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Review Article

A Review Article on Antioxidant Activity of *Syzygium aromaticum* [Clove]

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ABSTRACT

Clove (Syzygium aromaticum) is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes. Clove is native of Indonesia but nowadays is cultured in several parts of the world including Brazil in the state of Bahia. Clove may be looked upon as a champion of all the antioxidants. The Oxygen Radical Absorption Capacity (ORAC) test is a scale developed by U.S. Department of Agriculture for comparing antioxidant activity. Health benefits from the use of clove have been known for the centuries. It is beneficial as a home remedy in curing several ailments / diseases. In addition to its culinary uses, the clove buds have an abundance of medicinal and recreational uses. The ORAC score, of clove is over 10 million. Eugenol is the main constituent responsible for the medicinal properties of the clove bud. In the light of above, we thought it worthwhile to compile an up-to-date review article on clove covering its, synonyms, chemical constituents, phytopharmacology and medicinal uses. 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

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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). The effects of heat treatment and extraction solvents (pure/aqueous acetone, ethanol, methanol) on antioxidant activity (AA) and components of clove (Syzygium aromaticum Linn) were studied. Clove was subjected to dry heat treatment (microwave and roasting) and the AA measured by free radical scavenging activity (FRSA), reducing power (RP), and phospho-molybdenum assay (TAA). The present study evaluates the antioxidant potential of clove of copper against an induced a lipid peroxidation protein oxidative modification and iron induced damage to DNA 1,1- Diphenyl 2- picryl hydrazine free radical scavenging capability of methanol. The clove exhibited a concentration and dependent activity. 56% of DPPH radicals were scavenged with extract equivalent to 100µg Clove. Clove has low riboflavin [1.5µg/g] content but high ascorbate [985.6µg\g] and content tocopherol [660.6µg/g].

INTRODUCTION

Spices as clove, oregano, mint, thyme and cinnamon have been employed for centuries as food preservatives and as medicinal plants mainly due to its antioxidant and antimicrobial activities. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants. Clove in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices [1].

Syzygium aromaticum (S. aromaticum) (synonym: Eugenia caryophylata) commonly known as clove, is a median size tree (8-12 m) from the Myrtaceae family native from the Maluku islands in east Indonesia. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region [2]. A wide range of disease process including atherosclerosis, diabetes, pulmonary fibrosis, neurodegenerative disorders, can be correlated with oxidative stress [3]. The free radical lipid peroxidation is an important issue in the food in the food industry also where the manufacturing process in the nutritional quality of food [4]. Based on this database, 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 [5]. Cloves are the aromatic flower buds of a tree in the family Myrtaceae, Syzygium aromaticum. They are native to the Maluku Islands (or Moluccas) in Indonesia and are commonly used as a spice.

COMMON NAME:

Cloves, Carophyllus, Clovos, Caryophyllus

Botanical Names

Eugenia caryophyllus, Syzygium aromaticum

Names in Indian languages: -

Sanskrit: Bhadrasriya, Devakusuma, Devapuspa, Harichandana, Karampu, Lavanga, Lavangaka, Lavangam, Varala.

Hindi: Laung, Laumg, Lavang.

Telugu: Devakusumamu, Lavangamu, Lavangalu, Kaaravallu

Synonyms: - Clove buds, Clove flowers.

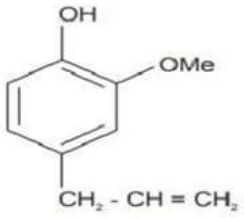
Biological Source: - Clove consists of the dried flower buds of Eugenia caryophyllus Thumb., belonging to family Myrtaceae.

Geographical Source: - Clove tree is a native of Indonesia. It is cultivated mainly in Islands of Zanzibar, Pemba, Brazil, Amboina, and Sumatra. It is also found in Madagascar, Penang, Mauritius, West Indies, India, and Ceylon.

Antioxidative activity has been reported in aroma extract of clove buds especially eugenol and eugenol acetate (6).

1. Chemical compounds isolated from clove

Clove represents one of the major vegetal sources of phenolic compounds as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids and hydroxyphenyl propens. Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material [7].



