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# EVALUATION OF ANTIOXIDANT ACTIVITY OF CLOVE (*SYZYGIUM AROMATICUM*)

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## ABSTRACT

Clove (*Syzygium aromaticum)* is a nutrient dense food rich in beneficial phytochemicals.The objective of this study was to elucidate in detail the antioxidant 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.

**Key words**: Clove, Antioxidant activity, Total phenol, Total flavonoids.

## INTRODUCTION

Cloves (*Syzygium aromaticum*) are dried aromatic unopened floral buds of an evergreen tree 10-20 m in height belonging to the family *Myrtaceae*, indigenous to India, Indonesia, Zanzibar, Mauritius and Ceylon[1]. They are esteemed as a flavouring agent and also used as a spice for scenting, chewing tobacco and an ingredient of betel chew. Cloves have many therapeutic uses: anti-inflammatory[2], antioxidant[3] and antifungal[4]. The antioxidant contents of fruits and vegetables increase because of natural antioxidant consumption, which has been found to be related to reduced risk of cancer and heart diseases.

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Medicinal plants play important roles in preventing various diseases and have received much attention from many researchers over the last few decades. Studies on the antioxidant contents of fruits and vegetables are increasing because natural antioxidant consumption has been found to be related with decreased risk for cancer and heart diseases[5]. Similar to other, fruits is also a natural source of macronutrients (carbohydrates and proteins) and micronutrients vitamins A and C. A significant relationship was also found to exist between the intake of these antioxidant-containing plants and reduced mortality attributed to the aforementioned diseases[6]. Natural antioxidants require more scientific analysis because they contain free radicals that may lead to chronic degenerative diseases[7]. The objective of this study was to determine the phenolic compounds antioxidants activity in different parts of the Cloves (*Syzygium aromaticum).*

## EXPERIMENTAL

### Materials and methods

### Sample collection and preparation of clove extract

The stem and fruits of clove (*Syzygium aromaticum)* were obtained from the market in Thi-Qar city, Iraq. The stem and fruits of clove were cleaned and then oven dried at 50$^{o}$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. All extracted samples were centrifuged by using table top centrifuge for 10 min. The supernatants were collected for further analysis.

### Total phenolic content (TPC)

The amount of total phenolics content (TPC) in colve was determined with the Folin-Ciocalteu reagent base on[8]. 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 ($Na_2CO_3$) 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).

### Total flavonoid content (TFC)

The TF content was determined by the colorimetric method as described by Mohamed et al.[9] A total 0.5 mL of the extract was mixed with 2.25 mL of distilled water in a

test tube, followed by the addition of 0.15 mL of 5% (w/v) $NaNO_2$ solution. After 6 min, 0.3 mL of a 10% $AlCl_3 \cdot 6H_2O$ 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).

**Radical-scavenging activity (DPPH)**

The DPPH free radical scavenging assay was measured using the method reported by Brand-Williams et al.[10] 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:

The percentage of DPPH scavenging activity was calculated using the following equation:

$$\text{Radical scavenging (\%)} = [(A0 - A1/A0) \times 100]$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts.

**Ferric reducing antioxidant power (FRAP)**

The antioxidant capacity of each sample was determined by the method, given by Benzie and Strain.[11] 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 $FeCl_3.6H_2O$ 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).

### 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 test[12].

## RESULTS AND DISCUSSION

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[13]. A 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 wormwood[14]. Indeed, the phenolic fraction of plant extracts has been linked to their antioxidant capacity and antimicrobial activities[15]. 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[16].

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[17]. 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 (Figure 3 and 4). 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 DW, respectively.

Clove stem and fruit pulps had relatively higher total flavonoids 22.53 mg/100 g FW than other medicinal plants such as *Alceakurdica*, *Stachyslavandulifolium*, *Valeriana officinalis*, *Lavandula officinalis* and *Melissa officinal* (0.22-10 mg CE/g DW)[18]. However, other studies have shown that phospholipids had the lower rate of antioxidant activity. Comparing antioxidant activity of this study and other published data is difficult due to the fact that content of antioxidant compounds can be influenced by extracting solvent, cultivar and location.

