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Evalution of Antioxidants Activity of Herbal Extracts (Clove)

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Abstract: *CLOVE (Syzygium aromaticum)*
It is a nutrient dense food rich in beneficial phytochemicals.The objective of this study was to elucidate in detail theantitioxidant capacity of Syzygium aromaticum from the different parts of clove including their stem and fruits as determined by total phenol content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH). For these purposes, acetone extracts (50%) were prepared. This study showed that the selected parts of the clove plant varied significantly. The results showed that the highest antioxidant activity through TPC and TFC were observed in fruit (247.61 and 141.70 mg/100 dry weight) followed by stem (209.48 and 126.50 mg/100 g), respectively. On the other hand, fruits exhibited a significant higher scavenging effect compared to stem sample. Interestingly, both FRAP and DPPH also showed that fruits samples had the highest antioxidant content (437.29 mg TE/100 g dry weight and 87.50%, respectively).
The antioxidant activities of different parts clove extracts indicate that the consumption of the whole fruit and stem supplies the important quantities of numerous necessary nutrients for human diet, which includes vitamins high phenolic compounds content (TPC, TFC) and antioxidant activity (FRAP, DPPH). In brief, when all the parameters measured were taken into account antioxidants were highly remarkable in the sequence of clove fruits > clove stem.

Keywords: Clove, Antioxidant activity, Total phenol, Total flavonoids

I. INTRODUCTION

Antioxidants are natural or synthetic substances that protect cells from damage caused by free radical unstable molecules produced during metabolism—thereby reducing risks of diseases like cancer and heart disease. Found abundantly in fruits, vegetables, and nuts, they act as "free radical scavengers" to prevent oxidative stress.

Key Aspects of Antioxidants

- Functions: They neutralize free radicals to prevent or delay cell damage. They are essential in managing oxidative processes that lead to diseases of aging.
- Sources:
- Dietary (Exogenous): Fruits (blueberries, apples), vegetables (broccoli, spinach), nuts (walnuts, pecans), and legumes are rich in vitamins C and E, beta-carotene, and polyphenols.
- Body-produced (Endogenous): The body produces its own antioxidants, such as glutathione and enzymes like superoxide dismutase (SOD) and catalase (CAT). Examples: Beta-carotene, Lutein, Lycopene, Selenium, Vitamin A, Vitamin C& E.

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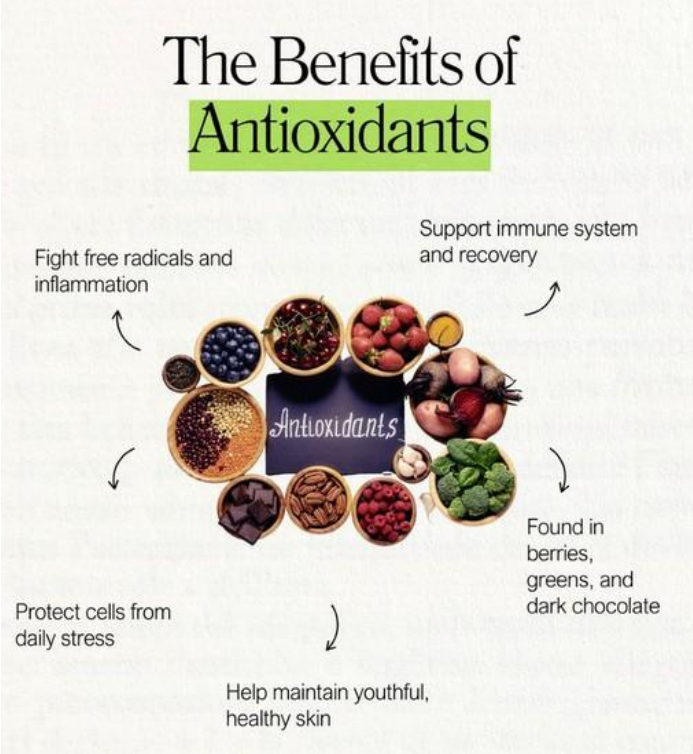

Antioxidant herbal plants, such as clove, rosemary, oregano, thyme, and sage, are potent sources of phenolic compounds, flavonoids, and vitamins that combat oxidative stress and reduce chronic disease risk. These plants often surpass cultivated fruits in antioxidant capacity, with species like Purple Dead Nettle acting as early-spring nutrient powerhouses.