Eugenol

2. Biological activities

Clove is an important medicinal plant due to the wide range of pharmacological effects consolidated from traditional use for centuries and reported in literature. A review of several scientific reports of the most important biological activities of clove and eugenol.

a. Antioxidant activity

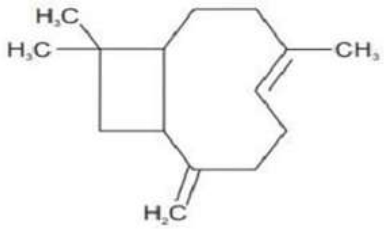
The antioxidant activity of aqueous extracts of clove has been tested by different in vitro methods

CULTIVATION AND COLLECTION

Clove tree is evergreen and 10 to 20 m in height. The plant requires moist, warm and equable climate with well-distributed rainfall. It is propagated by means of seeds. The seeds are sown in well-drained suitable soil at a distance of about 25 cm. The young clove trees are protected from sun even for a longer period by planting banana trees in between. The drug can be collected every year starting from 6 years old till they are 70 years old [8].

CHEMICAL CONSTITUENTS:

The other constituents present are the eugenol, acetyl eugenol, gallotannic acid, and two crystalline principles: α- and β- caryophyllenes, methyl furfural, gum, resin, and fibre. Caryophyllin is odourless component and appears to be a phytosterol, whereas eugenol is a colourless liquid [9].



Caryophyllene

as 2,2- diphenyl-1-picrylhydrazyl (DPPH); 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), oxygen radical absorbance capacity, ferric reducing antioxidant power, xanthine oxidase and 2-deoxiguanosine. Clove and plants as pine, cinnamon, and mate proved its enormous potential as, and mate proved its enormous potential as potential as food preservative among the other 30 plants analysed [10].

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 kind of foods.

Ethanol and aqueous extracts of clove and lavender at concentrations of 20, 40 and 60 µg/mL showed inhibitions up to 95% when tested as metal quelants, superoxide radical capture and scavenging of the DPPH radical. The powerful antioxidant activity of both extracts may be attributed to the strong hydrogen donating ability, metal chelating ability and scavenging of free radicals, hydrogen peroxide and superoxide [11]

Compared to butylated hydroxyanisole, BHT, Trolox and α-tocopherol, eugenol presented higher antioxidant activity in most of the methods tested, DPPH, ABTS, N, N-dimethyl-p-phenylenediamine, CUPRAC and ferric reducing assay. It was remarked that plant polyphenols are multifunctional in the sense that they can act as reducing agents, hydrogen atom donators, and singlet oxygen scavengers. Eugenol allows the donation of an hydrogen atom and subsequent stabilization of the phenoxil radical generated forming stable compounds that do not start or propagate oxidation.

Antioxidants are important compounds for treatment of memory deficits caused by oxidative stress [12].

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay is a typical ET-based method that measures the reduction of ferric ion (Fe^{3+})-ligand complex to the intensely blue-coloured ferrous (Fe^{2+}) complex by antioxidants in an acidic medium. Antioxidant activity is determined as increase of absorbance at 593 nm, and results are expressed as micromolar Fe2+ equivalents or relative to an antioxidant standard. Unlike other ET-based methods, the FRAP assay is carried out under acidic pH conditions (pH 3.6) in order to maintain iron solubility and, more importantly, drive electron transfer. Antioxidants can either reduce the Fe3+ in the solution to Fe2+, which binds the ferricyanide to yield Prussian blue, or reduce the ferricyanide to ferrocyanide, which binds the free Fe3+ in the solution and forms Prussian blue.

$$\text{Antioxidant} + Fe^{3+} \rightleftarrows Fe^{2+} + \text{oxidizedantioxidant}$$

$$Fe^{2+} + Fe(CN)_6^{3-} \rightleftarrows Fe[Fe(CN)_6]^{-}$$

The FRAP assay, in general, as an ET-based nonradical method, has been argued to have little relationship with the radical quenching process (HAT mechanism) occurring in lipid systems, and it has poor correlation with other antioxidant activity measurements. It is, therefore, suggested that this assay could be used in combination with other methods in distinguishing dominant mechanisms for different antioxidants.

