



### Evaluation of Antioxidative Potential of Ethanol Extract of *Syzygium Aromaticum*

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#### ABSTRACT

This study evaluated the phytochemical composition and antioxidant potential of *Syzygium aromaticum* (clove) extract. Total phenolic content (TPC), total flavonoid content (TFC), and in vitro antioxidant activities, including DPPH, ferric reducing antioxidant power (FRAP), nitric oxide (NO), and hydroxyl radical scavenging assays, were determined. The extract exhibited high phytochemical content, with TPC of 328.70 mg gallic acid equivalents (GAE)/g extract and TFC of 262.80 mg quercetin equivalents (QE)/g extract, indicating a rich presence of bioactive compounds. Antioxidant activity increased in a concentration-dependent manner. DPPH scavenging activity rose from 44.08% at 100 µg/mL to 78.10% at 800 µg/mL, while FRAP values ranged from 122.00 to 315.40 µM Fe<sup>2+</sup>, demonstrating strong reducing capacity. Nitric oxide and hydroxyl radical scavenging activities also showed significant inhibition, reaching 109.02% and 92.70%, respectively, at higher concentrations. The observed antioxidant effects may be attributed to the high levels of phenolic and flavonoid compounds, particularly eugenol, known for its free radical scavenging properties. In conclusion, *Syzygium aromaticum* extract demonstrates significant phytochemical richness and potent antioxidant activity, supporting its potential as a natural agent for managing oxidative stress-related conditions.

Keywords: *Syzygium aromaticum*, antioxidant, Total phenolic, total flavonoid, phytochemical

#### INTRODUCTION

Antioxidants are molecules that inhibit oxidation, a chemical reaction that can produce free radicals leading to cellular damage. The balance between antioxidants and pro-oxidants is crucial for maintaining cellular health, and an imbalance can result in oxidative stress, which is implicated in various diseases including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders [1,2]. Plants are a significant source of natural antioxidants, and numerous studies have focused on their potential health benefits. Among these plants, *Syzygium aromaticum*, commonly known as clove, has garnered considerable attention due to its rich phytochemical composition and potent antioxidative properties.

*Syzygium aromaticum* is a flowering plant in the family Myrtaceae, native to the Maluku Islands of Indonesia and widely cultivated in many tropical regions [3,4]. The dried flower buds, commonly referred to as cloves, are used as a spice and have a long history of use in traditional medicine [5]. Cloves are particularly valued for their high eugenol content, a phenolic compound recognized for its anti-inflammatory, analgesic, and antimicrobial properties [6]. Beyond eugenol, cloves contain various other bioactive compounds, including flavonoids, tannins, and phenolic acids, which contribute to their overall antioxidative potential [7,8,9].

The antioxidative capacity of *Syzygium aromaticum* has been evaluated through various in vitro methods. For instance, studies have demonstrated that clove extracts exhibit significant free radical

scavenging activity, reducing the levels of reactive oxygen species (ROS) and inhibiting lipid peroxidation [9,10,11]. The capacity to scavenge free radicals is often assessed using assays such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the ferric reducing antioxidant power (FRAP) assay, and the total antioxidant capacity (TAC) assay, which measure different aspects of antioxidative potential [12]. These studies indicate that the ethanol extract of clove possesses strong antioxidative properties, which may contribute to its therapeutic efficacy in preventing oxidative damage.

The therapeutic applications of *Syzygium aromaticum* extend beyond its antioxidative potential. Clove extracts have been reported to exhibit anti-cancer properties, with studies indicating that eugenol can induce apoptosis in various cancer cell lines [13,14]. Additionally, clove has demonstrated hypoglycemic and hypolipidemic effects in animal models, highlighting its potential role in managing metabolic disorders [15,16]. These findings reveal the importance of exploring the multifaceted health benefits of *Syzygium aromaticum* and its potential use as a functional food ingredient.

Moreover, the method of extraction can significantly influence the bioactivity of plant extracts. Ethanol is commonly used as a solvent due to its effectiveness in extracting a wide range of phytochemicals, including polyphenols and flavonoids, which are responsible for the antioxidative activity [17]. Previous studies have indicated that ethanol extracts of various plant species generally exhibit higher antioxidant activity compared to those obtained through other solvents, such as water or methanol [18,19,20]. This suggests that the choice of extraction solvent is crucial for maximizing the bioactive compound yield and enhancing the antioxidative capacity of the extract.

Despite the promising results associated with *Syzygium aromaticum*, there remains a need for comprehensive studies to further evaluate its antioxidative potential and elucidate the mechanisms underlying its effects. This study aims to investigate the antioxidative potential of the ethanol extract of *Syzygium aromaticum*, focusing on its ability to scavenge free radicals and protect against oxidative stress. Understanding the antioxidative properties of this plant may provide insights into its potential applications in preventing oxidative damage and promoting health.