Top Antioxidant Herbs and Spices :

- Clove: Holds the highest mean antioxidant value, far exceeding many other herbs.
- Oregano: Extremely high in antioxidant activity (ORAC test), particularly when dried.
- Rosemary: Known for high antioxidant activity (DPPH assay) due to phenolic compounds.
- Thyme & Sage: Rich in polyphenols and flavonoids that provide anti-aging protection.
- Basil: Contains eugenol and other compounds that reduce cellular inflammation.
- Peppermint: A top-tier antioxidant source, often used for cardiovascular health.

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Key Antioxidant Compounds in Herbs

- Phenolic Compounds: Phenolic acids, flavonoids, and tannins are the most abundant antioxidants found in these herbs.
- Vitamins and Minerals: Many herbs are rich in Vitamin C, Vitamin E, and carotenoids, which help lower oxidative stress.
- Eugenol: Found in basil, this compound provides anti-inflammatory and cardiovascular benefits.
- Mechanism: Antioxidants work by hydrogen-atom transfer (HAT) or single electron transfer (SET) to neutralize free radicals and terminate chain reactions.
- Health and Therapeutic Effects: They provide anti-aging, anti-inflammatory, and anticancer properties. They also aid in protecting against illnesses related to oxidative stress-mediated complications.

Common Uses

These herbs can be consumed as teas, used as garnishes in salads, or incorporated into meals to boost nutritional value. They are easy to grow in containers or gardens, providing a sustainable source of protective compund

- Other Uses: They are used in industrial products like lubricants and fuels to prevent oxidation.
- Risks of Supplements: High-dose antioxidant supplements can be harmful, with studies linking them to increased risk of lung cancer in smokers and potential interference with, rather than aiding, cell defense.
- Mechanism: Antioxidants work by hydrogen-atom transfer (HAT) or single electron transfer (SET) to neutralize free radicals and terminate chain reactions.
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AIM AND OBJECTIVE

Aim: Evaluation of Antioxidants activity of Herbal Extract (Clove).

Objectives:

1. Combating Oxidative Stress & Disease Prevention.
2. To identify and quantify plant-derived compounds.
3. Analytical Screening & Standardization.
4. Developing Natural Therapeutics and Cosmetics.

CLOVE

Syzygium aromaticum is a tree whose aromatic dried flower buds are known as cloves.

When it comes to phenolic chemicals, such as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenyl propane, clove is a major plant source of these substances.

Finding out how effective clove buds are as antioxidants was the driving force behind this study's GC-MS investigation and computational discoveries.

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Methods: This inquiry into clove pods focused on the chemical composition of clove using the GC-MS technique, as well as its antioxidant qualities and computational modeling.

They highly esteemed spice clove, formally known as S. aromaticum (L.) (Merr. and L.M. Perry) has a long and celebrated history of use.

The medicinal properties of cloves were known to ancient cultures. One of many spices used to flavor meals, it is also famous for alleviating pain, often targeting toothache.

Still, it has many other benefits, the most known of which involve improving immunity and safeguarding from infections.

It is believed that cloves' anti-inflammatory and anti-free-radical properties may help alleviate various illnesses, supporting good lung function

CHEMICAL COMPOSITION

Clove (Syzygium aromaticum) is primarily composed of 14–21% volatile oil, with eugenol (60–90%) being the major active constituent responsible for its anesthetic, antiseptic, and antioxidant properties.