The FRAP assay is simple, fast, and cost-effective and does not require specialized equipment. It was originally employed to measure the reducing power in plasma, but its use has been extended for assessing antioxidant activity in other biological fluids, foods, and plant extracts. However, Pulido, Bravo, and Saura-Calixto (2000) reported that FRAP results might vary depending on the analysis time as observed for the reaction between antioxidants and Fe^{3+}, which ranged from several minutes to several hours. Therefore, a single-point absorption end point may not represent a complete reaction, since different antioxidants require different reaction times for detection.

b. Antimicrobial activity

The antimicrobial activities of clove have been proved against several bacterial and fungal strains. Tested the antimicrobial activity of different

Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove [13]. The only sampled that showed complete bactericidal effect against all the food-borne pathogens tested Escherichia coli (E. coli), Staphylococcus aureus and Bacillus cereus was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action.

In another work published by [Dorman and Deans], the antibacterial activity of black pepper, geranium, nutmeg, oregano, thyme and clove was tested against 25 strains of Gram positive and Gram-negative bacteria [14].

The anticandidal activity of eugenol and carvacrol was tested in a vaginal candidiasis model, microbial and histological techniques were employed to compare the samples with the controls. The results suggest that eugenol and carvacrol could be a promising antifungal agent for treatment and prophylaxis of vaginal candidiasis [15].

Beta-cyclodextrin inclusion complexes containing eugenol and clove bud extracts were tested against two common foodborne pathogens, Salmonella enterica serovar Typhimurium LT2 and Listeria innocua [16]. Clove products have a great potential as food additives since they are very effective and for being natural products are preferred for consumers. Moreover, the solubility and the delivery are improved with the encapsulation process.

c. Antiviral

The antiviral activity of eugeniin, a compound isolated from S. aromaticum and from Geum japonicum, was tested against herpes virus strains being effective at 5 µg/mL, and it was deducted that one of the major targets of eugeniin is the viral DNA synthesis by the inhibition of the viral DNA polymerase [17].

In another study, aqueous extracts of S. aromaticum (L.) Merr. et Perry and other plants as Geum japonicum Thunb., Rhus javanica L., and Terminalia chebula Retzus among others showed strong antiherpes simplex virus type 1 (HSV-1) activity when combined with acyclovir.

d. Cytotoxicity of eugenol

The anti-oxidative, cytotoxic and genotoxic effects of eugenol and borneol were tested as the ability to modulate resistance against the damaging effects of H2O2 on DNA of different strains of human cells: malignant HepG2 hepatome cells, malignant Caco-2 colon cells and non-malignant human VH10 fibroblast. It was also evidenced that the citotoxic effects of eugenol were stronger than those of borneol. With regard to toxicity, eugenol presented strong genotoxic effects (DNAdamaging) on human VH10 fibroblast, medium genotoxic effects on Caco-2 colon cells and non-DNA-damaging effects on HepG2 hepatome cells [18].

Although there are many reports of the antioxidant activity of eugenol, at high concentration eugenol could be prooxidant. The cytotoxicity, reactive oxygen species (ROS) production, and intracellular glutathione levels in a human submandibular cell line (HSG cells) of eugenol and isoeugenol was studied by Atsumi et al [19]. It was found that in the absence of oxidative stress eugenol acts as an antioxidant at low concentrations but acts as a prooxidant at high concentrations. In the presence of oxidative stress eugenol increased ROS levels at low concentrations [5-10 µmol/ L] but decreased them at high concentrations [500 µmol/L]. Therefore, it can be concluded that the cytotoxicity of eugenol

occurs in a ROS-independent manner in the presence of oxidative stress.

3. Toxicity and pharmacokinetics

On the other hand, the World Health Organization (WHO) established that the daily quantity acceptable of clove per day is of 2.5 mg/kg of weight in humans [20]. The toxicity of clove oil was tested in two aquarium fish species, Danio rerio and Poecilia reticulata the medium lethal concentrations (LD50) at 96 h were (18.2+or-5.52) mg/mL Danio rerio and (21.1+or-0.8) mg/mL in Poecilia reticulata [21].

