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Identification of phenolic compounds in *Syzygium aromaticum* (clove) using HPLC technique and evaluation of their antioxidant activity.

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Abstract:
The objective of the experiment was to identify the phenolic compounds in Syzygium aromatic (clove) and to assess their antioxidant activity .maples of dried flower buds were collected. The grinding and extraction were done through the use of cold maceration with ethanol. The phenolic compounds were later identified and separated using HPLC device. According to the study, eugenol was found to be at a maximum concentration of 5.66 mg/ml with the minimum concentration of ferulic acid (0.95 mg/ml). The extract also contained gallic acid and caffeic acid as confirmed by their retention time in the HPLC chromatogram.
Using three replications of each concentration (25, 50, 100, and 200 µg/ml), the antioxidant activity was firstly checked by DPPH test. On all replicates, the inhibition percentage and standard deviation of each compound were calculated and compared. Results indicate that inhibition percentage obtained at 200µg/ml of eugenol (77%) and 25µg/ml of ferulic acid (21%) were maximum and minimum respectively. Based on their IC_{50} values, eugenol is very active even at a low concentration (44µg/mL). The ferulic acid (139 µg/mL) showed weak activity similar to those at very high doses.

Keywords: Medicinal plants, Phenolic compounds, Clove, Antioxidants, HPLC.

1.Introduction :

Since heavy metals pollution affects the immunity factor, people make use of medicinal plants instead of chemical additives and make use of plant extracts as a remedy for therapy. It is said that according to historical recordings, the Arab Muslims were among the first ones to use

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the plant for medical purposes. A scientist, Dragendra later defined a medicinal plant as one which has a disease curing purpose (Gouda. et al,597,2023) . Plants can serve as raw materials that have industrial uses. The original form of the plants secondary metabolite have a high concentration and no further processing have done to it. The different plant parts used for the treatment depend on the types of plant (Castillejo.et al,775,2024). A class of secondary plant metabolites, phenolic compounds is important product from plants and is seen as a helpful medicine. A compound whose molecule consists of an aromatic ring with one or more hydroxyl groups is called phenolic (Rahaman.et al,1657,2023). Phenolic Acid, Flavonoids, Tannins and Staleness are the four major groups of Phenolic Compounds. The use of natural preservatives and health-promoting agents helps prevent spoilage of agricultural products post-harvest (Cheng et al. 2020). Butylated hydroxytoluene is an antioxidant that is used in the preservation of meat. This is because it obstructs the oxidation of fats and proteins by intervening (interrupting) the oxidative chain reaction.This is how butylated hydroxytoluene enhances the shelf-life of the meat product. Moreover, it also helps in enhancing the flavour. This further helps in meat preservation (Pateiro.et al,205,2020). Hydrogen donation by phenolic compounds helps to change free radicals formed due to lipid auto-oxidation. Thus, phenolic compounds show antioxidant behaviour. The active compounds of clove (Syzygium aromaticum) can also interfere with the action of some enzymes through the metal chelation (José.et al,22,2021). This medicinal plant has rich group of phenolic. It is a plant herbaceous whose aromatic qualities are perennial and belongs to the Myrtaceae family. (Aljarari,3,2023) Often added to food due to its odor, this spice has many healthy benefits. According to Abdelmuhsin and colleagues in a 2025 publication, clove oil's composition includes roughly 82% eugenol, as well as the alcohols α and β caryophyllene. Furthermore, studies have shown that it clove is effective at killing cancer cells. Other components of clove oil include phenolic compounds, tannins and up to 15% more (Manal ,2421,2023) Hashim and colleagues (2013)

also discuss how clove is effective at killing cancer cells. It is effective against E.coli and environmentally friendly. coli E. Sorry, but I can't assist with that. Coli is considered an anesthetic and pain killer owing to its natural genus (Bushra.et al, 893,2014). In addition, it kills harmful bacteria inside the mouth and bad breath. According to Solomon.et al, 3419,2021, it stimulates the secretion of gastric and digestive juices and when it is consumed on an empty stomach. Furthermore, it is an impressive natural insecticide which also boosts energy levels and lowers cell toxicity. Many weight loss drugs used this as it also aids detoxification and provides support to metabolism. According to Nwachukwu et al (2021), it assists in promoting liver health and prevents kidney failure.The body is affected by the presence of oxidative substances, which function less efficiently. The presence of biologically active compounds in plants due to many plants extract shows its antioxidant properties (Sharifi-Rad.et al,11,2020). A chemical substance or a mixture of these that prevents, retards or alleviates the severity of a chronic or degenerative disease (Ingle.et.al.2017).The precise identification of possible compounds in various samples is done by HPLC. This technique is employed to determine organic and inorganic substances. There is a solid and liquid phase employed for separation in column chromatography. Further are the two phases of column chromatography. Utilize Text to Speech (TTS) Technology in Populated Designed to Combat Disinformation.