Other significant components include eugenyl acetate, \(\beta \)-caryophyllene, and \(\alpha \)-humulene. It also contains triterpenes (oleanolic acid), flavonoids (kaempferol, quercetin), and gallotannic acid.

Key Chemical Constituents:

Essential Oil (14–21%):

1. Eugenol: The primary compound (60-90%) responsible for the characteristic aroma, therapeutic, and antimicrobial properties.
2. Eugenyl Acetate (Acetyleugenol): Typically 5–15%.
3. Caryophyllene: A major sesquiterpene, roughly 5–15%.
4. Humulene: Found in significant amounts.

Non-Volatile Compounds (Phytochemicals):

1. Triterpenes: Oleanolic acid, crategolic acid (maslinic acid).

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2. Phenolic Acids & Tannins: Gallotannic acid, caffeic acid, ferulic acid, and ellagic acid.
3. Flavonoids: Kaempferol, rhamnetin, and quercetin (often glycosylated).
4. Other Components: Gum, resin, fibre, and small amounts of vanillin

Key Pharmacological Drivers:The therapeutic effects of clove are attributed to its high concentration of phenolic compounds, specifically eugenol, which provide anti-inflammatory, analgesic, antiseptic, and antifungal activities

Cloves have several uses related to the respiratory system, including alleviating pain in the teeth and gums; warding off upper respiratory tract infections; and stopping the common cold, flu, cough, runny nose, and asthma attacks.

Relieving toothaches is one of cloves' most common uses. They have antibacterial and antiseptic properties, containing the anesthetic eugenol oil.

They also help to prevent and treat stomach ulcers, improve sexual health, and aid people with diabetes in controlling their blood sugar levels, reducing the likelihood of complications.

They are a good source of vitamin C, which the body uses to fight free radicals. They also help with digestion, alleviate dental pain, and have a significant impact on fertility. Cloves contain a major constituent compound called eugenol, which has been shown to act as a natural

antioxidant. Eugenol is clear or pale yellow, with a peppery taste and clove-like smell, and has been used to flavor food and drugs since the 19th century. It is used as a mild rubefacient in dentifrices and as an obtundent for hypersensitive dentine, caries, or pulp. The wide range of eugenol's activities include its antimicrobial, anti-inflammatory, analgesic, and antioxidant effects. It has also been used in dental cement, analgesics, anesthetics, and zinc oxide-mixed temporary dental fillings.



Research on clove buds has been focused on their great antioxidant and antibacterial action, which has made them extensively employed in traditional medicine and food preservation. Rich in bioactive terpenoids and flavonoids, clove buds are the principal source of clove essential oil.

MATERIAL AND METHOD

MATERIAL: Clove

METHOD: 1

Sample collection and preparation of clove extract

The stem and fruits of clove (Syzygium aromaticum) were obtained from the market. The stem and fruits of clove were cleaned and then oven dried at 50°C for 24 hr.

The dried sample was then pulverized using a mechanical grinder and passed through a 250 µm mesh and then stored at room temperature until use. In the extraction process, about 0.1 g of clove stem and fruits were weighed in universal bottles and 10 mL Solvent was added.

Solvents used were acetone 50%, samples were then homogenized using homogenizer.

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All extracted samples were centrifuged by using table top centrifuge for 10 min. The supernatants were collected for further analysis.

METHOD: 2

Antioxidant Activity

Antioxidant activity is the ability of compounds to inhibit, delay, or prevent the oxidation of substrates by scavenging free radicals and chelating metals, thus reducing cellular oxidative stress and inhibiting deterioration.

Key mechanisms include:

Hydrogen atom transfer,
Electron transfer,
Repairing damage.

Common natural sources include

Polyphenols,
Flavonoids, and
Vitamin C,
While BHA and BHT are used in food.