Eugenol is easily absorbed when administrated by oral route reaching rapidly plasma and blood with mean halflives of 14.0 h and 18.3 h, respectively. A cumulative effect has been hypothesized and associated to relieve of neuropathic pain after repeated daily administrations [22].

4. Agricultural and larvicidal uses

Eugenol, eugenol acetate and beta-caryophyllene were effective in repellency of red imported fire ants Solenopsis invicta (Hymenoptera: Formicidae), being eugenol the fastest acting compound [23].

The most appropriate dose to anesthetize the angelfish was determined by Hekimoglu and Ergun [24]. This study will help in the transportation and handing of this fish which is one of the most stressful aquarium species.

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, 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

Materials

The spice used for the study, whole clove (Syzygium aromaticum Linn.) was purchased from a supermarket in Mysore, India in a clean and packed form. Whole clove is the mature and dry bud from the tree, which is shelf stable and used in whole or powdered form for cooking. All the chemicals purchased were of analytical grade from different firms namely, Sd Fine Chemicals, and Qualigens Ltd., Mumbai, India. DPPH (1,1-diphenyl-2-picrylhydrazyl) was procured from Glass double distilled water was used for all analyses.

Methods

A single batch of whole spice weighing 150 g was taken and divided into three parts for different treatments. Two sets were used for heat treatment and one set was left untreated which served as control. One set (50 g) was roasted on medium flame for 5 min with continuous stirring in a thick bottom pan. The end point was indicated by emission of a strong aroma typical of roasted clove. Another set was heated in microwave oven (Model number: BMO-700 T from BPL Sanyo Utilities and Appliances Ltd.) for a total of 2 min at high power (2450 MHz, 1200 watts) with intermittent stirring. The time of dry heat treatment was standardized after initial trials with each sample. The roasted spice was cooled and powdered immediately in a grinder to pass through a 40-mesh sieve and stored in airtight PET (polyethylene terephthalate) jars under refrigeration at 4 °C until further use. The

unheated spice set was also powdered and stored in PET containers under similar conditions.

Preparation of extract

Hence, different solvents namely ethanol, methanol and acetone, and the combination of solvent with water like 80 % ethanol, 80 % methanol and 80 % acetone were used for extraction of the spice as suggested [25]. The selection of extraction media was based on many earlier studies wherein authors have repeated a better and higher extraction of antioxidant components in polar and non-polar solvent [26].

Antioxidant activity of all extracts were measured by three standard techniques (reducing power, free radical scavenging activity by DPPH assay and total antioxidant activity by phosphomolybdenum complex assay). All extracts were made in duplicate, and all analyses were conducted in triplicate. Hence, the results represent average and standard deviations of six determinations for all samples.

Determination of antioxidant activity

The reducing power (RP) was determined by the method [27]. In brief the procedure was as follows - 1.0 ml of extract was mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide and incubated at 50 °C for 20 min. Thereafter, 2.5 ml of 10 % trichloro acetic acid was added to the mixture, followed by centrifugation. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl3 solution and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increasing reducing power. Free radical scavenging activity (FRSA) was measured using the method [28]. A 1.0 ml solution of DPPH (0.1 mmol in methanol) was mixed with 3 ml of extract, incubated for 30 min and the absorbance measured at 517 nm.

The DPPH concentration in the reaction medium was calculated as percent free radical scavenging activity (control OD- sample OD/control OD× 100).

The total antioxidant capacity (TAA) is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH [29]. The antioxidant activity of extracts was expressed as equivalents of ascorbic acid.

Determination of antioxidant components

Antioxidant components namely, total phenols, tannins and flavonoids were measured following standard techniques in all solvent extracts of spice. Total phenols were measured by Folin-Ciocalteau method and concentration was calculated using tannic acid as standard. Results were expressed as mg tannic acid equivalents/100 g sample [30]. The total flavonoid content was determined using a standard curve with quercetin (0–100 mg/l) as the standard and expressed as mg of quercetin equivalents (QE)/100 g of extract following the method of Dowd [31]. Tannins were measured colorimetrically based on the measurement of blue colour formed by reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution, tannic acid was used as standard (AOAC 1970) [32].