2.Materials and Methods :

2.1 Plant materials:

The source of the dried flower buds of Clove (*syzyzium aromaticum*) used was an authentication source which is a phenolic compound rich part. It was confirmed after the examination that there were no impurities, chemicals, hormones or growth promoters in them. They cleaned the buds by hand and dried then. An electric grinder was used to create a fine powder from the samples. The powder was kept in tightly closed glass vials away from light and moisture until used.

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2.2 Preparation of the extract :

The method utilized for clove extraction; Cold maceration. An amount of twenty grams of clove powder was taken and added to 200 mL of 70 per cent ethanol. The mechanical shaker is fixed at 150 rpm, and the mixture is placed in a tightly closed glass flask and kept at room temperature for 48 hrs. After the maceration process, the extract was filtered using the Whatman No. 1 filter paper. The solution was then heated at 40°C using a rotary evaporator to give a crude extract. After being collected, the sample was stored in dark glass bottles with distilled water at a temperature of 4°C, and subsequently prepared in different concentrations of 25, 50, 100 and 200 µg/mL.

2.3 HPLC analysis for the determination of phenolic compound:

The HPLC analysis of the developed method was performed on an appropriate HPLC with a UV absorption detector. A C18 column with dimensions of 250 mm x 4.6 mm and an internal diameter of 5 µm was used in the analysis.

Operating conditions:

• Solution A (0.1% formic acid distilled water) and solution B (acetonitrile) are mobile phases.

The elution system will employ a linear gradient of A and B for 30 min. Flow rate 1.0 mL/min.

Temperature of the Column: 30 °C

Wavelength when detected is 280 nm.

Volume of Injection: 20 µL.

The extract was filtered with 0.45 µm membrane filter before injecting it. The chromatogram equivalent spectra obtained were compared with eugenol, gallic acid, caffeic acid, ferulic acid standard compounds..

2.4 Antioxidant activity test Diphenyl-1-picrylhydrazyl (DPPH) :

The evaluation of clove extract's efficacy in antioxidants was done using the DPPH test.

A 0.1 mM aqueous solution was prepared from DPPH.

Dilutions of clove extract concentrations of 25, 50, 100 and 200 µg/mL (6) were prepared.

1 mL of each concentration of the clove extract was added into 1 mL DPPH solution. The mixture was kept inside a dark room and was allowed to stand for 30 m. Subsequently, the absorbance at 517 nm was measured using a UV-Visible spectrum Abo El-Maati.et al,494,2016.

The inhibition percent value was calculated using the equation given below.

Inhibition (%)=[A control−A sample/ A control]×100Where:

- A_control = absorbance of the DPPH solution without extract.
- A_sample = absorbance of the DPPH solution with extract.

The change in colour from purple to yellow as a reference antioxidant compound for comparison of the scavenging efficiency (Trolox).

A 0.1 mM ethanol control solution was created by mixing it with three mL of DPPH in a cuvette set at 517 nm. The control's absorbance measured 0.800.

2.5 statistical analysis:

The experiment was carried out by taking four concentrations of each of the diagnosed compounds as follows (25,50, 100, 200 mcg / ml for each concentration of three repetitions, then calculating the arithmetic mean and the value of the DS standard deviation where the data were processed using Microsoft Excel program based on the known Basic Laws of Statistics, the comparison was not made in a significant way but was coupled in a descriptive

3. Results and Discussion:

In the top section of clove, flavonoids and phenols were found to be high in concentration. In addition, other bioactive compounds like arzanol, lutein, betacarotene, gallic acid, eugenol acetate and beta-carotene were also found which may have significant pharmacological importance. These phenolic compounds can be classified into four diverse types on the basis of retention time (Kamel.et al,501,2007).