ANTIOXIDANT ACTIVITY

Antioxidants protect the body against damage caused by free radicals or reactive oxygen species (ROS), which are unstable molecules produced during normal metabolism and environmental stress. These free radicals can cause oxidative stress by damaging important cellular components such as lipids, proteins, and DNA. Antioxidants work by donating an electron or hydrogen atom to free radicals, thereby stabilizing them and reducing their reactivity. This action interrupts the chain reaction of oxidation and prevents further cellular damage. As a result, antioxidants help protect cells and tissues, reduce inflammation, slow aging processes, and lower the risk of various diseases. Common antioxidants include vitamin C, vitamin E, flavonoids, and phenolic compounds.

Antioxidants neutralize free radicals.

They prevent oxidative damage.
They protect biomolecules like DNA, proteins, and lipids.
They reduce inflammation and cell injury.
Examples: Vitamin C, Vitamin E, flavonoids, phenolics.

Free Radicals / Reactive Oxygen Species (ROS)
↓
Cause Oxidative Stress in Cells (damage to lipids, proteins, DNA)
↓
Antioxidants Donate Electron or Hydrogen
↓
Free Radicals Become Stable and Less Reactive
↓
Chain Reaction of Oxidation Stops
↓
Protection of Cells and Tissues
↓
Reduced Cellular Damage & Aging

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The first step to quantify the antioxidant activity of a plant extract is to select the right method.
There is a large variety of methods to determine this parameter and the variability of experimental conditions found in the literature for each of the methods hinders such selection and the possibility of easily comparing the obtained results with those of other authors.
All this makes it difficult to hierarchize plants based on the antioxidant activity of their extracts.
The results of different methods for different species should be analyzed using descriptive procedures of multivariate statistical techniques to establish the best method that allows ordering or selecting the plant extracts according to their level of antioxidant activity.
The available methods to quantify antioxidant activity can be classified based on the mechanism of action by which the applied compounds stop chain-breaking reactions.
They can be divided into two groups:
Hydrogen-atom transfer (HAT) (hydrogen atom transfer reactions) and
Single electron transfer (SET) (compound reduction reactions through electron transfer from an antioxidant).
Among the SET methods, the most used are
2,2-di-phenyl-1-picrylhydrazyl (DPPH radical scavenging capacity assay),
Ferric reducing (FRAP) assay,
Trolox equivalent antioxidant capacity (TEAC or ABTS) assay,
copper reduction (CUPRAC) assay and reducing power assay (RP).
Hydrogen atom transfer reaction assays include the
Crocin bleaching assay, the total peroxyl radical-trapping antioxidant parameter (TRAP) assay,
Total oxyradical scavenging capacity (TOSC) assay,
Oxygen radical absorbance capacity (ORAC) assay.

EVALUATION

1. Total phenolic content

The amount of total phenolics content (TPC) in colve was determined with the
Folin-Ciocalteu reagent base.
About 0.5 mL of Folin-Ciocalteau (10%, v/v) was added to 0.1 mL of clove extract sample.
The mixture was swirled and allowed to stand for 6 min followed by the addition of 1 mL 7.5% (w/v) of sodium carbonate (Na2CO3) and samples were mixed.
Solutions were allowed to stand for 2 hr at room temperature and the absorbance were read at 765 nm wavelength using spectrophotometer.
The results were expressed as milligrams of gallic acid equivalents per 100 g of sample (mg GAE/100 g of DW).