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 singleton. 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 [33].

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.

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 [34].

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:

% *A*nti-radical activity=

$$\frac{[\text{Control Abs- Sample Abs}]}{\text{Control Abs}} \times 100$$

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

$$\% \text{ scavenged } [H_2O_2] = [(A0-A1)/A0] \times 100.$$

Where A0 is the absorbance of the control

A1 is the absorbance in the presence of each of the three samples of clove and standards [35].

Reducing power assay

The reducing power of each of the three different extracts of clove was determined. 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 50^0C 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 [36].

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 [37].

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. 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 hydro methanol) 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. Hydromethnolic 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.

Reducing power

The antioxidant activity has been reported to be associated with reducing power [38]. 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 [39]. It is reported that the antioxidant action of reductions is based on the breaking of the free radical chain by donating a hydrogen atom [40]. 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.

Discussion

The overall observation of RP shows that among all extracts of clove; 80 % acetone showed the highest activity. This was followed by 80 % methanol and 80 % ethanol. Pure solvents had lower reducing power values. This could be related to the nature of antioxidants present in clove, as stated earlier. Clove has both fat soluble and water-soluble bioactive constituents, hence aqueous solvents could solubilize a higher quantity of antioxidant components and exhibit higher antioxidant activity [41]. Clove is also recognized for a very high content of antioxidant components among spices [42,43] in their study on antioxidant activity and phenolic compounds of spices reported the presence of 8.96 mg of phenols as gallic acid equivalents/100 g of dry weight of clove. Aqueous extracts of clove have been shown to have very high phenolic content and strong antioxidant properties by many workers [44].

Correlation between antioxidants components and antioxidant activity of clove in different media

Since the analysis of extraction solvents revealed the presence of significant quantities of antioxidant components, it was interesting to see whether there was a correlation between the antioxidant activity and components and the correlation coefficients (R values) are presented. The RP assay showed positive correlation with phenols and flavonoids for all samples with R values in the range of 0.676–816. A positive correlation was also seen with FRSA for all samples (range of R value, 0.503–0.824). Tannins, though positive, exhibited a very weak association in RP assay (0.036–0.174) and medium association with FRSA (R value, 0.356–0.554). The correlation between antioxidant components and TAA showed low R values for control (0.161–0.359) and microwave heated sample (0.354–0.468). For tannins, all association with TAA was negative and for total phenols, roasted sample also showed negative R value. The results indicate better association between antioxidant activity of clove with phenols and flavonoids when assayed with RP and FRSA.

To summarize, results of the study clearly indicate that clove is a powerful antioxidant demonstrating various levels of antioxidant activity in different solvents. These activities can be attributed to their antioxidant components which showed good correlation with the activities. Heat treatments were shown to have a positive effect in liberating some of these components, thus resulting in increased antioxidant activities in the heated samples. Hence clove has the potential to be used as an antioxidant in foods in heated form and processing has a positive effect on antioxidant properties of clove.

Effect of dry heat treatments on antioxidant activity of clove

The dry heat-treated extracts in all solvents showed slightly higher values which were marginally significant in relation to sample without heat treatment. The absorbance at 700 nm in different solvents for roasted and microwaved sample (at 0.8 mg concentration) was in the range of 0.751–1.442 and 0.771–1.448 respectively and followed the ascending order for acetone, ethanol, methanol, 80 % ethanol, 80 % methanol and 80 % acetone respectively. The absorbance of control sample (not heated) extracted with different solvents was in the range of 0.742 to 1.178 at 700 nm (acetone and 80 % acetone, respectively) and followed a similar order, though with significantly lower values than heat treated samples.