## MATERIALS AND METHOD

### Equipment and Chemicals

Spectrophotometer, refrigerator (Haier thermocool), weighing balance, measuring cylinder, glassware (pyrex), and sample containers. All the chemicals and reagents used in this research were of the purest analytical grade commercially available.

### Sample Collection:

*Syzygium aromaticum* was procured from Relief market and was botanically identified by Dr. C. Duru of the department of Environmental Biology, Federal Polytechnic Nekede Owerri.

### Preparation of *Syzygium aromaticum* Extract

The extraction was done using a continuous hot extraction process using a Soxhlet apparatus with 70% (v/v) ethanol. The ethanol extract was filtered and concentrated under reduced pressure to obtain a semi-solid residue.

### Evaluation of In Vitro Antioxidant Activity

Free radical scavenging effect of the extract was determined using the stable scavenger 2, 2-diphenyl-1-picrylhydrazyl (DPPH) with slight modifications of the method described by Brand-Williams et al. [21]. The reducing powers of the extract was carried out following the method of Moein *et al.*, [22]. Scavenging activity of hydroxyl radical by the plant extract was determined by the method of Peng *et al.*, [23]. Nitric oxide radical scavenging activity was determined according to the method of Mou *et al.*, [24].

### Statistical analysis

The experiments were conducted in triplicates. The data were represented as Mean  $\pm$  S.E. and One-way analysis of variance (ANOVA) was performed by Tukey's Multiple Range test using software SPSS (version 16.0, SPSS Inc.). Significant differences between means were determined and  $p < 0.05$  was a significant value.

## RESULTS AND DISCUSSION

### Phytochemical Composition of *Syzygium aromaticum*

The total phenolic and flavonoid contents of *Syzygium aromaticum* extract are presented in Table 1. The extract exhibited a high total phenolic content (TPC) of 328.70 mg GAE/g extract and a total flavonoid content (TFC) of 262.80 mg quercetin equivalents (QE)/g extract, indicating a rich presence of bioactive phytochemicals.

**Table 1**

*Total Phenolic and Flavonoid Content of Syzygium aromaticum Extract*

Parameter	Result
Total Phenolic Content (mg GAE/g extract)	328.70
Total Flavonoid Content (mg QE/g extract)	262.80

Values are mean $\pm$ SD of triplicate determination

### Antioxidant and Radical Scavenging Activities

The radical scavenging activities of *Syzygium aromaticum* extract at varying concentrations (100–800  $\mu$ g/mL) are shown in Table 2. The extract demonstrated a concentration-dependent increase in antioxidant activity across all assays. DPPH radical scavenging activity increased progressively from 44.08  $\pm$  3.00% at 100  $\mu$ g/mL to 78.10  $\pm$  2.40% at 800  $\mu$ g/mL. Similarly, ferric reducing antioxidant power (FRAP) values increased from 122.00  $\pm$  2.10  $\mu$ M Fe<sup>2+</sup> to 315.40  $\pm$  5.10  $\mu$ M Fe<sup>2+</sup>, indicating enhanced reducing capacity with increasing concentration. Nitric oxide radical scavenging activity also showed a marked increase, ranging from 62.10  $\pm$  7.40% at 100  $\mu$ g/mL to 109.02  $\pm$  3.00% at 800  $\mu$ g/mL. Likewise, hydroxyl radical scavenging activity increased from 56.10  $\pm$  1.72% to 92.70  $\pm$  2.62% across the same concentration range.

**Table 2: Radical Scavenging Activity of *Syzygium aromaticum* Extract**

Concentration ( $\mu$ g/mL)	DPPH (%)	FRAP ( $\mu$ M Fe <sup>2+</sup> )	Nitric Oxide Radical (%)	Hydroxyl Radical (%)
100	44.08 $\pm$ 3.00	122.00 $\pm$ 2.10	62.10 $\pm$ 7.40	56.10 $\pm$ 1.72
200	52.40 $\pm$ 1.80	186.70 $\pm$ 4.20	71.50 $\pm$ 2.04	63.40 $\pm$ 2.07
400	61.05 $\pm$ 3.80	270.10 $\pm$ 2.60	86.20 $\pm$ 4.60	74.30 $\pm$ 1.80
800	78.10 $\pm$ 2.40	315.40 $\pm$ 5.10	109.02 $\pm$ 3.00	92.70 $\pm$ 2.62

Values are mean $\pm$ SD of triplicate determination

### Discussion

Medicinal plants rich in phenolics and flavonoids are widely recognized for their ability to combat oxidative stress through multiple biochemical mechanisms. Therefore, assessing these parameters provides insight into the therapeutic relevance of clove as a natural antioxidant source. The present study evaluates the phytochemical composition and antioxidant potential of *Syzygium aromaticum* (clove), with emphasis on total phenolic content (TPC), total flavonoid content (TFC), and various radical scavenging activities. The current study results demonstrate that *Syzygium aromaticum* (clove) possesses significant phytochemical richness and potent antioxidant activity. The total phenolic content (TPC) was found to be 328.70 mg GAE/g extract, and the total flavonoid content (TFC) reached 262.80 mg Quercetin/g extract. These values indicate that clove extract is a superior source of bioactive compounds when compared to many other medicinal plants.