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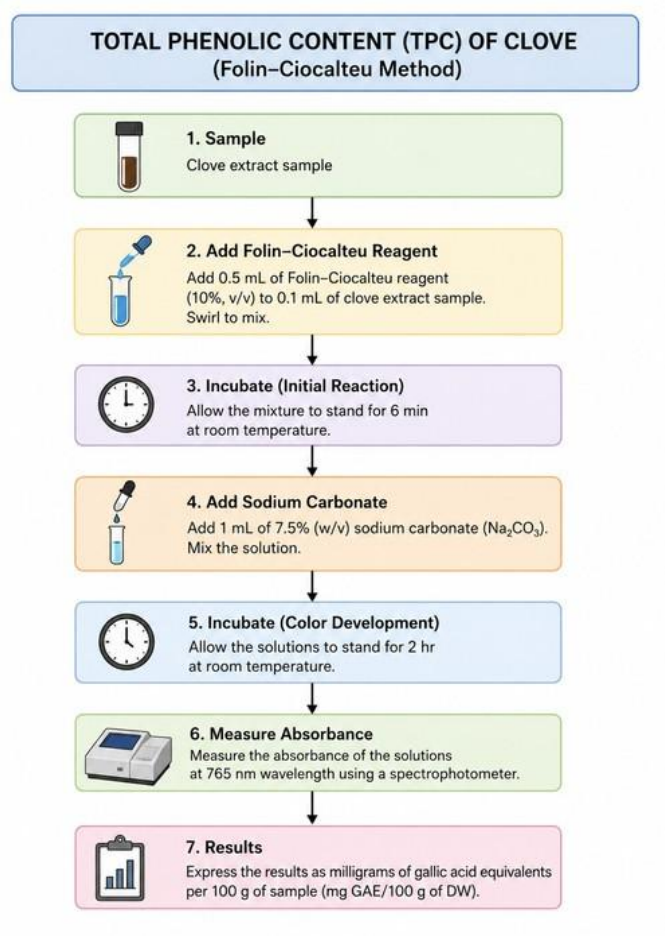

Total flavonoid content (TFC)

The TF content was determined by the colorimetric method as described by Mohamed.

A total 0.5 mL of the extract was mixed with

2.25 mL of distilled water in test tube, followed by the addition of 0.15 mL of 5% (w/v) NaNO2 solution.

After 6 min, 0.3 mL of a 10% AlCl3·6H2O solution was added, and the reaction was allowed to stand for another 5 min before

1.0 mL of 1 M NaOH was added.

The mixture was mixed well by vortexing, and the absorbance was measured immediately at 510 nm using a spectrophotometer.

The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of fresh sample (mg QE/100 g of FW).

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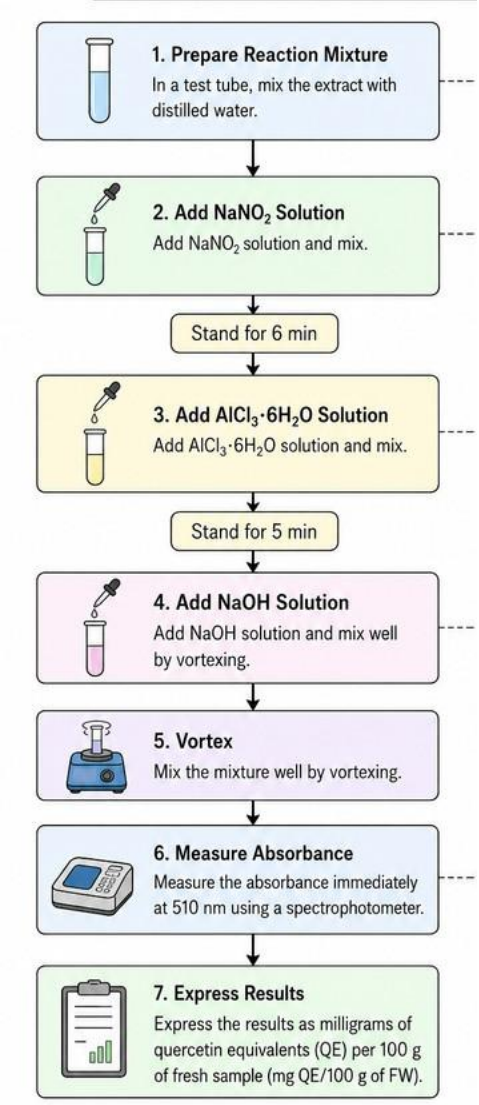

Radical-scavenging activity (DPPH)

The DPPH free radical scavenging assay was measured using the method reported by Brand-Williams.