When the TAA was measured in clove sample using phosphomolybdenum assay, the control sample had least activity in acetone followed by

methanol, 80 % ethanol, ethanol, 80 % acetone and 80 % methanol. The heated samples however followed a different order with least value in methanol and showed higher activity than control which was highly significant for all except acetone extract of microwave clove sample. The spread of values for all samples were between 196.16 and 307.941 mmol ascorbic acid/g, with varied differences among different solvent extracts.

When the TAA was measured in clove sample using phosphomolybdenum assay, the control sample had least activity in acetone followed by methanol, 80 % ethanol, ethanol, 80 % acetone and 80 % methanol (Table 2). The heated samples however followed a different order with least value in methanol, and showed higher activity than control which was highly significant for all except acetone extract of microwave clove sample. The spread of values for all samples were between 196.16 and 307.941 mmol ascorbic acid/g, with varied differences among different solvent extracts.

Antioxidants components in dry heat-treated clove extracted in different solvents

Total phenols, tannin and flavonoid content of unheated and dry heat-treated samples, extracted in different solvents are presented and discussed below. The extraction efficiency of solvents was different for each of the components and a large variation could be observed in the amount of antioxidant components extracted. The results are discussed for each of the component for ease of comprehension.

The extraction of tannins was highest in 80 % acetone for control sample (16,441 mg/100 g), in 80 % ethanol for microwaved sample (19,558 mg/100 g) and in methanol for roasted sample (15,823 mg/100 g). The acetone and 80 % acetone media extracted higher tannins from control compared to heat treated sample (range, 13,706–14,950 mg/100 g). In ethanol and 80 % ethanol extracts, the microwave treated sample had significantly higher tannin content than control. Roasted sample also showed a higher extraction of tannins in comparison to control, and it was marginally significant for ethanol, and not significant for 80 %ethanol.

Values indicate mean ± standard deviations. Significant difference between heat treated sample and control on application of Students T Test. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant

MEDICINE USES

Clove is used as an antiseptic, stimulant, carminative, aromatic, and as a flavouring agent. It is also used as anodyne, antiemetic. Dentists use clove oil as an oral anesthetic and to disinfect the root canals. Clove kills intestinal parasites and exhibits broad antimicrobial properties against fungi and bacteria and so it is used in the treatment of diarrhea, intestinal worms, and other digestive ailments. Clove oil can stop toothache. A few drops of the oil in water will stop vomiting, eating cloves is said to be aphrodisiac. Eugenol is also used as local anaesthetic in small doses. The oil stimulates peristalsis; it is a strong germicide, also a stimulating expectorant in bronchial problems.

Applications

S. aromaticum (Myrtaceae) commonly known as clove, is a median size tree (8-12 m) native from the Maluku islands in east Indonesia. The clove tree is frequently cultivated in coastal areas at maximum altitudes of 200 m above the sea level. The production of flower buds, which is the commercialized part of this tree, starts after four years of plantation. The collection could be done manually or chemically mediated using a natural

phytohormone which liberates ethylene in the vegetal tissue, producing precocious maturation.

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 Fe3+ to Fe2+ 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 1000µg/mL of free radical scavenging DPPH assay. Hydroethanolic and aqueous activity [% inhibitory] of 92.94%, 62.12%, 58.8% & 48.32 respectively] at the highest concentration of 1000µg/ml of free radical activity DPPH assay. Clove for the production of valuable drugs which can enhance the treatment of a wide range of diseases.

Clove is a medicinally important drug, reported to have a variety of different applications like antioxidant, antifungal, antiviral, antibacterial, anti-inflammatory, antithrombic, antipyretic, analgesic, anticonvulsant, antimycotic, insecticidal, antimutagenic, antiulcerogenic etc. Clove is used to treat various health conditions, including intestinal parasites, migraine headaches, colds, impotence, and gastrointestinal problems such as nausea, vomiting, diarrhoea and gas. There is a great scope for researchers to develop efficacious formulations using clove, based on the information presented, it could be concluded that clove represents a very interesting plant with an enormous potential as food preservative and as a rich source of antioxidant compounds. It's proved biological activities suggest the development of medicinal products for human and animals uses and confirm why this plant has been employed for centuries.

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