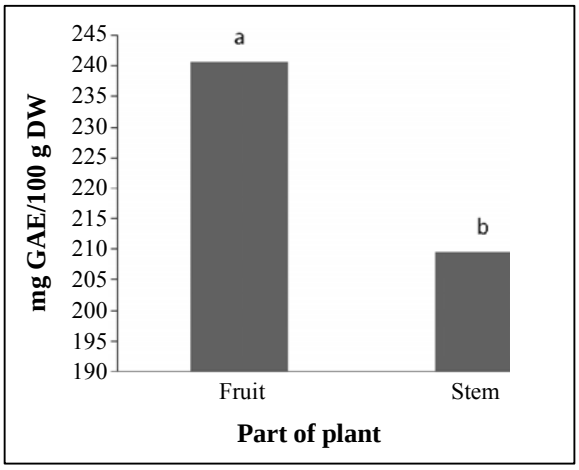



**Fig. 1: Total phenolic content of clove stem and fruit**

[a-b]Mean with different letters within each column are significantly different ($P < 0.05$)

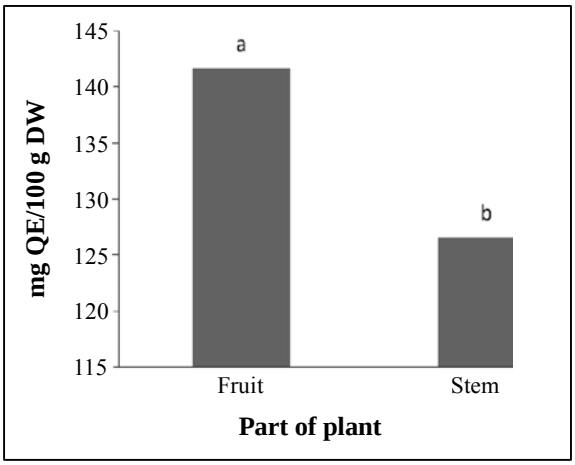



**Fig. 2: Total flavonoids content of clove stem and fruit**

[a-b]Mean with different letters within each column are significantly different ($P < 0.05$)

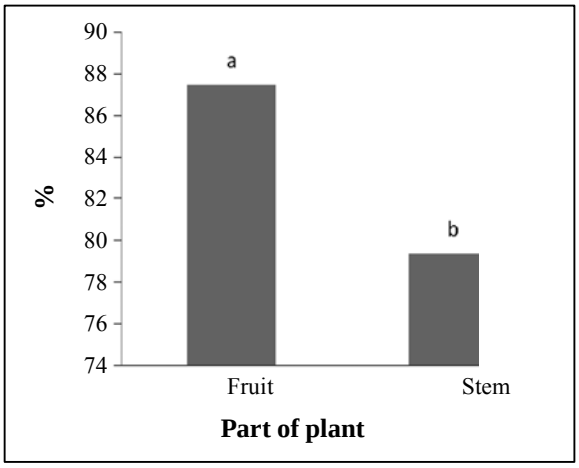
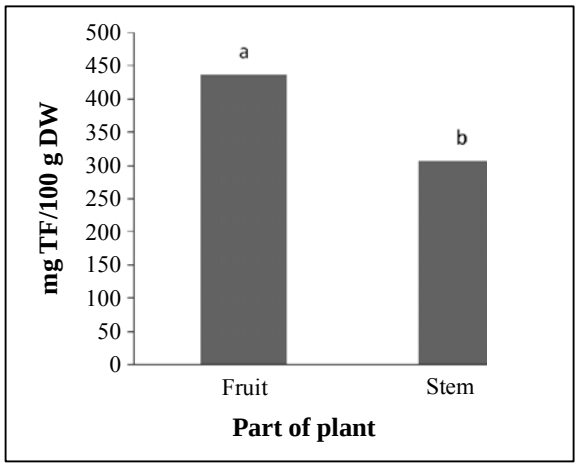



**Fig. 3: Radical-scavenging activity (DPPH) of clove stem and fruit**

[a-b]Mean with different letters within each column are significantly different (P < 0.05)




[a-b]Mean with different letters within each column are significantly different (P < 0.05)

**Fig. 4: Ferric reducing antioxidant power (FRAP) of clove stem and fruit**

## 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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