The 2,2-diphenyl-1-picrylhydrazyl was dissolved in methanol to prepare the DPPH solution.

The DPPH solution was diluted several 42 times with methanol to obtain 0.9 absorbance at 516 nm, using spectrophotometer.

1 mL of DPPH solution was added to 100 µL of clove extract solution.

The mixture was shaken in a vortex and kept for 2 hr in dark place.

After 2 hr, the mixture was transferred to micro plate plastic and absorption of DPPH solution after the addition of the sample was measured at 516 nm using the spectrophotometer.

The changing in absorption of each sample computed as difference between the blank and sample readings.

The following equation calculates the percentage of DPPH scavenging activity:

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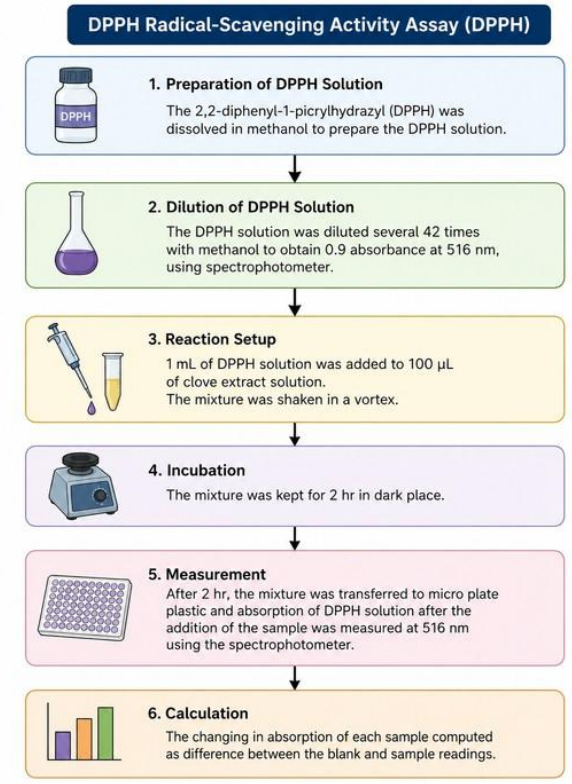
The percentage of DPPH scavenging activity was calculated using the following equation:
Radical scavenging (%) = [(A0 – A1/A0) × 100]
Where A0 is the absorbance of the control and A1 is the absorbance of the sample exracts.



Ferric reducing antioxidant power (FRAP)

The antioxidant capacity of each sample was determined by the method, given by Benzie and Strain.

FRAP reagent was prepared by using 300 mM acetate buffer, (pH 3.6; 3.1 g of sodium acetate trihydrate, plus 16 mL glacial acetic acid and the distilled water made up to total volume of 1 L).

10 mM TPTZ (2,4,6-tri (2-pyridyl)-striazine), in 40 mM HCl and 20 mM FeCl3.6H2O in the ratio of 10:1:1. Freshly prepared FRAP reagent
(1000 µL), warmed at 37°C, was mixed with 100 µL sample, standards.

Samples were kept for 30 min and after that the mixture was transferred to micro plate plastic.

The absorbance was measured at 595 nm wavelength using spectrophotometer.

The result was express as milligrams of Trolox equivalents per 100 g of sample (mg TE/g of DW).

