (Clove).

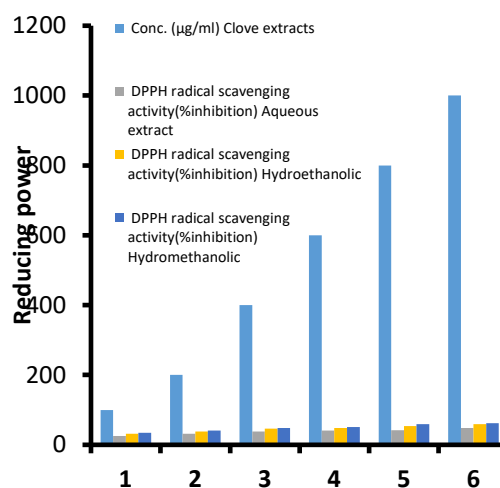


Fig. 2. Reducing power of various extracts of *Syzygium aromaticum* (clove).

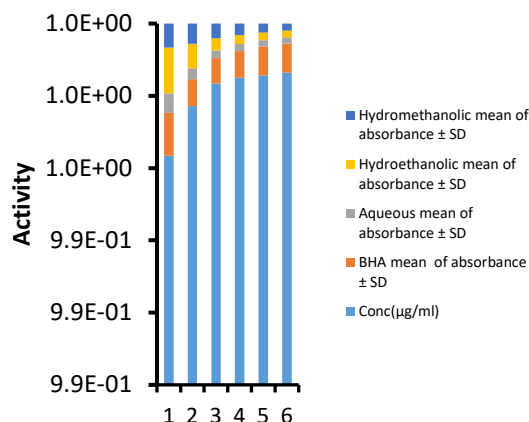


Fig. 3. Reducing powers of different clove extracts using potassium ferricyanide reduction method.

CONCLUSION

Many substances consumed by man either through foods, drinks or inhalation may be destructive to the health and thus, shortening the life span of man by giving chances to free radicals and pathogenic micro-organism, when generated in the body system of man, causes damage to which eventually leads to death at the short period of time. Clove is an aromatic plant that is a source of fragrances, flavor, cosmeceuticals, health beverages and chemical terpenes. Medicinal plants are important for pharmaceutical research and drug development. The result of the present research showed that all the three different extracts of clove exhibited noticeable activity in neutralizing the free radicals and other toxic substances in the body of humans due to its ability to scavenge the free radicals, reduction of Fe^{3+} to Fe^{2+} and hydrogen peroxide. The comparison of all the three different extracts of clove with standard (BHA) finally concluded that Hydro methanolic extract was found to be higher active than the other Hydroethanolic and Aqueous with total activity (% inhibitory) of 92.94%, 62.12%, 58.8% & 48.32% respectively) at the highest concentration of 1000 μ g/mL of free radical scavenging DPPH assay. Accordingly, the findings of this work can be considered to suggest that *Syzygium aromaticum* (Clove) could be important as a potential source of natural antioxidant and anti-carcinogenic agents for preventing oxidative stress and related degenerative diseases such as cancer, rheumatoid arthritis, diabetes, premature menopause, Alzheimer disease, heart disease etc. This research serves as preliminary work for the isolation of some important compounds present in Clove for the production of valuable drugs which can enhance the treatment of a wide range of diseases. The study also serves as preliminary work for the isolation of specific antioxidants which can be used in the food industry to produce important food additives rich in antioxidants to neutralize free radicals.

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