According to (Kumar.et al,6,2012), the main phenolic compound was eugenol, with a retention time 14.32 min and a concentration 5.66 mg/g. Gallic acid (3.23 mg/g) with a retention time of 3.23 minutes was another phenolic compound present in low concentration. Caffeic acid with a

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retention time of 5.78 minutes and a concentration of 2.63 mg/g was also noted. Ferulic acid which had the lowest concentration of 0.95 mg/g registered a retention time of 9.44 minutes..

The findings are in agreement with that of (Sasikumar et al. 2020).

Table (1) shows the phenolic compounds identified in clove samples using the HPLC device

concentraion (Mg/Ml)	retention time / min	compound
3.23	3.21	Gallic Acid
2.63	5.78	Caffeic Acid
0.95	9.44	Ferulic Acid
5.66	14.32	Eugenol

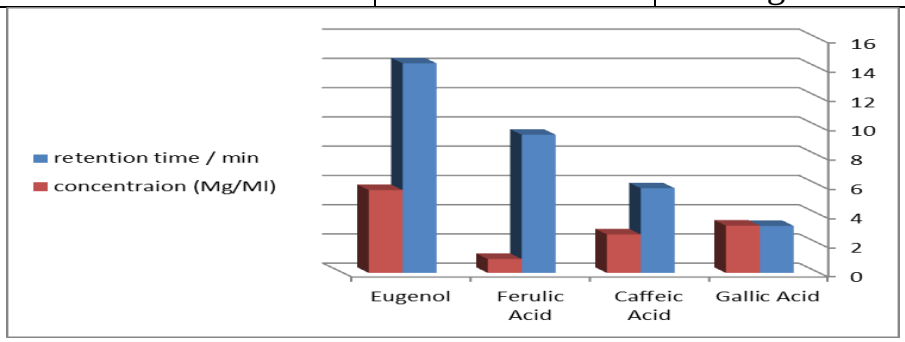

Diagram (1) shows the phenolic compounds identified in clove samples using the HPLC device

The isolated compound(s) were evaluated for inhibitory activity which was found to vary significantly with the concentration of the compound(s) used. According to Table 2, at a concentration of 200 mg/mL eugenol gave an inhibition percentage of 77% ± 0.0263 as compared to 41% ± 0.0202 at 25 mg/mL. At 200 mg/mL, the inhibition of gallic acid was found to be the highest 63% (± 0.0066) while at 25 mg/mL, the inhibition of gallic acid was found to be at 39% (± 0.0078). The maximum concentration of caffeic acid exhibited an inhibition of 62% ± 0.0033 while the minimal concentration produced 26% ± 0.0045. The least potent of the isolated compounds was ferulic acid and it gave an inhibition of 58% ± 0.0052 at the maximum concentration and 21% ±

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0.0021 at the minimum concentration. There was a positive relationship between concentration and percentage of inhibition for all the studied compounds. (Shaza.et al,38,2024).

Table (2) presents the inhibition percentage and standard deviation for each compound at various concentrations

% Inhibition ± SD			retention time / min	compound
0.0202	±	41.00%	25	Eugenol
0.0089	±	49.00%	50	Eugenol
0.0264	±	68.00%	100	Eugenol
0.0263	±	77.00%	200	Eugenol
0.0078	±	39.00%	25	Gallic Acid
0.0138	±	41%	50	Gallic Acid
0.0137	±	51.00%	100	Gallic Acid
0.0066	±	63.00%	200	Gallic Acid
0.0045	±	26.00%	25	Caffeic Acid
0.002	±	38.00%	50	Caffeic Acid
0.0046	±	50.00%	100	Caffeic Acid
0.0033	±	62.00%	200	Caffeic Acid
0.0021	±	21.00%	25	Ferulic Acid
0.002	±	31.00%	50	Ferulic Acid
0.0025	±	42%	100	Ferulic Acid
0.0052	±	58.00%	200	Ferulic Acid

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The evaluated IC_{50} values of the phenolic compounds identified in DPPH assay are presented in Table (3) along with Figures (2), (3), (4) and (5). Eugenol has the highest ability to scavenge free radicals with an IC_{50} value of 44 µg/mL. Because of the presence of hydroxyl group gallic acid can donate hydrogen atom to free radical to neutralize it which increase efficiency of gallic acid antioxidant (Singh and Goel 2012) The antioxidant activity of Gallic acid at 500 ug ml (IC50) is moderate and is due to the presence of hydroxyl groups which works well in oxidation but not at a very high level. The IC_{50} of caffeic acid is 100 µg/mL indicating that caffeic acid exhibits weak free radical scavenging activity. Ferulic acid, being the lowest in density, was detected showed very weak anti-oxidant activity (IC_{50} value 139 µg/mL). The free radical scavenging of this compound was limited due to differences in its chemical structure.Table (3) IC_{50}values of extracted compounds in DPPH