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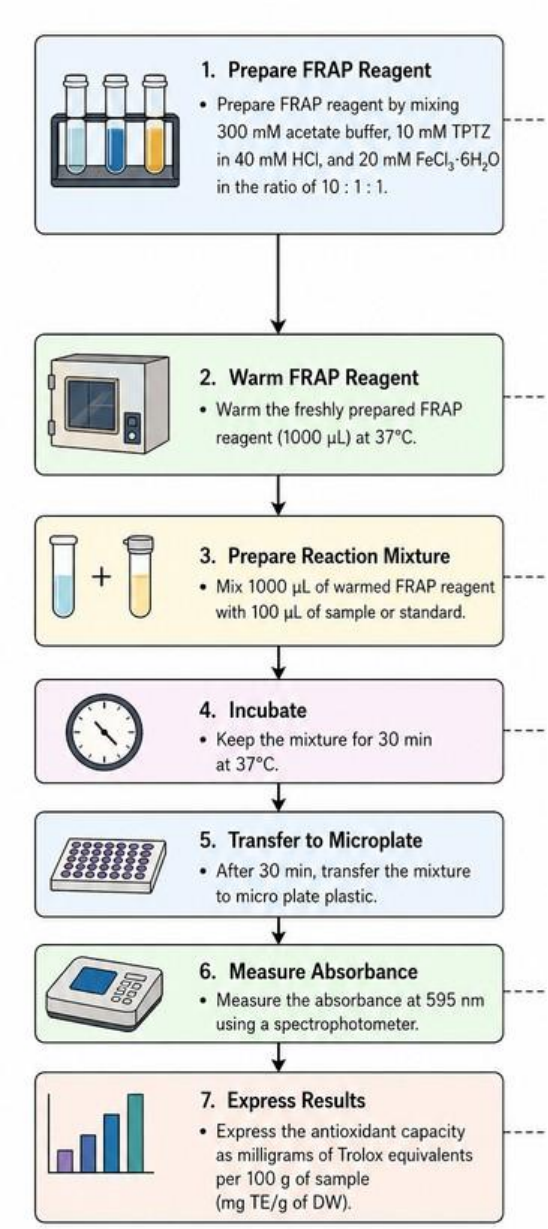

Statistical analysis

The experiment was carried out in triplicate.

Statistical analysis of the data was performed by one-way ANOVA using (SPSS 19 software).

Significant differences

($P < 0.05$) between the two part of clove were analyzed by Duncan triplicates range test12.

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LITERATURE REVIEW

1 Introduction to Clove and Its Bioactive Composition

Clove (Syzygium aromaticum), a member of the Myrtaceae family, is native to the Maluku Islands in Indonesia and is widely cultivated for its aromatic flower buds. It is traditionally used for culinary, medicinal, and aromatic purposes. The dried buds contain significant concentrations of essential oils, primarily eugenol, which has been identified as the main contributor to clove's pharmacological effects. In addition to eugenol, other compounds such as eugenol acetate, ß-caryophyllene, flavonoids, tannins, and triterpenoids also contribute to its bioactivity (Chaieb et al., 2007).

Clove has held an esteemed position in traditional medicinal systems for centuries, particularly in Asia and the Middle East. In Ayurveda, it has been prescribed for conditions such as indigestion, cough, and toothache, often in powdered or oil form. Similarly, Traditional Chinese Medicine uses clove to stimulate the kidney meridian meridian and stimulating properties to the spice. In Unani medicine, clove is used to alleviate inflammation and oxidative conditions, such as chronic headaches and

Clove has held an esteemed position in traditional medicinal systems for centuries, particularly in Asia and the Middle East. In Ayurveda, it has been prescribed for conditions such as indigestion, cough, and toothache, often in powdered or oil form. Similarly, Traditional Chinese Medicine uses clove to stimulate the kidney meridian meridian and improve yang energy, attributing warming joint pain. The convergence of its traditional uses with modem pharmacological findings especially in reducing oxidative stress highlights the ethnopharmacological wisdom behind its historical applications. These systems, though based on observational knowledge, identified clove as an effective remedy long before the discovery of free radical theory, showing a compelling alignment between ancestral practices and contemporary scientific validation (Gupta et al., 2015).