When comparing these findings to other studies, there is a clear evidence that *Syzygium aromaticum* possess notable phytochemicals. Abdelmuhsin et al. [25] reported a TPC of  $7.25 \pm 0.12$  mg GAE/g and a TFC of  $57.22 \pm 0.41$  mg QE/g for clove extract. Similarly, Al Mashkor [26] observed TPC and TFC values in clove fruits at 247.61 mg/100 g (approximately 2.47 mg/g) and 141.70 mg/100 g (approximately 1.41 mg/g), respectively. Furthermore, Bikheet et al. [27] found that a 1% ethanolic clove extract in a milk beverage matrix contained 58.70 mg/100 g (0.587 mg/g) of TPC and 46.15 mg/100 g (0.461 mg/g) of TFC. Though the results presented in this study (328.70 mg GAE/g and 262.80 mg QE/g) are substantially higher. However, the current results agree qualitatively with Lumingkewas and Unitley [28], as it identified the frequency of flavonoids, tannins, and other phytochemicals in clove methanol extracts as "many" (+++), confirming the high concentration of secondary metabolites in this species. The significant variation in phytochemical levels can be attributed to several scientific factors. The choice of extraction solvent, such as ethanol versus water, significantly affects the yield of phenolic compounds due to higher solubility in organic solvents [29,27]. This current study likely utilized a more efficient extraction process or a higher purity bud extract compared to the flavored milk beverages used in Bikheet et al. [27]. Additionally, the composition of secondary metabolites is heavily influenced by physiological variations, environmental conditions, geographical origin, and soil nutrients [28].

The antioxidant capacity of clove extract in this study showed a strong, dose-dependent relationship. The DPPH radical scavenging activity increased from 44.08% at 100  $\mu\text{g/mL}$  to 78.10% at 800  $\mu\text{g/mL}$ . This finding agrees with Abdelmuhsin et al. [25], who reported an IC<sub>50</sub> of 0.08 mg/mL (equivalent to 80  $\mu\text{g/mL}$ ) for DPPH scavenging. Since the current study achieved approximately 44% inhibition at 100  $\mu\text{g/mL}$ , it aligns closely with the IC<sub>50</sub> values reported in their investigation. The results also agree with Al Mashkor [26], who recorded a high DPPH scavenging effect of 87.50% in clove fruits, supporting the conclusion that clove is a "highly remarkable" antioxidant source. The FRAP values in the current study, ranging from 122.00 to 315.40  $\mu\text{M Fe}^{2+}$ , further support the strong reducing power of the extract, though they are presented in different units than the Trolox equivalents used by Al Mashkor [26].

Scientific explanations for this potent antioxidant activity are rooted in the chemical profile of *Syzygium aromaticum*. Clove is one of the most essential sources of phenolic compounds, predominantly eugenol (70–90%), eugenyl acetate, and  $\beta$ -caryophyllene [30]. These phenolic and flavonoid compounds are effective antioxidants because they have the capability to donate hydrogen atoms or electrons and scavenge free radicals [25]. Phenols neutralize free radicals to reduce oxidative stress, while flavonoids defend against reactive oxygen species (ROS) [26].

The current study found exceptional results for Nitric Oxide (NO) and Hydroxyl radical scavenging, with the 800  $\mu\text{g/mL}$  concentration reaching 109.02% and 92.70% inhibition, respectively. This agrees with the general pharmacological overview provided by Yadav et al. [30], who characterize clove as possessing a "wide spectrum of biological activity" including potent free radical scavenging. Abdelmuhsin et al. [25] highlight that these properties make clove extract a promising candidate for treating oxidative stress-related diseases.

The mechanism for such high radical scavenging, such as the high phenolic content, exerts microbicidal and protective action by disrupting harmful oxidative pathways. The fact that the extract performed exceptionally well against Nitric Oxide and Hydroxyl radicals suggests a high concentration of eugenol, which is identified as a potent molecule capable of interfering with multiple cell signaling pathways to reduce inflammation and oxidative damage [29].

## CONCLUSION

The findings of this study confirm that *Syzygium aromaticum* is a rich source of phytochemicals with powerful antioxidant properties. While the quantitative TPC and TFC results were significantly higher when compared to previous studies, the qualitative trend of high antioxidant capacity and dose-dependent radical scavenging is in strong agreement across all reviewed literature. These discrepancies are likely due to differences in extraction methods, plant parts, and geographical factors. The high levels of eugenol and flavonoids identified in the sources provide a clear scientific basis for the extract's ability to neutralize diverse free radicals and serve as a potential natural therapeutic agent.

## Competing interests

The authors declare that they have no competing interests

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