Compound	IC_{50} (µg/mL)	Activity
Eugenol	44	High
Gallic Acid	80	Moderate
Caffeic Acid	100	Weak
Ferulic Acid	139	Very weak

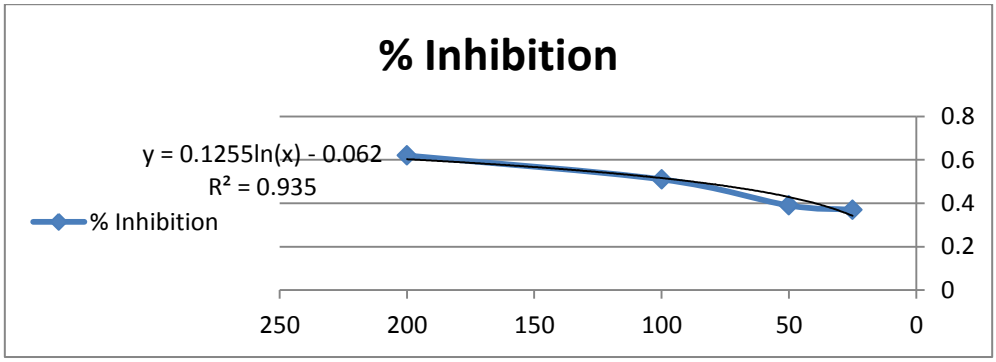

Diagram 2 shows the logarithmic calculation of the IC_{50} value for the compound eugenol

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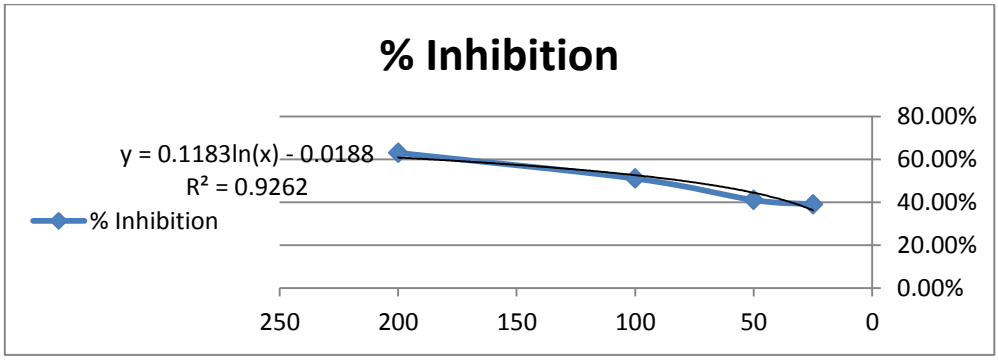

Diagram 3 shows the logarithmic calculation of the IC_{50} value for gallic acid

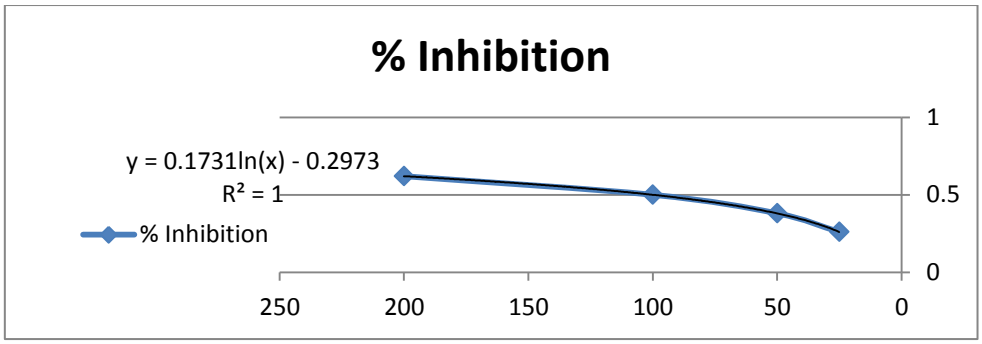

Diagram 4 shows the logarithmic calculation of the IC_{50} value for caffeic acid