2. Botanical Description of the Clove Plant

Syzygium aromaticum is an evergreen tree belonging to the Myrtaceae family, typically growing to a height of 10-20 meters. It thrives in tropical climates with high humidity and rainfall, predominantly cultivated in Indonesia, Madagascar, Sri Lanka, and Tanzania. The tree has large, glossy green leaves arranged oppositely and small crimson flowers arranged in terminal clusters. The commercially valuable part of the plant is the unopened flower bud, which turns reddish-brown upon drying. These buds are nail-shaped hence the name "elove," derived from the Latin word clavus. Clove is propagated mainly through seeds or stem cuttings and begins yielding harvestable buds in its fifth year of growth, with peak productivity at around 15 to 20 o 20 years. Botanically, the essential oil is stored in the bud's hypanthium and calyx, which gives the spice its potent aroma and pharmacological potential (Trease & Evans, 2009).

3. General Culinary and Medicinal Uses of Clove

Clove has been widely used in both culinary and traditional medicinal contexts. As a spice, clove is valued for its strong, pungent flavor and is commonly used in seasoning meats, baked goods, and beverages across many cultures. Beyond its culinary role, clove has held a central place in traditional medicine for its analgesic, anti-inflammatory, antiseptic, and carminative properties. In dental care, clove oil is frequently used to alleviate toothache and oral infections due to its local anesthetic effect. Additionally, clove extracts are employed in herbal teas and ointments to treat gastrointestinal discomfort, Crespiratory ailments, and skin disorders Scientific studies support many of these uses, attributing clove's therapeutic effects to its high eugenol content and other phenolic constituents with bioactive properties (Chaieb et al., 2007).

RESULT:

A large number of methods have been developed to evaluate antioxidant capacity of food and dietary supplements, herbal extracts or pure compounds. Nevertheless, few of them have been used widely due to the difficulty of measuring total antioxidant capacity owing to limitations associated with methodological issues and free radical sources. A

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comparison between stem and fruit of clove in terms of the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (FRAP, DPPH) is illustrated in Fig. 1, 2, 3 and 4.

The fruit showed different trends with regard to total phenolic content and total flavonoid content. The TPC and TFC were higher in fruits (240.70 mg GAE/100 g DW and 209.48 mg QE/100 g DW, respectively) than in stem (209.48 mg GAE/100 g DW and 126.5 mg QE/100 g DW, respectively) The high contents of total phenolic compounds and total flavonoids in this species contribute to important antioxidant activity of wormwood14. Indeed, the phenolic fraction of plant extracts has been linked to their antioxidant capacity and antimicrobial activities. Flavonoids are a group of polyphenolic components synthesized

by plants with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action, reduce blood-lipid and glucose and to enhance human immunity.

Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components. Recently, interest has considerably increased in finding naturally occurring antioxidant to replace synthetic antioxidants, which were restricted due to their side effects such as carcinogenesis. Two different and

complementary assays: the DPPH• (2,2-di-phenyl-1-picrilhydrazyl) free radical scavenging and the FRAP (Ferric Reducing Antioxidant Power) were used to evaluate in vitro antioxidant activities of the obtained clove stem and fruits and acetone extracts for stem and fruits. Based on DPPH and FRAP tests, acetone extracts present the higher antioxidant activities than clove stem and fruits extracts in both stem and fruits. Furthermore, fruits

exhibited the highest antioxidant activities according to both used tests, DPPH and FRAP. For acetone extract, the free radical scavenging varied 87.50% in fruits to 79.41% in stem.

Concerning FRAP test, for clove stem and fruits extract, the higher ferric reducing antioxidant Power was observed in fruits and stem 437.29 and 306.42 mg TE/100 g Int. J. Chem. Sci.

II. CONCLUSION

These results highlighted that different parts of clove (Syzygium aromaticum) i. e. Stem and fruit contained significantly different amount of antioxidant capacity and activity. According to the results, fruits of clove brought about higher phenolic compounds and higher antioxidant activity. It can be concluded that, clove stem and fruit can be considered as an excellence source of antioxidant compounds.

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