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Total phenols content, Antioxidant activity and Antibacterial activity of some medicinal plants in Yemen

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Abstract

The aim of this research was to extract total phenolics from clove seeds, ginger rhizomes, and Tecoma flowers using various polar solvents, followed by quantification using the Folin method. Subsequently, the antioxidant activity was evaluated using DPPH assay, and the antibacterial activity against two strains of Gram-positive bacteria, Staphylococcus aureus, and Gram-negative bacteria, Escherichia coli, was assessed using the disc diffusion method. The results demonstrated the superiority of clove seed extract over other extracts in terms of total phenolic content (816.47 mg GAE/10g), antioxidant activity (73.8%), and the inhibitory concentration of 50% DPPH (0.015 mg/mL). Plant extracts exhibited efficacy against Gram-positive bacteria, while no activity was observed against Gram-negative bacteria. These findings suggest the potential use of total antioxidants extracted from clove seeds and ginger rhizomes in the medical or industrial fields as alternatives to synthetic antioxidants.

Keywords: *Clove, Ginger, Tecoma, Antioxidant, Antibacterial activity.*

1. Introduction

Plants possess numerous bioactive compounds with the ability to scavenge free radicals and act as antioxidants. Among these, phenolic compounds are prominent natural antioxidants, including flavonoids, tannins, carotenoids, and phenolic acids. Phenolics are aromatic compounds bearing one or more hydroxyl groups and are found in all parts of plants such as leaves, flowers, and especially colored fruits (Vermerris and Nicholson, 2006). Phenolic compounds are widely distributed in the plant kingdom, considered secondary products of photosynthetic metabolism. Moreover, these compounds serve as a dietary source and have various physiological effects, acting as antioxidants and anticoagulants, reducing blood cholesterol levels, enhancing estrogen activity, and aiding in plant resistance to numerous diseases, especially bacterial infections. Antioxidants play a crucial role in preventing undesirable changes in food flavor and natural odor, as well as in resisting or reducing the incidence of chronic diseases such as cancer, diabetes, and inflammation (Trilaksani, 2003). The aim of this study was to evaluate the total phenolics content, antioxidant activity, and antibacterial efficacy of three plant species: clove, ginger rhizomes, and Tecoma flowers.

2. Materials and Methods

2.1. Plant Samples: Three plant species, namely Cloves (*Syzygium aromaticum*), Ginger rhizomes (*Zingiber officinale*), and Tecomar flowers (*Tecome stans*), were collected. Plant classification was verified in the plant laboratory at the Faculty of Agriculture, Ibb University, Yemen.

2.2. Chemicals: High-purity chemicals of analytical grade were used, procured from BDH Chemical Ltd and Sigma, which were Folin–Ciocalteu phenol reagent (Merck, Germany), Ethanol (Smart Lab, Indonesia) Na2CO3 (Merck, Germany), Gallic Acid (Sigma Aldrich, Germany), and DPPH (1.1 diphenyl,2 picrylhydrazyl).

2.3. Sample Preparation: Samples were washed with distilled water, air-dried at room temperature for 4 weeks until a constant weight was achieved, ground, and stored in polyethylene bags in the dark until extraction.

2.4. Extraction: Extraction was performed using a mixture of solvents (acetone, ethanol, methanol, propanol, and distilled water) according to the method described by (Wang et al., 2011).

2.5. Total Phenolics Estimation: Total phenolics in plant extracts were estimated using the Folin-Ciocalteau method (FC) as described by (Škerget et al., 2005).

2.6. Antioxidant Activity Assessment: Antioxidant activity in plant extracts was assessed using the DPPH method described by (Hassan et al, 2011).

2.7. Antibacterial Activity Assessment: Antibacterial activity in the studied extracts was evaluated using microbial isolates (Escherichia coli & Staphylococcus aureus) obtained from the microbiology laboratory, Faculty of Science, University of Ibb, following the method described by (Salem et al, 2013).

2.8. Statistical Analysis: All results were analyzed using a completely randomized design (C.R.D) with SAS 9.4-2021 statistical software.

3. Results and Discussion

3.1. Total Phenolic content (TPC) :

The results in Table 1 indicate a significant superiority of clove flower extract over the other plant extracts studied, with a total phenolics content of 816.47 mg GAE/10g. These findings align with previous studies (Małgorzata and Kinga, 2021), which also reported the superiority of clove flower extract in total phenolics content compared to other 13 plant species studied. Several studies have highlighted the high content of total phenolics in clove flowers (Przygodzka et al., 2014; Turgay and Esen, 2015; Elhussein et al., 2018; Asseffa et al., 2018). The results also showed that the total phenolics content in ginger rhizomes was 20.09 mg GAE/10g, consistent with findings by Mahmudati et al. (2022), who reported total phenolics content in ginger extract ranging from 21.22 to 23.58 mg GAE/10g, higher than that found by Ghasemzadeh et al. (2010). Tecomar flowers contained the lowest amount of total phenolics compared to the other studied plants, with a content of 17.73 mg GAE/10g. These values were lower than those reported by Govindappa et al. (2011), Salem et al. (2013), and Thaís et al. (2022).