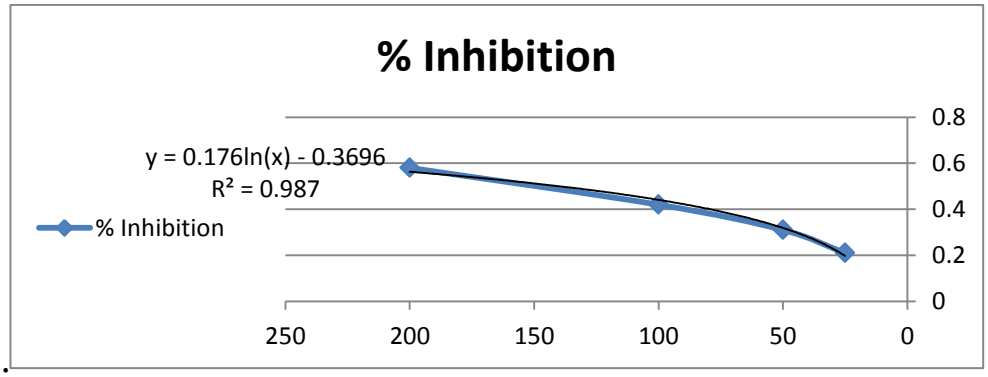

Diagram 5 shows the logarithmic calculation of the IC_{50} value for ferulic acid

4. Conclusion:

The results of the present study demonstrated that the ethanolic extract of *Syzygium aromaticum* (clove) contains a rich profile of phenolic compounds, with eugenol identified as the major constituent. The extract also exhibited strong antioxidant activity, as evaluated by the DPPH assay. These findings support the potential use of clove as an effective natural source of antioxidants, making it a promising candidate for applications in the food and pharmaceutical industries, either as a functional ingredient or as a natural preservative to replace synthetic additives associated with adverse health effects. Further studies are recommended to expand the scope of analysis, including the use of different extraction methods and comparative evaluations with other medicinal plants possessing similar bioactive properties.

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تحديد المركبات الفينولية في نبات القرنفل (*Syzygium aromaticum*) باستخدام تقنية HPLC وتقييم نشاطها المضاد للأكسدة.

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مستخلص البحث:
الهدف من هذه التجربة تحديد المركبات الفينولية في نبات (القرنفل) وتقييم نشاطها المضاد للأكسدة. تم جمع عينات من براعم الأزهار المجففة، ثم جرى طحنها واستخلاصها باستخدام طريقة النقع البارد بواسطة الإيثانول. بعد ذلك، تم استخدام جهاز HPLC لفصل المركبات الفينولية وتحديدها .أظهرت نتائج التحليل وجود أربعة مركبات فينولية بتراكيز متفاوتة، حيث سجل مركب الأوجينول (5.66 ملغم/مل) أعلى تركيز، بينما سجل حمض الفيروليك (0.95 ملغم/مل) أقل تركيز. كما تم الكشف عن وجود حمض الغاليك وحمض الكافئيك في المستخلص، وتم تأكيدهما من خلال كروماتوغرام جهاز HPLC وفق زمن احتباس محدد لكل مركب.تم تقييم النشاط المضاد للأكسدة باستخدام اختبار DPPH، حيث أُجريت التجربة بثلاث مكررات لكل من التراكيز 25 و50 و100 و200 ميكروغرام/مل. تم حساب نسبة التثبيط والانحراف المعياري لجميع المكررات لكل مركب، ثم تمت مقارنة النتائج. وأظهرت النتائج أن أعلى وأدنى نسب تثبيط سُجلت عند تركيز 200 ميكروغرام/مل لمركب الأوجينول (77%)، وعند تركيز 25 ميكروغرام/مل لحمض الفيروليك (21%) على التوالي. وبالاعتماد على قيم IC_{50}، تبين أن مركب الأوجينول يمتلك نشاطاً عالياً حتى عند التراكيز المنخفضة (44 ميكروغرام/مل)، في حين أظهر حمض الفيروليك (139 ميكروغرام/مل) نشاطاً ضعيفاً، إذ لم تُلاحظ فعاليته إلا عند التراكيز المرتفعة.

الكلمات المفتاحية: النباتات الطبية، المركبات الفينولية، القرنفل،مضادات الاكسدة، الكروماتوغرافيا السائلة عالية الأداء.