Table 1: Total content of phenolic compounds of plants extracts

Plants extracts	Total phenol content	LSD
Clove	816.47 mgG.A/10gm	768.69
Ginger	20.09 mgG.A/10gm	
Tecoma	17.73 mgG.A/10gm	

3.2. Antioxidant Activity

The results presented in Table 2 revealed significant differences in the inhibitory activity against DPPH radical, where clove extract surpassed the other extracts, exhibiting the highest values of 60.4%, 71.09%, and 73.8% for clove, ginger, and Tecomaria flowers, respectively. These findings corroborate with Arslan (2023), who reported high DPPH radical scavenging activity for clove extract (60.39%), particularly the aqueous extract. Similarly, Małgorzata and Kinga (2021) highlighted clove extract's superiority over other plants in DPPH scavenging activity. Consistent with previous studies (Ghasemzadeh et al., 2010; Turgay and Esen, 2015; Mahmudati et al., 2022), ginger extract exhibited significant DPPH scavenging activity. Moreover, Tecomaria extract also showed inhibitory effects on DPPH, as reported by Govindappa et al. (2011) and Salem et al. (2013). The high antioxidant activity of the studied plant extracts can be attributed to their high phenolic compound content. The antioxidant activity estimates aligned with total phenolic content results, where clove had the highest phenolic content, correlating with its superior antioxidant activity. The elevated antioxidant activity in plant extracts signifies the presence of numerous phenolic acids with antioxidant properties. Clove was found to contain phenolic compounds such as eugenol, eugenol acetate, kaempferol, quercetin, and gallic acid, while ginger contains gingerols, shogaols, and paradols. Additionally, Tecomaria was found to contain various active compounds, including saponins, flavonoids, alkaloids, phenols, steroids, anthraquinones, tannins, terpenes, phytosterols, triterpenes, hydrocarbons, resins, volatile oil, and glycosides (Raju et al., 2011; Thaís et al., 2022; Binuti and Lajubutu, 1994).

Table 2: Dteremenation of Scavenging activity for DPPH in plants extracts

Activity / Concentration		Scavenging activity %			
		Gallic Acid	Clove	Ginger	Tecoma
Concentration mg/ml	0.02	38	63	20.66	13.05
	0.04	49	66	56.95	20.8
	0.06	57	68	58.25	38.7
	0.08	75	71	65.16	56.0
	0.1	89	73.8	71.09	60.4

3.3. Inhibitory activity of 50% free Radical DPPH:

Table 3 illustrates the inhibitory concentrations required to inhibit 50% of the free radicals DPPH. Clove seed extracts exhibited a concentration of 0.015 mg/mL capable of inhibiting 50% of DPPH radicals, attributed to their high phenolic content. Meanwhile, ginger rhizome and Tecoma flower extracts showed inhibitory concentrations of 0.054 mg/mL and 0.16 mg/mL, respectively. In comparison, gallic acid, considered a standard substance, required 0.43 mg/mL to inhibit 50% of the free radicals. These results align with Tahir et al. (2020), demonstrating superior inhibitory activity of clove extract compared to other extracts.

Table 3:Dteremenation of Scavenging and inhibitory activity of 50% DPPH

Samples	Inhibition concentration (IC) mg/ml
Gallic Acid	0.43
Clove	0.015
Ginger	0.054
Tecoma	0.16

3.4. Antibacterial activity

Table 4 demonstrates the antibacterial activity of the plant extracts. The results indicate ineffectiveness against gram-negative bacteria (Escherichia coli), while a dose-dependent inhibition was observed against gram-positive bacteria (Staphylococcus aureus). Concentrations of 1200 µg/mL exhibited the highest inhibition zone (11, 9.4, and 9.3 mm) for clove, ginger, and Tecomaria, respectively. These findings are consistent with Lim et al. (2022), showing no activity against E. coli but significant activity against gram-positive bacteria for clove extracts. In contrast, conflicting results were found regarding the antibacterial activity of ginger and Tecomaria extracts against gram-negative bacteria, as reported by Turgay and Esen (2015). The vitality of plant extracts against bacteria correlates with their active compound content, particularly phenolic compounds. These plants are known for their various applications in traditional medicine and as food preservatives, indicating their potential as natural antioxidants and preservatives (Grant and Lutz, 2000; Peter, 2000; Tuzlaci et al., 2001; Liang, 1992; Chiozzi et al., 2022; Kalem et al., 2017; Arslan, 2023).

Table 4: Antibacterial Activity of plants extracts (Inhibition Zone,mm).

Plants extracts	Con.µg/ml Bact.	150	300	450	600	750	900	1050	1200
Clove	*E.coli*	0	0	0	0	0	0	0	0
	Staph.aureus	0	6.5	7	7.2	8	8.7	9.3	11
Ginger	*E.coli*	0	0	0	0	0	0	0	0
	Staph.aureus	0	0	6.7	7.4	8	8.6	9	9.4
Tecoma	*E.coli*	0	0	0	0	0	0	0	0
	Staph.aureus	0	6.5	6.9	7.4	7.9	8.5	8.9	9.3

4. Conclusions:

The research results revealed varying levels of total phenolics and antioxidant activity among the studied plants, with cloves exhibiting the highest total phenolic content and antioxidant activity, followed by ginger and Tecoma flowers. None of the plant extracts studied showed efficacy against gram-negative bacteria, while they were effective against gram-positive bacteria. This suggests the potential use of these plant extracts as natural antioxidants and preservatives instead of synthetic materials.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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combination with cinnamon (Cinnamomum burmanii) and Sappan wood (Caesalpinia sappan) as an anti-diabetic.

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